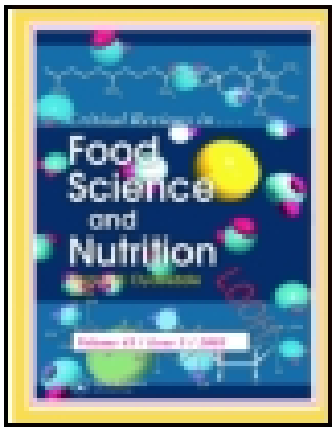

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Prebiotics: a potential treatment strategy for the chemotherapy-damaged gut?

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Prebiotics: a potential treatment strategy for the chemotherapy-damaged gut?

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Abstract

Mucositis, characterized by ulcerative lesions along the alimentary tract, is a common consequence of many chemotherapy regimens. Chemotherapy negatively disrupts the intestinal microbiota, resulting in increased numbers of potentially pathogenic bacteria, such as Clostridia and Enterobacteriaceae, and decreased numbers of "beneficial" bacteria, such as Lactobacilli and Bifidobacteria. Agents capable of restoring homeostasis in the bowel microbiota could therefore be applicable to mucositis. Prebiotics are indigestible compounds, commonly oligosaccharides, which seek to reverse chemotherapy-induced intestinal dysbiosis through selective colonization of the intestinal microbiota by probiotic bacteria. In addition, evidence is emerging that certain prebiotics contribute to nutrient digestibility and absorption, modulate intestinal barrier function through effects on mucin expression, and also modify mucosal immune responses, possibly via inflammasome-mediated processes. This review examines the known mechanisms of prebiotic action and explores their potential to reduce the severity of chemotherapy-induced mucositis in the intestine.

MUCOSITIS

The treatment of cancer by chemotherapy, sometimes in combination with radiotherapy, is associated with an array of acute side-effects, including alopecia, neutropenia and nausea (Logan et al., 2007). Furthermore, these treatments can also induce inflammation and deterioration of the mucosal membranes of the alimentary tract, a condition referred to as mucositis. Mucositis can occur throughout the entire gastrointestinal tract (Gibson et al., 2005); however, it most commonly affects the mucosa of the mouth (oral mucositis) and small intestine (gastrointestinal mucositis), with symptoms including pain, vomiting, bloating, and diarrhoea. Severe cases are often associated with secondary complications such as malnutrition and septicaemia, resulting in poor patient outcomes. Indeed, the pathobiology of mucositis is influenced by the nature of the causative agent (Logan et al., 2009), with temporal variations in pathogenesis, in addition to the location and severity of damage (Logan et al., 2009). Consequently, the multi-factorial nature of mucositis makes it a difficult condition to understand, and subsequently treat.

Despite mucositis being one of the most common side-effects of cancer treatment (Choi et al., 2007) its incidence and severity is highly variable, and is dependent on the treatment regimen and individual variation in terms of tolerability (Lalla et al., 2006). Approximately 40% of all patients undergoing chemotherapy develop mucositis (Gibson et al., 2002; Lalla et al., 2006) with this number rising to between 60% and 100% in patients receiving high-dose chemotherapy regimens for conditions such as the haematological malignancies (Gibson et al., 2002; Lalla et al., 2006).

To date, effective treatments for intestinal mucositis have been elusive. Potential treatment options are limited by the requirement for treatments to protect the mucosa and promote the repair process, without compromising cytotoxic effects on the neoplasm. Certain growth factors such as epidermal growth factor, transforming growth factor-α and betacellulin (Choi et al., 2008; Dahlhoff et al., 2008; Sukhotnik et al., 2008a; Sukhotnik et al., 2008b; Boukhettala et al., 2010; Kim et al., 2010) have the potential to promote differentiation and proliferation of epithelial cells, and 'prime' the intestine prior to chemotherapy, in addition to accelerating the repair process. Previously, keratinocyte growth factor-1 (KGF-1) has shown efficacy in the oral form of the condition (Blijlevens and Sonis, 2007), and decreased diarrhoea in a rat model of irinotecan-induced mucositis (Gibson et al., 2005), however, it is yet to be applied extensively in the treatment of intestinal mucositis in humans. Other growth factors, such as whey growth factor extract (WGFE) (Howarth et al., 1996; Clarke et al., 2002; Tran et al., 2003), insulin-like growth factor-I (IGF-I) (Howarth et al., 1998; Howarth, 2003; Cool et al., 2005) and fibroblast growth factor-20 (FGF-20) (Gibson et al., 2007) have also been investigated, demonstrating variable efficacy. Although in their infancy, nutraceutical agents have recently been tested in experimental models for efficacy against mucositis. These include plant extracts such as grape seed extract (Cheah et al., 2009) and Iberogast (Wright et al., 2009), and animal-sourced oils such as Lyprinol (Torres et al., 2008) and Emu Oil (Lindsay et al., 2010).

Development of Mucositis

Mechanisms underlying the development of mucositis are complex, and still not fully understood; however, it is hypothesized that the condition progresses through five stages. The

initiation of mucositis occurs directly after chemotherapy administration and is characterised by the production of reactive oxygen species (ROS) (Sonis et al., 2004; Lalla and Peterson, 2006; Logan et al., 2007). These then activate nuclear factor-κB, (NF-κB) (Logan et al., 2009), a transcription factor implicated in promotion of the inflammatory response in conditions including arthritis and the inflammatory bowel diseases (Mankan et al., 2009). NF-κB is thought to be directly responsible for the up-regulation of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 (Logan et al., 2009). These cytokines further amplify damage to the mucosal cells (Sonis et al., 2004). Mucositis first becomes clinically evident during the fourth phase, termed 'ulceration and inflammation' (Sonis, 2004a). It is during this stage that the epithelial layer increases in permeability, allowing the translocation of bacteria into the blood stream. This is accompanied by severe pain and discomfort, and sometimes secondary complications such as malnutrition and septicaemia (Logan et al., 2007). Further changes occur during the ulcerative stage, including crypt ablation and villus atrophy, leading to a decrease in the overall absorptive area of the intestine, a decrease in digestive enzymes, and an increase in intestinal permeability, with subsequent increases in bacterial translocation and alterations in the gut microbiota (Stringer et al., 2009b). Following the discontinuation of chemotherapy or radiotherapy, symptoms resolve and mucosal repair begins, characterised by proliferation and differentiation of tissues and cells (Sonis et al., 2004; Lalla and Peterson, 2006). Currently, our understanding of the mechanisms underlying these changes is incomplete, and a role for apoptosis as an early event has been proposed (Sonis, 2004b). Through a better understanding of the role of the microbiota in the progression and resolution of intestinal

mucositis, and the mechanisms by which these interactions can be influenced, it is possible that the local microbiota could be altered in such a way as to lessen the severity of the condition.

The Bowel Microbiota and Development of Intestinal Mucositis

The role of the gut microbiota in the development of intestinal mucositis awaits clear definition; although evidence is emerging that the symbiotic relationship between the microbiota and its host is negatively disrupted by chemotherapy (Stringer et al., 2009a; Stringer et al., 2009b). This may have important implications for both the severity of mucositis, and the overall health of the host. The microbiota may influence the development and severity of mucositis through at least five different mechanisms. These include influencing the inflammatory response, intestinal permeability, the composition of the mucin layer, epithelial repair, resistance to harmful stimuli, and the gut immune system (van Vliet et al., 2010). In mucositis, there is a shift in composition of the microbiota away from commensals and 'beneficial' bacterial species, such as *Bifidobacteria* spp. and *Lactobacillus* spp., towards more 'pathogenic' microbes such as Clostridia and Enterobacteriaceae (Stringer et al., 2009a). Recent studies have also demonstrated decreases in goblet cell numbers and mucin secretion associated with chemotherapy administration (Stringer et al., 2009b). Maintaining a healthy microbiota and mucin layer during chemotherapy treatment could minimise the likelihood of bacterial translocation and secondary infection arising from mucositis. Probiotic microorganisms hold potential in this context. Probiotics are defined as living microorganisms which when administered in adequate amounts, exert desirable health benefits on the host (Reid, 2005). A recent review by Prisciandaro et al. (2011) reported that certain probiotics such as Lactobacillus, Bifidobacterium genera,

Enterococcus and Streptococcus species may reduce the severity of chemotherapy-induced mucositis. Modalities capable of stimulating colonisation of the bowel by probiotic bacteria more globally could be a potential strategy to restore intestinal homeostasis. Prebiotics could represent such a strategy.

PREBIOTICS

The concept of prebiotics, and targeted modulation of the gut microbiota was introduced in 1994-1995 (Delzenne and Roberfroid, 1994; Gibson and Roberfroid, 1995), with the definition of prebiotics as compounds that 'selectively stimulate the growth and/or activity of microbial species in the gut microbiota and confer health benefits to the host' (Roberfroid et al., 2010). In order to be classified as a prebiotic, a given substance must be neither hydrolysed nor absorbed in the proximal gastrointestinal tract, must be selectively fermented by one or a limited number of beneficial bacteria in the intestine and be able to alter the colonic microbiota toward a healthier composition (Gibson and Roberfroid, 1995; Looijer-van Langen and Dieleman, 2009; Roberfroid, 2007). The fermentation of prebiotics in the colon produces short-chain fatty acids (SCFA), which support proliferation of the colonic microbiota (Yanahira et al., 1995), Oligosaccharides (OS) tend to predominate as compounds that meet the definition of a prebiotic (Roberfroid, 2007).

A wide variety of compounds have the potential to meet the requirements for definition as a prebiotic, although at this stage, not all have been studied sufficiently to classify them as such. The most intensively researched prebiotic compounds to date include inulin (Abrams et al.,

2005), a plant derived fructan, lactulose (Bovee-Oudenhoven et al., 2003), galacto-oligosaccharide (GOS) (Shoaf et al., 2006; Alliet et al., 2007), derived from milk and fructo-oligosaccharide (FOS) (Cherbut et al., 2003), also having plant origins. Other compounds studied for their prebiotic potential include mannan-oligosaccharides (MOS) (Yang et al., 2008b), β-1,4-mannobiose (MNB) (Ibuki et al., 2010), sialyl-oligosaccharides (SOS) (Sinclair et al., 2008), xylo-oligosaccharides (XOS) (Xu et al., 2009), germinated barley foodstuff (GBF) (Kanauchi et al., 2008), epilactose (Nishimukai et al., 2008; Watanabe et al., 2008), arabinoxylan-oligosaccharides (AXOS) (Maki et al., 2012) and chito-oligosaccharides (COS) (Liu et al., 2008).

Different prebiotics may exert an array of biological effects, including alteration of gut morphology (Leforestier et al., 2009), stimulation of mucosal and systemic immune responses (Eiwegger et al., 2010; Gopalakrishnan et al., 2012), alteration of local gut microbial communities, such as by enhancing the proliferation of probiotics (*Lactobacillus plantarum* L12 and *Bifidobacterium pseudocatenulatum* B7003) *in vitro* (Marotti et al., 2012), modulation of inflammation (Pilzner et al., 2012), changes in mucin production (Leforestier et al., 2009) and a decreased occurrence of infection in the first six months of life (Arslanoglu et al., 2007). Prebiotics have also been reported to alter the uptake of various nutrients and minerals, such as calcium, from the intestine (Abrams et al., 2005; Bakker-Zierikzee et al., 2005). Considered together, certain prebiotics could have the potential to combat specific aspects of mucositis pathogenesis.

Prebiotics and Intestinal Dysfunction

One of the earliest studies to investigate the influence of indigestible carbohydrates on gut dysfunction examined the effect of FOS ingestion in immunoglobulin A (IgA) deficient dogs with small bowel bacterial overgrowth (Willard et al., 1994). This study concluded that FOS ingestion altered the microbial communities in the small intestine towards fewer aerobic/facultative anaerobic species, although the exact mechanism and implications of these findings were not determined. The following year, Gibson and Roberfroid (1995) introduced the concept of a 'prebiotic' as it is defined today, and in later studies, introduced two other major postulates. Firstly, the concept of targeted modulation of the intestinal microbiota (Gibson, 1998) and secondly, the use of prebiotics and probiotics concurrently, which has come to be known as 'synbiotics' (Roberfroid, 1998).

This early work, and the establishment of set criteria for a 'prebiotic' resulted in a number of studies examining the effect of oligosaccharides on the microbiota (Yanahira et al., 1995) and digestibility studies on substances suspected of containing indigestible oligosaccharides (Brand-Miller et al., 1998). Recently, Bogusławska-Tryk et al. (2012) reported that inulin and FOS type fructans increased probiotic bacteria, while decreasing certain pathogens. Additionally, these investigators reported that inulin and FOS increased the length of the small and large intestines in chickens and this contributed to an increase in intestinal enzymatic activity, which in turn led to an improvement in nutrient digestibility and absorption (Bogusławska-Tryk et al., 2012).

De Souza Oliveira et al. (2012) suggested that one of the beneficial effects of the prebiotic inulin, during the fermentation process of skim milk by *Streptococcus thermophilus* and *Lactobacillus rhamnosus*, was due to the release of fructose, which could act as a carbon and energy source for the host. In the setting of clinical cancer treatment, this carbon and energy source could potentially be used to enhance the energy levels available to chemotherapy patients struggling with nutrient malabsorption due to mucositis.

These studies were followed by investigations into disease states characterised by an imbalance in the commensal microbiota, particularly in the colon. Certain prebiotics have revealed therapeutic promise in the treatment of inflammatory bowel disease (Kumar et al., 2012) and researchers are now investigating prebiotics for their potential to treat other gut disorders which include acute infantile diarrhoea (Agustina et al., 2007) and irritable bowel syndrome (Fedorak and Madsen, 2004). As mucositis is characterised by a disrupted intestinal microbiota it could be clinically rewarding to test the capacity for prebiotics to normalize the composition of the gut flora.

Prebiotics and Cancer

Some studies have indicated that prebiotics such as FOS may play an important role in cancer prevention (Daddaoua et al., 2007; Sung and Choi, 2008). Clark et al. (2012) recently reviewed the effect of prebiotics on biomarkers of colorectal cancer (CRC) and concluded that certain prebiotics had the potential to prevent colonic cancer. In their review, potential biomarkers of prebiotic efficacy on CRC were identified (Clark et al., 2012). These endpoints included

neoplasia occurrence, cell proliferation, gene expression, and DNA methylation, which were likely more indicative relating to CRC risk (Clark et al., 2012). Furthermore, it has been suggested that secondary markers, such as toxic compounds in the colon or rectum, protein degradation, and bacterial enzyme activity could also be considered in CRC prevention (Clark et al., 2012). Indeed, recurrence rate of adenomatous polyps was decreased after administration of non-digestible lactulose in humans (Roncucci et al., 1993; Clark et al., 2012). These endpoints could be used in the future study of prebiotics on mucositis. It has become evident that prebiotic-type compounds present in milk, such as GOS, play an important role in the development of the neonatal gut, and immune system (Fanaro et al., 2005; Arslanoglu et al., 2007). Modulating cancer-related biomarkers of proliferation, apoptosis and immune modulation via prebiotic administration could be equally applied in mucositis pathogenesis, although we await appropriately controlled studies in this context.

Prebiotics and the Microbiota

The mucosal barrier on the epithelial surface of the intestine consists, in part, of colonized bacterial communities, which may contribute to the defensive gut barrier and guard against other pathogen colonization and subsequently decrease the likelihood of bacterial translocation (Gori et al., 2011). However, chemotherapy administration can induce changes in bacterial communities to a more pathogenic composition (Stringer et al., 2009b). Microbiota composition in the small and large intestine were significantly altered by 5-FU injection in rats. Specifically, levels of *Clostridium* spp., *Lactobacillus* spp. and *Streptococcus* spp. were decreased and

Escherichia spp. increased in the jejunum, whereas, levels of *Enterococcus* spp., *Lactobacillus* spp. and *Streptococcus* spp. were decreased in the colon.

The ability for prebiotics to alter the commensal microbiota towards a beneficial composition has been demonstrated in several studies. For example, a prebiotic oligosaccharide mixture administered to humans improved gut microbiota composition, as measured by an increase in Bifidobacteria and a reduction in the levels of pathogenic Clostridia-related species (Gori et al., 2011). In broiler chickens, MOS and FOS affected the microbiota composition of the small intestine. Yang et al. (2008a) reported that MOS ingestion decreased mucosa-associated *Coliform* spp. in the jejunum in an *Escherichia coli* challenge model in broiler chickens (Yang et al., 2008a), and the same authors also demonstrated that FOS increased *Lactobacillus* spp. levels in the jejunum, and lactic acid levels in the ileum in both the *Escherichia coli* challenged and non-challenged model. Several other studies have shown similar results, with an increase in *Bifidobacteria* spp. and *Lactobacillus* spp. in both rat large intestine and human fecal material following administration of FOS (Lara-Villoslada et al., 2006; Ten Bruggencate et al., 2006). Moreover, when FOS was administered in conjunction with *L. paracasei* to gnotobiotic piglets, the increase in *Bifidobacteria* spp. and *Lactobacillus* spp. was prolonged and enhanced, compared to piglets administered the probiotic alone (Bomba et al., 2002). AXOS has also been reported to alter lactic acid bacteria and *Clostridium* sp. composition in sturgeon fish species (Geraylou et al., 2012). Recently, it has been reported that the administration of a specific prebiotic mixture (short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides, ratio 9:1) to newborn human babies resulted in higher levels of fecal bifidobacteria and

lactobacilli than the placebo group (Salvini et al., 2011). Together, these studies demonstrated that prebiotics have the potential to consolidate the beneficial bacterial composition of the mucosal barrier in the small and large intestine as well as altering the profile of the microbiota in fecal material.

The altered microbiota may in turn compete with pathogenic bacteria for adhesion sites in the gut. However, evidence is still lacking to confirm the capacity for prebiotics to normalise the composition of the microbiota in specific areas of the small and large intestine in mucositis. In addition, prebiotics are able to decrease gut pH and modulate the SCFA pattern, resulting in an inhospitable environment for pathogens (Eiwegger et al., 2010; Gori et al., 2011). Collectively, the stimulation of favourable strains of bacteria in the gut, and a reduction in gut pH, may also contribute to enhanced immune defences in the mucosal barrier. This could represent a fertile area for future research.

Nevertheless, not all prebiotic studies have resulted in clinical improvements. For example, the prebiotic, MOS, failed to improve growth performance in pigeons, nor improve the immune response against the Newcastle disease virus (Khan et al., 2012). This indicates that our understanding of the potential applications for prebiotics is limited and suggests that a range of factors, particularly the amount and type of prebiotic supplement used, the species and health status of the subject, as well as a range of other factors, all play an important role.

Further evidence is emerging that administration of prebiotics can alter gene expression in the gut through their fermentation products, in particular, butyrate (Sauer et al., 2007; Rodenburg et al., 2008). Butyrate has been identified as a potential anti-neoplastic agent in the colon (Scharlau et al., 2009), has been shown to induce apoptosis in gastric cancer cells (Matthews et al., 2007) and also plays an essential role in the regeneration of the mucosa (Sauer et al., 2007) and in inhibition of pro-inflammatory cytokine production in rat mesenteric lymph nodes (Looijer-van Langen and Dieleman, 2009).

Prebiotics and Mucins

The mucin layer is an integral component of barrier function in the intestine (Stringer et al., 2009b). Mucins form gels and generate a protective mucus blanket overlying the epithelial surface protecting the mucosa from bacterial overgrowth and/or penetration (Specian and Oliver, 1991). Mucins also produce a physical and chemical barrier that protects the epithelium from luminal agents such as enteric bacterial toxins (Specian and Oliver, 1991). In addition, mucins create an enormous repertoire of potential binding sites for microorganisms, such as commensal and pathogenic bacteria (Robbe et al., 2004).

It has been proposed that inhibition of bacterial adhesion occurs as a result of terminal sugars on prebiotics interfering with receptors on the bacteria that facilitate adhesion to the intestinal mucin layer (Forchielli and Walker, 2005). Sinclair et al. (2008) demonstrated that this interference was not unique to bacterial cells, with a study using sialyloligosaccharide shown to inhibit binding of the cholera toxin to its receptor on the cell. FOS and GOS have been linked to

increased mucin production (Ten Bruggencate et al., 2006; Leforestier et al., 2009) resulting in reduced bacterial translocation and consequently, a lower incidence of pathogenic infection (Looijer-van Langen and Dieleman, 2009). In contrast, chemotherapy agents significantly decreased epithelial mucin levels in the jejunum in rats treated with 5-FU (Stringer et al., 2009b) although, mucus secretion in the large intestine was increased in irinotecan treated rats (Gibson et al., 2003). Therefore, it is clear that chemotherapy can induce mucin changes in rats. However, there is still insufficient evidence to definitively predict mucin changes induced by different chemotherapy agents.

Prebiotics, on the other hand, could have the ability to prevent chemotherapy-induced damage to the gut by maintaining mucosal integrity through mucin modification. However, the capacity for prebiotics to alter mucin levels in the different intestinal regions in the mucositis setting requires future research.

Prebiotics and the Gut Immune System

The gut mucosal lining contains organized lymphoid tissues that support the initiation of anti-microbial immune responses (Rancez et al., 2012). Lymphoid cells can be orchestrated by chemokines, trafficking and positioning into different mucosa-associated lymphoid tissues, contributing to immune defence (Rancez et al., 2012). Prebiotics therefore have the potential to modulate lymphoid cells and stimulate the gut immune system (Forchielli and Walker, 2005; Daddaoua et al., 2007; Geraylou et al., 2012). For example, administration of FOS directly stimulated the mucosal immune system in mice and humans, in the form of increased IgA

production (Looijer-van Langen and Dieleman, 2009; Yasuda et al., 2012). IgA is the dominant antibody associated with mucosal immunity, and plays an important role in inhibition of pathogen adhesion to the intestinal wall and the activation of macrophages (Looijer-van Langen and Dieleman, 2009). Recently, Yasuda et al. (2012) reported that FOS decreased allergic peritoneal inflammation in mice through increased local synthesis of IgA. However, prebiotics such as FOS and inulin did not affect IgA levels in dogs (Patra, 2011) or in humans treated with AXOS (Walton et al., 2012).

In addition to the expression of IgA, a mixture of the prebiotics FOS and GOS enhanced delayed type hypersensitivity in an influenza vaccine model as a marker of T helper cell (T_h1) immunity (Vos et al., 2006) and induced an immunoglobulin profile considered beneficial in infants at high risk for allergy (van Hoffen et al., 2009). FOS has also been demonstrated to increase circulating levels of IgM and IgG in plasma, suggesting that the influence of prebiotics is not limited to the gut (Janardhana et al., 2009). Furthermore, GOS has recently shown the ability to reduce the severity of colitis in mice through increasing the activity of natural killer (NK) cells in the spleen and mesenteric lymph nodes (MsLN) (Gopalakrishnan et al., 2012). GOS also stimulated NK expression of the chemokine receptor CCR9, which is involved in lymphocyte trafficking to the gut, pre-infection in the blood, spleen and MsLN (Gopalakrishnan et al., 2012). GOS mixture has also been reported to improve NK cell activity in highly active antiretroviral therapy (HAART)-naïve HIV-infected human individuals (Gori et al., 2011). In addition, prebiotics such as GOS could produce SCFA during fermentation (Lamsal, 2012), which have been reported to induce NK cytotoxic activity in rats (Pratt et al., 1996).

FOS facilitated the proliferation of Lactobacilli, Bifidobacteria, both of which are involved in the induction of regulatory T cells (Treg), which play an important role in modulation of the immune system in C57BL/6J mice in response to Flu-vaccination (van't Land et al., 2010). FOS is also involved in the direct induction of Treg (van't Land et al., 2010). Therefore, FOS could modulate the chemotherapy-affected immune response through activation of suppressive T cell populations including Treg. To this end, bacteria, such as *Bifidobacterium infantis* 35624 induced to proliferate by certain prebiotics, may enhance the activity of indoleamin 2,3-dioxygenase which in turn may contribute to Treg production and T cell tolerance (Konieczna et al., 2012; Yasuda et al., 2012).

In addition, β-1,4-mannobiose has been reported to exert prebiotic activity by stimulating both systemic and innate immune responses in the small intestine in one-day-old chicks (Ibuki et al., 2010). MNB up-regulated the peptide transporter associated with antigen processing (TAP)-2 expression (Ibuki et al., 2010), an essential factor for expression of the major histocompatibility complex (MHC) molecule on cell surfaces (Panter et al., 2012). Moreover, MNB up-regulated TNFSF15 gene expression in healthy chicks, related to the differentiation, proliferation and apoptosis of immune cells (Zhang and Li, 2012). Likewise, MNB enhanced healthy chick immunity through up-regulated expression of BLB-1, BF-2, IRF-1, IRF-7 and TLR3 genes (Ibuki et al., 2010).

Furthermore, it has been shown that AXOS up-regulated the immune response in piglets by influencing the inflammatory and antibacterial protein pancreatitis associated protein (PAP) and down regulated *Escherichia coli* induced responses (Niewold et al., 2012). Interestingly, the higher degree of polymerization of AXOS seems to have a greater beneficial impact on health, as measured by enhanced phagocytic activity of fish macrophages as well as improved alternative haemolytic complement activity and total serum peroxidase content (Geraylou et al., 2012). As chemotherapy-induced mucositis is associated with a compromised immune system, prebiotics may have the potential to beneficially modify the immune response.

Prebiotics and Inflammation

The inflammatory process associated with mucositis is complex, and is characterised by a number of different mechanisms, including the production of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, nitric oxide (NO) and ROS (Abdelouhab et al., 2012; Liu et al., 2012). Administration of prebiotics such as inulin, FOS and germinated foodstuff have demonstrated efficacy in models of inflammatory intestinal disorders, including dextran sulphate sodium (DSS)-induced colitis, dimethylhydrazine-induced colon cancer, and chemotherapy (5-FU) induced rats mucositis (Araki et al., 2000; Kanauchi et al., 2003; Lara-Villoslada et al., 2006; Winkler et al., 2007; Smith et al., 2008; Abdelouhab et al., 2012).

Initially, the beneficial effects demonstrated by prebiotics were believed to be due solely to their interaction with the commensal intestinal microbiota. However, recent indications suggest that prebiotic administration may have independent effects. A study in obese mice revealed that

oligofructose ingestion stimulated secretion of glucagon-like peptide-2 (Cani et al., 2009), a potent intestinal growth factor, in a rat model of DSS-induced colitis (Brubaker et al., 1997). Sung and Choi (2008) found that cyclooxygenase-2 expression in rats treated with dimethylhydrazine, was decreased following FOS administration, and it was proposed that this could represent a potential mechanism of action for certain prebiotics.

Recently, inulin has been shown to significantly reduce colonic lesions in DSS-induced colitis in rats (Abdelouhab et al., 2012). Moreover, FOS ameliorated allergic peritoneal inflammation induced by ovalbumin (OVA), partially through the suppression of interleukin-5 (IL-5), and chemokine eotaxin in mice (Hosono et al., 2003; Yasuda et al., 2012). The IL-5 gene cluster and eotaxin are concomitant with the infiltration of eosinophils, neutrophils, mast cells and T lymphocytes into the airways of mice contributing to inflammation, particularly in Th2 type chronic inflammatory disease (Pilzner et al., 2012). Thus, it is likely that FOS influences cell populations such as T cells, basophils, mast cells, and epithelial cells (Hosono et al., 2003; Yasuda et al., 2012).

GOS has demonstrated efficacy in inhibiting the adherence of pathogenic bacteria to Caco-2 and HEp-2 cells in culture (Shoaf et al., 2006) and *in vitro* tests also demonstrated that GOS, in the absence of bacteria, stimulated sucrase activity in a Caco-2 cell line (Leforestier et al., 2009). More recently, it has been reported that the prebiotic, inulin has the ability to normalise increased NO levels, in peritoneal macrophage cultures from Swiss mice (Abdelouhab et al., 2012). In addition, prebiotics could mediate the secretion of pro-inflammatory cytokines such as TNF-α

via modification of Toll-like receptor (TLR) gene expression. Trevisi et al. (2008) found that FOS in combination with *Bifidobacterium animalis* increased expression of the TLR-2 encoding gene in the lymph nodes of piglets. The authors further described that TLR-2 and TLR-4 encoding gene expressions were significantly associated with TNF-α encoding gene expression (Trevisi et al., 2008). On the other hand, Fukuda et al. (2011) revealed that germinated barley food stuff (containing prebiotics) decreased TLR-4 expression by increasing butyrate production and decreasing cyclooxygenase 2 mRNA expression in azoxymethane treated F344 rats. Furthermore, the consumption of XOS in combination with inulin decreased blood lipopolysaccharide (LPS) concentrations and attenuated LPS-induced increases in gene expression following IL-1β and LPS-induced decreases in gene expression of IL-13 in the blood of healthy humans (Lecerf et al., 2012).

LPS can be recognized by TLR-4 with the MD-2, CD14 and LPS-binding protein (LBP). Initiation of this TLR signaling has also been involved in proteins of MyD88, Tirap, Trif, and Tram (Yamamoto et al., 2002; Yamamoto et al., 2003; Shigeoka et al., 2007). Ultimately, this LPS-TLR signaling leads to the induction or suppression of genes that orchestrate the inflammatory response through both MyD88 dependent and independent expression (Yamamoto et al., 2002; Yamamoto et al., 2003; Shigeoka et al., 2007). However, it is still not clear if prebiotics increase the immune response of intestinal epithelial cells by modifying TLR-4 in the MyD88 dependent, independent or other pathways. Future studies should focus on the effects of prebiotics on TLR-4 modification and its signaling pathways compared to LPS, as well as the key proteins involved.

In a recent commentary, Howarth (2012) proposed that prebiotics have the potential to influence inflammasomes and modulate intestinal inflammation and function. Inflammasomes are a group of protein complexes built around several proteins including NLRP3, NLRC4, AIM2 and NLRP6 (Strowig et al., 2012). These inflammasomes can recognize a wide range of microbial, stress and damage signals and following the activation of caspase-1, subsequently induce the secretion of pro-inflammatory cytokines such as IL-1 and IL-18 (Strowig et al., 2012). Inflammasomes can also induce pyroptosis, a form of cell death (Strowig et al., 2012). Furthermore, inflammasome-mediated processes play an important role in microbial infections, regulating metabolic processes, and mucosal immune responses in human diseases (Strowig et al., 2012). Indeed, a recent study by Qu et al. (2012) found that inflammasomes NLRC4 phosphorylation (NLRC4 phospho-Ser 533) successfully inhibited the activation of caspase-1 and pyroptosis in response to *Salmonella typhimurium*. This indicated that NLRC4 phosphorylation could be a decisive point for NLRC4 inflammasome activation and host innate immunity (Qu et al., 2012). Future studies could investigate the potential for prebiotics to modify inflammasomes through different signaling pathways. This would further our understanding of prebiotics and their direct and indirect effects on inflammation in the chemotherapy-damaged intestine (Howarth, 2012).

The influence of prebiotics on inflammation in mucositis requires further investigation. Future studies should not only focus on histological or inflammatory cytokine related responses, but also on molecular responses, such as gene expression and DNA methylation (Clark et al., 2012).

The journey to identify the key genes related to chemotherapy-induced inflammation, and whether prebiotics could control or alter this inflammation at the gene or protein level, promises to be an exciting and worthwhile focus for the future.

Prebiotics and Synbiotics as Potential Treatment Strategies for Mucositis

To date, there have been few rigorous scientific investigations into the interactions of prebiotics in the healthy and diseased small intestine. In particular, the area of intestinal mucositis remains largely unexplored, with only one published study investigating FOS in a rat model of 5-Fluorouracil (5-FU) induced mucositis (Smith et al., 2008). This study demonstrated that FOS was able to marginally reduce myeloperoxidase activity, an indicator of neutrophil infiltration, in the proximal small intestine of chemotherapy-treated rats. However, no other beneficial effects were evident in the other parameters measured. Similarly, there is only a single report of a synbiotic investigation in mucositis, whereby FOS and the new probiotic *Lactobacillus fermentum* BR11, was administered to rats (Smith et al., 2008). Nevertheless, their combination did not seem to exert a further improvement in the severity of mucositis than administered *L. fermentum* BR11 to rats alone (Smith et al., 2008). More recently, the combination of GOS and Bifidobacterium strain (*B. longum* subsp. *longum* PCB133) significantly inhibited pathogenic *Campylobacter jejuni* levels, a pathogen responsible for food-borne gastroenteritis in humans (Baffoni et al., 2012). Considered together, investigations of prebiotics and synbiotics provide a fertile area for further research in disorders of the small bowel, such as intestinal mucositis.

When used in combination, prebiotics and probiotics may complement each other to achieve a synergistic or additive effect in an attempt to ameliorate mucositis (Geraylou et al., 2012).

Probiotics have been studied extensively in relation to gut health (Parnell and Reimer, 2012; Shimizu et al., 2012). However, only recently have prebiotics been examined specifically in relation to chemotherapy-induced mucositis (Smith et al., 2008). Although it remains unknown whether they can be considered as new agents for the treatment of chemotherapy-induced mucositis, prebiotics and synbiotics require investigation in relation to factors such as composition, dose, molecular mechanisms and gene expression, both *in vitro* and *in vivo,* specifically as they relate to the different mechanisms underlying mucositis pathogenesis.

In conclusion, prebiotics are demonstrating efficacy as growth promoters, anti-inflammatory agents and microbial modulators making them promising candidates for the potential treatment of chemotherapy-induced mucositis. The challenge for the future will be the development of predictive *in vitro*, animal model and clinical trial systems to specifically identify the most efficacious probiotics, prebiotics and synbiotic combinations for this debilitating condition.

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