British Journal of Nutrition (2002), **87**, Suppl. 2, S231–S239 DOI: 10.1079/BJN/2002542

Non–digestible oligosaccharides and defense functions: lessons learned from animal models

R. K. Buddington[1,2]*, K. Kelly-Quagliana[3], K. K. Buddington[1] and Y. Kimura[4]

[1]*Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762-5759, USA*
[2]*College of Veterinary Medicine, Mississippi State, MS 39762, USA*
[3]*Department of Animal and Dairy Sciences, Mississippi State, MS 39762, USA*
[4]*Otsu Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd., 31-13 3-Chome Saigawa Otsu, Shiga 520-0002 Japan*

Animals are constantly exposed to a diversity of health challenges and the gastrointestinal tract (GIT) is a major, if not the principal, site of exposure. Animal models and a limited number of human clinical studies have shown that the assemblages and metabolic activities of the resident bacteria are important determinants of the effectiveness of the various host defense mechanisms and thereby influence the ability of animals to respond to health challenges. The assemblages of bacteria resident in the GIT provide a first line of defense that can exclude invading pathogens, reduce the proliferation of opportunistic pathogens already resident in the GIT, and reduce the availability, carcinogenicity, or toxicity of noxious chemicals. The mucosa of the GIT is a second, multilayered line of defense that includes the mucous and other secretions, the epithelial cells, and immune-associated cells scattered within and under the epithelium. The final line of defense contends with pathogens or noxious chemicals that transcend the mucosal barrier and enter the host and consists of the innate and acquired components of the systemic immune system and the xenobiotic metabolizing enzymes. The lactic acid producing bacteria (LAB) are considered to be immunomodulatory and directly or indirectly influence the GIT and systemic defense functions. Corresponding with this, supplementing the diet with inulin, oligofructose, or other nondigestible oligosaccharides that increase the densities and metabolic capacities of the LAB enhances defense mechanisms of the host, increases resistance to various health challenges, and accelerates recovery of the GIT after disturbances.

β-fructans: Inulin: Oligofructose: Oligosaccharides: Immune: Lactic acid bacteria: Gastrointestinal tract

Introduction

The internal and external environments of animals and humans provide constant and varying challenges to health and well-being. To cope with these challenges, animals have evolved a wide diversity of defense mechanisms. These include physical and functional barriers to invasions, the abilities to recognize and eliminate harmful organisms that do invade, and transformation of potentially harmful substances, while tolerating self and beneficial organisms and substances.

Host defense functions are dependent on adequate intakes of energy and nutrients (Cunningham-Rundles & Lin, 1998), particularly after surgery, trauma, and other inducers of stress (Bengmark & Jeppsson, 1995). Fiber is recognized as a critical component of the diet and is necessary to promote the most effective defenses (Frankel *et al.* 1995; Spaeth *et al.* 1990). Fermentable forms of fiber, such as inulin, oligofructose and other nondigestible oligosaccharides (NDO), represent a nutrient source to the resident bacteria and indirectly provide nutrition to the host as short-chain fatty acids (SCFA), vitamins, and other bacterial metabolites. Supplementing the diet with some NDO identified as prebiotics changes the proportions and metabolic characteristics of the resident bacteria (Gibson & Roberfroid, 1995). The specific responses of the host and the magnitude of benefits vary among the amounts and sources of fiber, and involve complex interactions between the host and the bacteria resident in the gastrointestinal tract (GIT).

This review examines if and how, supplementing the diet with fiber, particularly NDO, and more specifically

Abbreviations: GIT, gastrointestinal tract; LAB, lactic acid producing bacteria; NDO, nondigestible oligosaccharides; SCFA, short-chain fatty acids.
Note: For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.
* **Corresponding author:** Dr R. K. Buddington, tel +0 662 325 7580, fax +0 662 325 7939, email rkb1@ra.msstate.edu

inulin and oligofructose, influences the defense functions of animals and thereby increases resistance to pathogens, environmental contaminants, and other health challenges. The components of the defense system are considered to include the bacteria resident in the GIT, the various defense functions of the GIT that limit invasion, and the systemic defense functions that respond to organisms or compounds that do manage to escape the primary GIT defenses and enter the body. In addition to the innate and acquired components of the immune system, the xenobiotic metabolizing enzymes of the host provides animals with a defense against numerous compounds that are detrimental to normal host physiology. These compounds include the secondary metabolites of plants that have evolved to reduce herbivory and anthropogenic sources of dangerous chemicals, which are more recent health risks. The natural and man-made noxious compounds are often not recognized by the immune system, but must be eliminated or detoxified. This is accomplished by the Phase I and II xenobiotic metabolizing enzymes that are expressed in the GIT and other tissues.

The complex interactions between the GIT bacteria and the various defense functions of the host diet are modulated by diet composition, including the amounts and types of fiber. The interactions are difficult to explore and understand in humans, especially when genetic and phenotypic variation are considered (Szilagyi, 1997). Animal models have been, and continue to be, instrumental in providing much needed information and insights. There is general agreement that NDO enhance resistance to some health challenges, but only a very few studies have directly examined if and how NDO enhance defense mechanisms. The objective of this review is to provide readers with an understanding of topics relevant to how diets supplemented with NDO might enhance defense functions, with the emphasis on inulin and oligofructose. We first provide examples demonstrating that NDO increase resistance to a variety of health challenges. Subsequent sections describe how the increased resistance to health challenges conferred by NDO may involve different components of the integrated defense systems of animals. Because of the limited number of studies that have directly examined the interactions among dietary fiber, defense mechanisms, and resistance to health challenges, readers are cautioned that some of the findings and interpretations must be considered as speculative. The papers selected for this review will provide interested readers with a 'starting point' toward understanding if and how inulin, oligofructose and other NDO enhance defense functions.

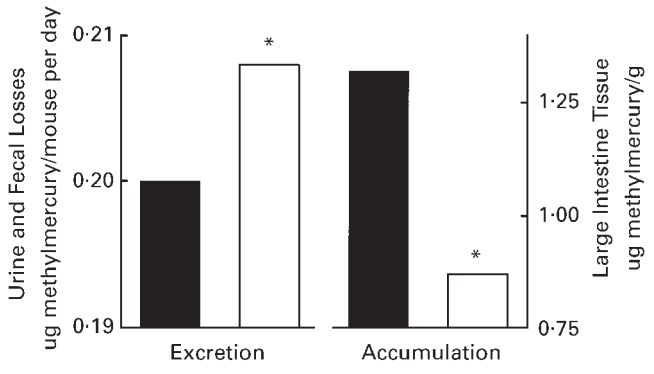

Fig. 1. Methylmercury excreted in the urine and feces and accumulated in the large intestinal tissue of mice fed diets with 100 g/kg fiber as cellulose (■) or inulin (□) and exposed to methylmercury daily for 2 weeks (from Kimura *et al.* 2002). The asterisks indicate mice fed the diet with inulin excreted more and accumulated less methylmercury than those fed the diet with cellulose ($P<0{\cdot}05$).

Resistance to health challenges conferred by inulin, oligofructose and other nondigestible oligosaccharides

Resistance to lumenal pathogens

The mature GIT ecosystem is resistant to colonization by invading species or to overgrowth by opportunistic pathogens that are already present (Bengmark, 1998). The protection provided by the resident bacteria is highlighted by the much lower infective dose of pathogens required to kill gnotobiotic mice compared to those with a GIT harboring even simple assemblages of bacteria (Zachar & Savage, 1979). Additional protection can be obtained by encouraging higher densities of lactic acid producing bacteria (LAB), either by supplementing the diet with NDO or probiotics. Corresponding with this, supplementing the diets of chickens, pigs, and rats with oligofructose and other NDO reduces fecal densities of *Salmonella* (Letellier *et al.* 2000; Fukata *et al.* 1999; Bovee-Oudenhoven *et al.* 1997; Bailey *et al.* 1991). Similarly, supplementing the diet of mice with inulin and oligofructose reduces densities of *Candida* in the small intestine of mice 7 d after infection (Buddington *et al.* 2002), increases colonization resistance to *Clostridium difficile* after antibiotic treatment (Gaskins *et al.* 1996; May *et al.* 1995), and protects gnotobiotic quail inoculated with bacteria associated with necrotizing enterocolitis (Catala *et al.* 1999).

The benefits of protective diet components, such as NDO, are more profound for young animals that have an immature GIT, an undeveloped immune system, and unstable bacterial assemblages (Dai & Walker, 1999). Breast milk confers greater resistance to pathogens than to commercially available formulas and this is partly attributed to the higher densities and proportions of LAB in the GIT of breast-fed infants. These findings have led to a search for compounds that increase the abundance of LAB in the GIT of infants dependent on foods other than breast milk. Although NDO are added to some infant formulas, studies are lacking about the influence on colonization resistance and defense functions.

A major, and frontline, mechanism of host defense is the mucosal barrier. Dietary inputs are the principal source of energy and nutrients for the GIT, and as a result patients and animals dependent on parenteral nutrition have a reduced mucosal barrier. As a consequence they suffer a higher incidence of bacterial translocation. Combining fiber with enteral nutrients enhances the barrier functions (Frankel *et al.* 1995; Deitch *et al.* 1993; Spaeth *et al.* 1990) and can accelerate recovery from the detrimental influences of 5-fluorouracil (Deng *et al.* 1999) and γ-radiation (Ishizuka *et al.* 2000). Although the majority of studies have used cellulose or other fibers that are poor nutrient

sources for the resident bacteria, the limited information available indicates inulin, oligofructose, and other NDO further enhance the mucosal barrier (Chinery *et al.* 1992). The ability of probiotic lactobacilli to reduce the extent of GIT damage caused by the chemotherapeutic agent methotrexate (Mao *et al.* 1996) suggests the beneficial responses to NDO involve the associated changes in bacterial assemblages.

Resistance to noxious chemicals in the diet

Dietary inputs can expose animals to noxious substances. Of particular interest are environmental contaminants, drugs, pesticides, and other potentially detrimental compounds of anthropogenic origin. Supplementing a mouse diet with inulin increases fecal losses and thereby reduces accumulation of methylmercury compared to a diet with cellulose or lacking fiber (Fig. 1). Interestingly, the diet with inulin did not diminish the absorption, increase fecal losses, or reduce accumulation of retinol (Kimura *et al.* 2002). Adding wheat bran to the diet of mice also reduces mercury retention (Rowland *et al.* 1986), and the viscous indigestible polysaccharides sodium alginate and guar gum reduce the accumulation of pentachlorobenzene by rats (Ikegami *et al.* 1994). Using rice bran, corn, soybean, spinach, and burdock as sources of fiber in rat diets increases excretion and reduces accumulation of polychlorinated dibenzofurans, polychlorinated dibenzo-*p*-dioxins, and polychlorinated biphenyls (Morita *et al.* 1995, 1993).

Inulin, oligofructose, and other NDO increase resistance to carcinogens and reduce the growth of spontaneous and transplantable tumors (Pool-Zobel *et al.* 1996; present volume). However, the inability of β-fructans to reduce the incidence of lung tumors in mice after challenge with B16F10 tumor cells (Buddington *et al.* 2002) indicates that protection is not provided against all such challenges.

Surviving systemic challenges with pathogens

Translocation of GIT pathogens into the interior or systemic circulation of the host is a cause of morbidity and mortality and often results when the mucosal barrier is damaged or diminished. Mice infected systemically with virulent strains of *Listeria monocytogenes* and *Salmonella typhimurium* after being fed a diet with inulin and oligofructose (at 100 g/kg) had lower mortality than mice fed a diet with cellulose as the source of fiber (Buddington *et al.* 2002). Interestingly, inulin provided greater resistance than oligofructose to the systemic infections, significantly so for the *Listeria* challenge.

Accelerating recovery of disturbed gastrointestinal tract ecosystems

Diarrhea disturbs the physical, functional, and biotic characteristics of the GIT, much like floods disturb river systems. After a disturbance, the recovery of the GIT ecosystem is dependent on restoring the normal balance of bacterial species. However, undesirable species, which often are opportunistic pathogens, usually recover faster than commensal species, often leading to secondary infections. Although the majority of attention has focused on accelerating restoration of the LAB, other commensal bacterial groups need to be considered (Rembacken *et al.* 1999). Probiotics hasten recovery from diarrhea by re-establishing the populations of LAB and decreasing the densities of potential pathogens (Guandalini *et al.* 2000; Vanderhoof *et al.* 1999). Probiotics consisting of LAB extend similar benefits to patients suffering from Crohn's disease (Malin *et al.* 1996) and other inflammatory bowel diseases (Shanahan, 2000). Adding oligofructose to an oral rehydration solution accelerates recovery of LAB species endemic to the GIT, reduces the magnitude of increase in enterics (Oli *et al.* 1998), and hastens restoration of the structural and functional characteristics of the GIT (Chandra *et al.* 1996). Similarly, the enteropathy induced by indomethacin is less severe when rats are provided with the NDO lactosucrose (Honda *et al.* 1999). There is also evidence that supplementing the diet with NDO helps dogs and cats recover from large intestinal diseases (Simpson, 1998).

Defense mechanisms and responses to inulin, oligofructose, other nondigestible oligosaccharides and other sources of dietary fiber

Inulin, oligofructose and other NDO are not considered to be immunogenic and apparently do not directly induce the expression of various enzyme systems associated with xenobiotic metabolism. Instead, the majority of influences of NDO on defense functions, and thereby health, can be attributed directly or indirectly to changes in the population and metabolic characteristics of the bacterial assemblages present in the GIT (Bengmark, 1998). In addition, dietary fiber can adsorb some noxious chemicals, increase microbial mass and stool volume, and shorten residence time of digesta, and thereby decrease exposure to the host (Berdanier, 1994; Cummings *et al.* 1992).

The complex, multi-species assemblages of bacteria characteristic of humans and 'normal' animals are hard to study and this has complicated attempts to examine if and how diets supplemented with NDO influence defense functions. Probiotics and the use of gnotobiotic and genetically modified animals have provided valuable insights into the interactions between the GIT bacteria and defense functions of the host (Fuller & Perdigon, 2000; Umesaki & Setoyama, 2000; Erickson & Hubbard, 2000; Falk *et al.* 1998). Perhaps the most dramatic example is the lack of resistance gnotobiotic animals have to lumenal pathogens (Zachar & Savage, 1979). Simply establishing a limited number of species from the 'normal' commensal flora provides protection against invasions by pathogens. The importance of the GIT bacteria led to the development of the Altered Schaedler Flora, which is used to improve the health and disease resistance of rodent models (Dewhirst *et al.* 1999). The use of gnotobiotic animals associated with one or more species of bacteria has led to the recognition of *Bifidobacteria* and other LAB as potential therapeutic agents (Duffy *et al.* 1999). It is likely that many or most of the mechanisms of immunomodulation demonstrated for probiotic species will be

shared with β-fructans and other NDO that encourage the growth and metabolism of LAB already resident in the GIT.

The research with animal models has revealed three lines of host defense. The first is the assemblages of bacteria resident in the different regions of the GIT. The second line of defense is the multilayered mucosal barrier that acts as a selective filter for absorption of water, nutrients, and electrolytes, but must exclude pathogens, hazardous chemicals, and other potential challenges to health. The third line of defense consists of the systemic mechanisms that must recognize and eliminate any potentially harmful organisms or chemicals that manage to transcend the mucosal barrier. The defense mechanisms are generally considered to include those that act against antigens that can be recognized and eliminated by the innate and acquired components of the immune system. This review also includes the mechanisms of defense associated with the xenobiotic metabolizing enzymes that are important for transforming and eliminating compounds that are not recognized by the immune system, yet pose a risk to health. The relationship among the GIT bacteria, host defense, and health is well recognized and the modulation by inulin, oligofructose, and other NDO has been the subject of numerous reviews. Therefore, the following sections will focus on the latter two lines of defense, the associated mechanisms, and the potential and realized responses to diets supplemented with inulin, oligofructose, and other NDO.

Defense mechanisms of the gastrointestinal tract

Before a pathogen or toxin present in the GIT can enter the host, it must first cross the multilayered mucosal defenses. The availability of genetically manipulated animals has assisted in understanding how the GIT bacteria modulate mucosal defenses. Exemplary is the protection provided by LAB to interleukin 10 knockout mice that are predisposed to inflammatory bowel disease (Madsen *et al.* 1999). Probiotic LAB have been considered as immunomodulators because they enhance mucosal defense functions and reduce bacterial translocation in animal models (Lee *et al.* 2000; Herias *et al.* 1999), with similar findings for humans (Hove *et al.* 1999). Since inulin, oligofructose and other NDO increase LAB densities, it is likely they provide health benefits that are similar to those obtained with LAB-based probiotics.

Bacterial regulation of various GIT immune functions is well established (Fuller & Perdigon, 2000; Neish *et al.* 2000; MacDonald & Pettersson, 2000; Lefrancois & Goodman, 1989). The co-evolution of bacteria with host animals (Carman *et al.* 1993) has resulted in GIT immune responses that are able to differentiate between commensal and exotic species of bacteria (Berg & Savage, 1975). The relationship is obvious from the changes in immune cell populations and proportions of immune-related cells of the GIT following microbial colonization of gnotobiotic animals, with the responses varying among the species that are introduced. (Falk *et al.* 1998; Imaoka *et al.* 1996; Helgeland *et al.* 1996). Gnotobiotic and newborn animals have functional immune systems, but the cell populations are smaller, are present in different proportions, are not fully activated. As a consequence, the responses to viruses and other pathogens differ from those of animals with diverse and established assemblages of bacteria (Cebra, 1999).

The interactions between the GIT bacteria and the mucosal defenses are more pronounced during infancy when the bacterial populations are undergoing successional changes and there is a greater risk of bacterial translocation leading to systemic disease (Duffy, 2000). Postnatal successional changes in the resident bacteria coincide with development of GIT structural and functional characteristics. The resident bacteria influence GIT synthesis of protein and DNA (Muramatsu, 1990), are critical for development of the defense functions (Kalliomäki *et al.* 2001; Kirjavainen & Gibson, 1999; Cebra, 1999), and influence the risk of bacterial translocation (Berg, 1995).

The mucous secreted by epithelial cells is the first layer of mucosal defense and consists of a combination of immunoglobulin A (IgA), antimicrobial peptides, and a complex mixture of glycosylated proteins that are coded for by several genes. The various glycoproteins in mucous are capable of binding pathogens, preventing them from adhering to the epithelium (Mack & Sherman, 1991), can inhibit replication of some viruses (Yolken *et al.* 1994), and may provide a nutrient source to LAB and other commensal bacteria. It is not surprising that disruption of mucous secretion is associated with inflammatory bowel disease (Shirazi *et al.* 2000). Mucous secretion is dependent on lumenal nutrients (Spaeth *et al.* 1994), but supplementing the diet with fiber increases secretion (Sharma & Schumacher, 1995; Bengmark & Jeppsson, 1995), with the magnitude of increase varying among sources of fiber (Vahouny *et al.* 1985).

The immunoglobulin A (IgA) secreted by B-lymphocytes (plasma cells) in the lamina propria of the mucosa is directed to specific antigens present in the lumen of the GIT, including antigens associated with the commensal bacteria (Macpherson *et al.* 2000). Fibers that are fermented by the LAB increase secretion of IgA into the GIT (Kudoh *et al.* 1999), and this may reduce development of allergic reactions to food antigens (Cross & Gill, 2001; Kirjavainen & Gibson, 1999).

The antimicrobial peptides secreted by the GIT are another important component of the innate immune system (Hancock & Scott, 2000). At the present time, the relationship among the antimicrobial peptides, the GIT bacteria, and diet are poorly understood, and to our knowledge nothing is known about the influence of supplementing the diet with NDO. However, the increased activity of other innate defenses (e.g. macrophages and NK cells) in response to LAB (reviewed by McCracken & Gaskins, 1999) and NDO (our own unpublished findings) suggest that constituitive expression of antimicrobial peptides may be modulated by changes in the GIT bacteria.

Another level of mucosal defense is provided by the epithelial cells, which are capable of antigen presentation, secretion of cytokines, and can recruit immune cells (e.g. macrophages). The high rates of epithelial cell proliferation and turnover, though costly, remove compromised cells and limit the movement of pathogens and noxious

chemicals from the lumen to the host. Oligofructose stimulates higher rates of colonocyte proliferation than cellulose and other NDO (Howard *et al.* 1995), without increasing the total amount of mucosa. Similarly, soy polysaccharide increases proliferation of enterocytes and colonocytes and enhances mucosal structure and functions (Chinery *et al.* 1992). These findings partly explain why different sources of fiber provide varying resistance to health challenges.

The final layer of the GIT defense that protects against pathogens includes the B-lymphocytes, CD4 and CD8 T-lymphocytes in the organized lymphoid tissues in the wall of the GIT (e.g. Peyer's patches), and the immune associated cells scattered throughout the lamina propria and amongst the epithelial cells. Secretion of IgA by the B-cells provides lumenal protection, whereas the T-cells respond to bacterial translocation and other health threats (Lee *et al.* 2000; Gautreaux *et al.* 1994). The patterns of immune function modulation in the GIT vary among sources of fiber (Lim *et al.* 1997) and the regions and tissue layers of the GIT. Feeding dogs a diet with fermentable fiber increases the ratio of $CD4^+$:$CD8^+$ cells in the lymphoid tissues, but increases the proportion of $CD8^+$ in the lamina propria and intraepithelial cell populations (Field *et al.* 1999). The same diet resulted in lower numbers of circulating B-cells, but did not affect the activity of natural killer cells associated with the GIT. These findings are shared with other species (McCracken & Gaskins, 1999) and suggest diets with fermentable fiber do not change the types and functions of immune cells, but instead alter the distribution, relative abundance, and specific responses.

The xenobiotic metabolizing enzymes expressed by the epithelial cells of the GIT provide a mechanism of protection against some environmental toxins. The enzymes of the epithelial cells mainly catalyze oxidative reactions and expression is responsive to changes in diet and the GIT bacteria (Ilett *et al.* 1990). The highest activities are measured in the jejunum. Activities of the epithelium are lower in the colon, where bacterial transformation dominates (Peters *et al.* 1991; Ilett *et al.* 1990), and the processes are mainly reductive (Rowland *et al.* 1986). Despite the importance of this defense mechanism, it has received little attention, and even less is known about the possible modulation of xenobiotic metabolizing enzymes by dietary supplements of inulin, oligofructose, and other NDO.

Systemic host defense mechanisms

Animals have evolved defense mechanisms to respond to pathogens or to noxious compounds that transcend the mucosal barrier and enter the host. The types and magnitude of responses by the systemic defenses to antigens differ somewhat from those of the GIT. However, the ability of probiotics to enhance systemic defenses, increase resistance to health challenges, and potentiate vaccine responses (Fuller & Perdigon, 2000; McCracken & Gaskins, 1999) demonstrates that there are interactions between the GIT bacteria and systemic immune functions. Systemic immune responses vary according to the source of fiber (Cavaglieri *et al.* 2000; Schiffrin, 1997). Feeding mice diets supplemented with inulin and oligofructose increased activities of natural killer cells and phagocytes (our unpublished data) and enhanced T-lymphocyte functions (Pierre *et al.* 1999) more so compared to mice fed diets with cellulose or lacking fiber. These results are consistent with the observation of heightened resistance to systemic infections with *Listeria* and *Salmonella* (Buddington *et al.* 2002), the lower incidence and growth of tumors after exposure to carcinogens (Reddy, 1999) and transplanted tumor cells (Taper *et al.* 1998), and are in agreement with enhanced innate and acquired immune functions provided by *Lactobacillus* and other LAB (Fuller & Perdigon, 2000; Miake *et al.* 1985; Sato, 1984). Interestingly, circulating white blood cell counts were lower in mice fed the diets with inulin and oligofructose. Ratios for $CD4^+$ relative to $CD8^+$ T-lymphocytes and T- relative to B-lymphocytes from the spleen and thymus did not differ between mice fed the diets with β-fructans and cellulose (our unpublished data). These findings are suggestive of a shift to a greater dependence on cell-mediated immunity, a Th1 state (Pratt *et al.* 1996; Taga & Kishimoto, 1995; Thompson, 1994). Corroborating this contention, supplementing diets with NDO changes cytokine concentrations and profiles (Maassen *et al.* 2000; Lim *et al.* 1997), increases blastogenic activity of mesenteric lymph node lymphocytes, decreases the responses of T-lymphocytes isolated from Peyer's patches, and lowers IgE synthesis (Field *et al.* 1999).

Transformation of noxious chemicals by the liver and other tissues augments the activities of the GIT bacteria and mucosa. The combined activities of the xenobiotic metabolizing enzymes of the GIT, the resident GIT, and the liver and other tissues influence the route of excretion, with water soluble metabolites eliminated in the urine and lipid soluble metabolites recycled to the GIT by the bile. Hepatic transformation of mutagenic metabolites of carcinogens can be modulated by fiber (Helsby *et al.* 2000) and by the densities of LAB in the GIT (Nugon-Baudon *et al.* 1998), with the responses varying among the numerous xenobiotic metabolizing enzymes. These findings suggest diet may be an important determinant of the mechanisms of host defenses that are associated with the transformation, disposition, and accumulation of noxious chemicals.

Signaling between the gastrointestinal tract bacteria and host defenses

The ability of LAB and other GIT bacteria to influence defense functions can be indirect. This would include the host's responses to SCFA and other bacterial metabolites (Sakata *et al.* 1999). For example, mucin secretion by the rat colon is stimulated by an increase in lumenal concentrations of SCFA, but not by lactate or succinate (Shimotoyodome *et al.* 2000). Furthermore, butyrate, a SCFA of particular importance to the GIT, enhances defense functions of both the small and large intestine (Wachtershauser & Stein, 2000) and stimulates mucosal growth by increasing the proliferation of enterocytes and colonocytes (Ichikawa & Sakata, 1998). In addition to serving as an important source of energy, SCFA trigger the secretion of several GIT trophic hormones (Tappenden

& McBurney, 1998; Tappenden *et al.* 1997, 1996; Reilly *et al.* 1995). Supplementing diets with inulin, oligofructose and other NDO should increase production of SCFA, and particularly butyrate, and can be predicted to strengthen mucosal defenses and enhance responses to health challenges. In addition to metabolites, components of bacterial cells (e.g. lipopolysaccharides) are well known as potent modulators of immune functions.

The interactions between the bacteria and host defenses can be direct and involve attachment of GIT bacteria to receptors of host cells, thereby permitting 'cross-talk'. The best-known examples are those involving the interactions between pathogens and epithelial cells causing changes in functions of the cells and the GIT (Uzzau & Fasano, 2000; Pothoulakis, 2000). Some species of GIT bacteria induce specific patterns of gene expression that alter the local environment (Bry *et al.* 1996). Adherence of *Lactobacilli* to receptors of epithelial cells can induce expression of genes coding for variants of intestinal mucins that inhibit the adherence of enteropathogenic *E. coli* to the epithelium (Mack *et al.* 1999). The secretion of regulatory cytokines that influence host defenses is modulated by bacteria, with host responses depending on the types and proportions of the secreted cytokines (Maassen, 2000; Pothoulakis, 2000; Chen *et al.* 1999). The cytokines secreted from the GIT provide a signaling mechanism to influence systemic defenses. In addition, lymphocytes of the GIT enter the systemic circulation after interacting with antigen and then differentiate and mature in the germinal vesicles of lymphoid follicles (Lim *et al.* 1997).

Perspectives

Although the LAB modulate immune functions, not all species of LAB elicit the same type and magnitude of responses (reviewed by Fuller & Perdigon, 2000 and McCracken & Gaskins, 1999). Similarly, not all sources of fiber, nor all NDO, provide the same benefits or increase resistance to health challenges (Alles *et al.* 1999). There is a need to identify specific NDO (alone or in combination) that provides the greatest resistance to health challenges. Inter-individual variation in the assemblages of GIT bacteria will influence the types and magnitude of responses to NDO. This will complicate attempts to identify a 'universal' NDO supplement. Getting past the perception of prebiotics as 'conbiotics' will require additional research and a better understanding of the interactions among NDO, the GIT bacteria, and the multiple defense mechanisms of the host. Obviously, animal models will continue to play an important role.

Immunosuppressed patients are at greater risk of infections originating from the GIT. Yet, to our knowledge, the possible benefits such patients may gain from diets supplemented with NDO have yet to be investigated. There is also a need to better understand, if and how, NDO can be used to reduce accumulation of environmental contaminants and other noxious chemicals.

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