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The immune-enhancing effects of dietary fibres and prebiotics

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The gastrointestinal tract is subjected to enormous and continual foreign antigenic stimuli from food and microbes. This organ must integrate complex interactions among diet, external pathogens, and local immunological and non-immunological processes. It is critical that protective immune responses are made to potential pathogens, while hypersensitivity reactions to dietary antigens are minimised. There is increasing evidence that fermentable dietary fibres and the newly described prebiotics can modulate various properties of the immune system, including those of the gut-associated lymphoid tissues (GALT). This paper reviews evidence for the immune-enhancing effects of dietary fibres. Changes in the intestinal microflora that occur with the consumption of prebiotic fibres may potentially mediate immune changes via: the direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine; the production of short-chain fatty acids from fibre fermentation; or by changes in mucin production. Although further work is needed to better define the changes, mechanisms for immunomodulation, and the ultimate impact on immune health, there is convincing preliminary data to suggest that the consumption of prebiotics can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation. Future protocols on the physiological impact of consuming prebiotics should be designed to include assessments of the gut microflora, gut physiology and the function and composition of the various regions of GALT.

Dietary fibres: Inulin: Short-chain fructo-oligosaccharides: Lymphocytes: Gut associated lymphoid tissue

Introduction

Dietary components and their digestion products are in intimate contact with the vast immune system of the intestine (gut-associated lymphoid tissue, GALT) and the presence of food in the small intestine may be necessary for adequate function and development of GALT (Ruthlein *et al.* 1992). Although specific nutrients are known to be important in the development and function of the immune system (Alexander, 1995), less is known about the potential of dietary fibres to impact on immune function. However, studies demonstrating a lower incidence of bacterial translocation across the gut barrier with the administration of dietary fibre (Deitch *et al.* 1993; Spaeth *et al.* 1994; Frankel *et al.* 1995; Xu *et al.* 1998) suggest that this dietary nutrient modulates immunity. This review will summarise evidence for the immune-enhancing effects of dietary fibres and assess potential mechanisms by which changes in the gut microflora may impact on the immune system.

Overview of the immune system

The immune system is defined as the host's defence against destructive forces from both outside (e.g. bacteria, viruses, parasites) and within (e.g. malignant and autoreactive cells) the body. Immune responses are generally classified as either innate (inborn components of the immune system) or acquired (adaptive). The components and cells that comprise these two arms of the immune system are presented in Table 1.

The innate immune system provides immunity to invading organisms without the need for prior exposure to these antigens and includes physical barriers such as the skin and mucous membranes, cell-mediated barriers, including phagocytic cells, inflammatory cells, dendritic cells, and natural killer cells, and soluble mediators such as cytokines, complement and acute-phase proteins (Delves & Roitt, 2000*a*). This arm of the immune system provides the early phases of host defence that protect the organism during the 4–5 days it takes for lymphocytes to become

Abbreviations: APC, antigen-presenting cell; GALT, gut-associated lymphoid tissue; IEL, intraepithelial lymphocytes; Ig, immunoglobulin; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; SCFA, short-chain fatty acid; TNF, tumor-necrosis factor.

Note: For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.

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Table 1. The immune system

Arm of immune system	Defences	Components	Functions
Innate immune system	Physical barriers	Skin	Prevent the entry of antigens into systemic circulation
		Mucous membranes	
	Cell-mediated barriers	Phagocytic cells, e.g. neutrophils, macrophages	Engulf foreign antigens
		Inflammatory cells, e.g. basophils, mast cells	Release inflammatory mediators, e.g. histamine, prostaglandins
		Natural killer cells	Destroy infected or malignant cells
		Dendritic cells	Present antigens to lymphocytes
	Soluble factors	Cytokines	Activate/recruit other cells
		Complement	Enhance phagocytosis
		Acute-phase proteins	Promote repair of damaged tissue
Acquired immune system	B-lymphocytes	Plasma cells	Secrete antibody
	T-lymphocytes	CD4+ T-cells	Induce activation of lymphocytes
		Th1 cells	Promote cell-mediated responses
		Th2 cells	Promote humoral (antibody) responses
		CD8+ T-cells	
		Cytotoxic T-cells	Destroy infected or malignant cells
		Suppressor T-cells	Suppress activity of lymphocytes

activated. Macrophages and their precursor monocytes and the polymorphonuclear leucocytes (neutrophils) make up the major cellular component of the innate immune system (Table 1). Macrophages are essential not only in directly destroying organisms but also in processing and presenting antigens to helper T-cells to initiate acquired immune defences (Delves & Roitt, 2000*a*). Natural killer cells are effective against self cells that have been transformed by viruses or DNA damage, and are also key players in the innate immune system (Delves & Roitt, 2000*a*).

The acquired, or adaptive, immune system develops over an individual's lifetime. Immune responses by this system generally occur after those of the innate immune system; they are antigen-specific, and are more efficient upon secondary exposure to the pathogen (Goust & Bierer, 1993). Lymphocytes are an important cellular component of this arm of the immune system that modulate the function of other immune cells or directly destroy cells infected with intracellular pathogens (Table 1). Each developing T- or B-cell generates a unique receptor, or recognition molecule, by rearranging its receptor genes, such that a set of cells expressing a vast array of diverse receptors is produced, allowing immune cells to selectively eliminate virtually any foreign antigen that enters the body (Delves & Roitt, 2000*a*). B-cells, abundant in lymph nodes, recognise foreign antigen through membrane-bound antibodies, or immunoglobulins, and upon activation become antibody-secreting plasma cells to effectively remove soluble bacteria/antigens (Delves & Roitt, 2000*a*). Antibodies are secreted in soluble form and bind foreign particles to facilitate clearance by phagocytes (Delves & Roitt, 2000*b*). B-cells can also serve as antigen presenting cells and in this respect influence T-cell function (Delves & Roitt, 2000*a*).

T-cells express a T-cell receptor that recognises foreign antigen presented in complex with a major histocompatibility complex (MHC) molecule on the surface of an antigen-presenting cell (APC) (Delves & Roitt, 2000*a*). Subpopulations of T-cells include the helper T (Th) cells, which are identified by the presence of the membrane glycoprotein CD4, and cytotoxic/suppressor T-cells that express the CD8 glycoprotein (Delves & Roitt, 2000*a*). $CD4^+$ cells recognise antigen in complex with MHC class II molecules (found primarily on APC such as macrophages and dendritic cells), whereas $CD8^+$ cells recognise antigen in complex with MHC class I molecules (most nucleated cells in the body express MHC class I) (Delves & Roitt, 2000*a*). $CD4^+$ cells secrete a number of cytokines that are important in the activation of B- and other T-cells, as well as cells of the innate immune system. Based on the types of cytokines these $CD4^+$ cells produce, they are classified into a number of Th types (0, 1, 2 or 3) (Chen *et al.* 1994; MacDonald, 1998; Delves & Roitt, 2000*a*). Th1 cells generally promote cell-mediated inflammatory responses, whereas Th2 cells support antibody (humoral) responses (Delves & Roitt, 2000*b*). Less is known about the function of Th0 and Th3 type cells. $CD8^+$ cells include T-cells with cytotoxic or suppressor function. Cytotoxic T-cells destroy (by releasing granules or inducing apoptosis) intracellular bacteria/virus-infected cells and tumour cells (Delves & Roitt, 2000*b*). Less is known about $CD8^+$ suppressor cells, but they are believed to suppress the activation or activities of other immune cells, and may play a role in immunological tolerance, such as the tolerance to foreign antigens encountered in the gut (Bloom *et al.* 1992; Green & Webb, 1993) (Fig. 1). Although there is considerably less known about these suppressor cells, they have recently been classified into different subtypes, based on the cytokines they produce (Fitch *et al.* 1995).

The gut-associated lymphoid tissues (GALT)

A mucosal immune system (containing defences of both the innate and the acquired immune systems) is strategically placed in areas where external pathogens and antigens may gain access to the body. This includes the mucosal associated lymphoid tissues, which protect sites such as the respiratory, urinary and reproductive tracts, and GALT, which protects the intestine. As the intestine is the first line of defence from the environment, and

must integrate complex interactions among diet, external pathogens, and local immunological and non-immunological processes, it is critical that protective immune responses are made to potential pathogens, yet it is equally important that hypersensitivity reactions to dietary antigens are minimised. Although there is little anatomical data available, it has been estimated that approximately 25 % of the intestinal mucosa is made up of lymphoid tissue (Kagnoff, 1987). GALT is composed of aggregated tissue in the form of Peyer's patches and solitary lymphoid follicles, and non-aggregated cells in the lamina propria and intraepithelial regions of the intestine, as well as mesenteric lymph nodes (Langkamp-Henken *et al.* 1992) (Fig. 1).

Peyer's patches are aggregates of lymphoid follicles found throughout the mucosa and submucosa of the small intestine. These patches contain both $CD4^+$ and $CD8^+$ T-cells, as well as naïve B-cells, plasma cells, macrophages and dendritic cells (Langkamp-Henken *et al.* 1992). Overlying the Peyer's patches are specialised epithelial cells known as M-cells, which endocytose, transport and release antigens from the gut into the Peyer's patches, where these antigens are presented on APC to T- and B-cells (Langkamp-Henken *et al.* 1992; Kagnoff, 1993). Upon activation, B-cells undergo class-switching to produce IgA antibodies, a process that is facilitated by both activated $CD4^+$ and $CD8^+$ cells (Langkamp-Henken *et al.* 1992; Kagnoff, 1993). Activated immune cells exit the Peyer's patches via the mesenteric lymph nodes, enter the systemic circulation by way of the thoracic duct, and then specifically home back to populate the lamina propria and intraepithelial regions of the intestine (Langkamp-Henken *et al.* 1992; Kagnoff, 1993; DeWitt & Kudsk, 1999). Thus, Peyer's patches represent a major 'sampling' site for intestinal antigens. Solitary lymphoid follicles are present throughout the length of the intestinal tract, particularly in the colon and rectum, and have M-cells associated with the overlying epithelium (Laissue & Gebbers, 1992). At present it is assumed that these follicles are functional equivalents of Peyer's patches (Laissue & Gebbers, 1992).

The lamina propria consists of a diffuse population of T- and B-cells, plasma cells, mast cells and macrophages (Langkamp-Henken *et al.* 1992). The majority of T-cells within the lamina propria are $CD4^+$. Ninety per cent of the plasma cells (mature B-cells) in the lamina propria secrete IgA (Laissue & Gebbers, 1992). Most of the IgA is secreted into the gut lumen, and takes the form of secretory IgA, distinct from serum IgA. Secretory IgA (sIgA) is a dimer of two monomeric IgA molecules with an attached secretory component (the cleaved extracellular domain of a transmembrane protein expressed on intestinal epithelial cells). The secretory component facilitates the transport of IgA through the epithelium and into the gut lumen and protects IgA from degradation by intestinal enzymes and toxins (Laissue & Gebbers, 1992). The main function of IgA is to prevent the attachment of intestinal pathogens (Kagnoff, 1993).

Intraepithelial lymphocytes (IEL) are located in the interstitial spaces of the mucosal epithelium in a ratio of approximately one lymphocyte for every six to ten epithelial cells (Langkamp-Henken *et al.* 1992), making IEL the largest immunocompetent cell pool in the body. Although IEL line both the small and large intestine from the crypt base to the villus tip (Abreumartin & Targan, 1996), their exact biological function in the mucosal immune system is not known. They are in continuous contact with luminal antigen through the epithelial layer, and it has been suggested that IEL may be the first compartment of the immune system that responds to gut-derived antigens (McKay & Perdue, 1993). Furthermore, as IEL are comprised primarily of $CD8^+$ T-cells (Kagnoff,

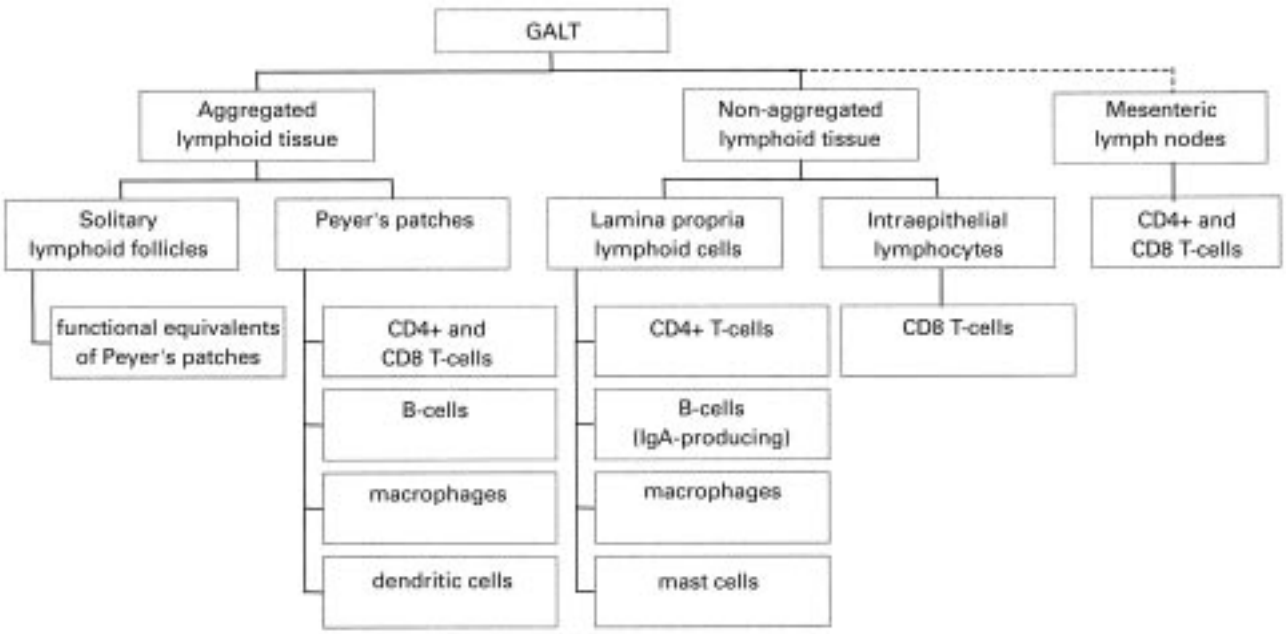

Fig. 1. The gut-associated immune system. A schematic illustration of the components and cells that comprise the gut associated lymphoid tissues.

1993), they may be functional suppressor cells with a role in oral tolerance (Trejdosiewicz, 1992).

Although not situated within the intestinal mucosa, the mesenteric lymph nodes are considered as part of the GALT. Mesenteric lymph nodes are composed of immune cells leaving and entering the gut and those that are part of the peripheral circulation. Immune cells drain to the intestinal lymphatics after differentiation in Peyer's patches, and pass through mesenteric lymph nodes en route to the thoracic duct and then again en route back to the lamina propria regions of the gut (Weiner, 1997).

Dietary fibre and immune function

There is increasing evidence that the addition of fermentable fibre to the diet alters the function and structure of the gut, modifies the production of gut-derived hormones, and is associated with improved whole body glucose homeostasis even in the absence of disease (Mortensen & Clausen, 1996; Massimino *et al.* 1998). To date, relatively few studies have been conducted on the effects of dietary fibre on GALT. It is not possible at this time to draw conclusions on the immune effects of different fibres. Therefore, we have reviewed altogether studies examining the immune effects of fermentable dietary fibres, grouping all fibres that can be metabolised by intestinal micro-organisms. The specific fibre sources used in the studies can be found in Table 2. Studies in our own laboratory conducted on adult dogs indicate that adding fermentable fibre to the diet can modulate the type and function of cells from different regions of the GALT (Field *et al.* 1999). In this study, sixteen adult dogs (23 ± 2 kg) were fed (for 14 days) in a randomised crossover design, two isoenergetic isonitrogenous diets containing 8·3 g/kg non-fermentable or 8·7 g/kg fermentable fibres for 2 weeks. The fermentable fibre diet was a mixture of plant fibres (beet pulp, oligofructose powder and gum arabic). The fibre content of the diet significantly altered the proportion of T-cells ($CD4^+$ and $CD8^+$) in GALT and their *in vitro* response to mitogens (Field *et al.* 1999). Specifically, we found that switching from a low to high fermentable fibre diet, as compared to cells from dogs switched from a high to a low fermentable fibre diet, resulted in higher ($P<0·05$) mitogen responses in the predominantly T-cell tissues (mesenteric lymph nodes and intraepithelial lymphocytes) and lower mitogen responses ($P<0·05$) in areas involving B-cell function (lamina propria and Peyer's patches). After consuming the high fermentable fibre diet there was a higher proportion of $CD8^+$ T-cells among the IEL, lamina propria and Peyer's patches, and a higher proportion of $CD4^+$ T-cells in the mesenteric lymph nodes and peripheral blood (Field *et al.* 1999). Apart from a higher CD4:CD8 ratio ($2·4 \pm 0·2$ *v.* $1·7 \pm 0·2$, $P<0·05$), switching to the high fermentable fibre diet did alter immune functions (^{3}H-thymidine uptake in response to mitogens or NK activity) in peripheral blood (Field *et al.* 1999). The observed effect of changes in the fermentable fibre content of the diet on the composition and function of GALT, but not peripheral immune cells, raises practical issues in assessing the role of diet on immune function since peripheral blood is the most frequently sampled immune site in human and large animal studies.

A limited number of studies assessing the effect of dietary fibre on immune function have been published (Table 2). Our results are consistent with recent studies demonstrating proportionately more $CD4^+$ T-cells in mesenteric lymph nodes of rats fed a diet containing 5 % w/w pectin as compared to cellulose (Lim *et al.* 1997), and with an increased proportion of $CD8^+$ IEL in rats fed a diet supplemented with sugar beet fibre, compared to a fibre-free diet (Nagai *et al.* 2000). Changing, or adding, fibre to the diet has yielded various other effects on immune function, including an increase in serum, mesenteric lymph node, and mucosal immunoglobulin production (Lim *et al.* 1997; Yun *et al.* 1997; Yun *et al.* 1998; Kudoh *et al.* 1999; Yamada *et al.* 1999), an increase in the number of Peyer's patches (Pierre *et al.* 1997), altered cytokine production in mesenteric lymph nodes (Lim *et al.* 1997; Yun *et al.* 1997; Yun *et al.* 1998), and altered leucocyte and lymphocyte numbers in tissues such as the spleen (Zusman *et al.* 1998; Kudoh *et al.* 1998), blood (Kaufhold *et al.* 2000), and intestinal mucosa (Gaskins *et al.* 1996; Madar *et al.* 1998; Kudoh *et al.* 1998; Kudoh *et al.* 1999). Although exploration in this area is still in its infancy, animal studies have clearly demonstrated that dietary fibre content and type can modulate measures of immune function.

Mechanisms for the effects of fibre on the immune system

The mechanism for the effect of fermentable dietary fibres on immune function in the gut has not been established. A number of interesting hypotheses have been proposed, and will be discussed (Table 3). A prebiotic fibre is neither hydrolysed nor absorbed in the upper part of the gastrointestinal tract, and becomes a selective substrate for one or a limited number of beneficial colonic bacteria thereby altering the microflora of the gut (Gibson & Roberfroid, 1995). There is strong evidence indicating that consumption of prebiotic fibres (inulin and oligofructose) increase the proportion of beneficial lactic acid bacteria in the human colon (Mitsuoka *et al.* 1987; Gibson *et al.* 1995; Buddington *et al.* 1996; Bouhnik *et al.* 1996; Kleesen *et al.* 1997; Menne *et al.* 2000). Other oligomers that may be prebiotics but for which more evidence is required are lactulose, and oligosaccharides containing xylose, mannose, and galactose (Gibson, 1998). The few studies that have examined the effects of prebiotic fibres on the immune system are reviewed in Table 2. The studies conducted with recognised prebiotic fibres (oligofructose) have shown increased lymphocyte and/or leucocyte numbers in GALT (Gaskins *et al.* 1996; Pierre *et al.* 1997; Field *et al.* 1999) and peripheral blood (Kaufhold *et al.* 2000). Additionally, studies have documented that feeding lactulose is associated with increases in IgA secretion or IgA^+ cells in GALT (Kudoh *et al.* 1998; Kudoh *et al.* 1999), a decrease in the $CD4^+/CD8^+$ ratio in the spleen (Kudoh *et al.* 1998), and an increase in the phagocytic function of intraperitoneal macrophages (Nagendra & Venkat Rao, 1994).

Table 2. Immunomodulatory effects of dietary fibres

Reference	Subjects	Experimental fibre	Control diet/fibre	Immune effects
Field *et al.* 1999	Adult mongrel dogs	Fermentable fibre mixture (beet pulp, oligofructose*, gum arabic), 8·7 g/kg	cellulose, 8·3 g/kg	↑ CD8+ cells in IEL, PP and LP ↑ CD4+ cells in MLN and peripheral blood Higher T cell mitogen responses in MLN and IEL
Gaskins *et al.* 1996	C57BL/6NHsd mice	Oligofructose*, 30 g/l drinking water	Ensure® (low residue)	↑ cecal and colonic macrophages
Kaufhold *et al.* 2000	Veal calves	Oligofructose*, 10 g/d	whole-milk based cellulose, 5 % w/w	↑ eosinophil granulocytes in blood
Kudoh *et al.* 1998	Sprague-Dawley rats	Arabic gum, celfur, lactulose†, or purified indigestible dextrin, 5 % w/w		↑ κ -light chain- and IgA-positive cells in small intestine and cecal mucosa (all fibres) ↓ CD4+:CD8+ ratio in spleen (celfur, lactulose) ↓ CD3+ cells in spleen (arabic gum)
Kudoh *et al.* 1999	Sprague-Dawley rats	Celfur, glucomannan, curdlan, or lactulose†, 5 % w/w	cellulose, 5 % w/w	↑ IgA-positive cells in cecum (celfur, lactulose) ↑ IgA secretion into cecal contents (all fibres)
Lim *et al.* 1997	Sprague-Dawley rats	Pectin, konjak mannin, or chitosan, 5 % w/w	cellulose, 5 % w/w	↓ serum and MLN IgE (all fibres) ↑ serum IgA and IgG (pectin) ↑ MLN IgA and IgG (pectin, chitosan) ↑ CD4+ T cells in MLN (pectin) ↑ INF-γ in MLN (pectin)
Madar *et al.* 1998	Sprague-Dawley rats (tumour-bearing)	Cellulose, white grape, or tomato peel, 15 % w/w	cellulose, 3 % w/w	↑ area of lymphoid infiltrates in colonic mucosa close to tumor (white grape, tomato peel) ↑ Ki-67+ cells in colonic mucosa and tumors (all fibres)
Nagai *et al.* 2000	Wistar/ST rats	Sugar beet fibre, 10 % w/w	fibre-free	↑ CD8+ IEL in colorectum
Nagendra & Venkat Rao, 1994	Wistar rats	Lactulose †, 0·5 % of energy	infant formula	↑ phagocytic function of intraperitoneal macrophages
Pierre *et al.* 1997	*Min* mice	Oligofructose (from sucrose)*, wheat bran, or resistant starch, 5·8 % w/w	cellulose, 2 % w/w	↑ number of PP in small intestine (short-chain FOS)
Yamada *et al.* 1999	Sprague-Dawley rats	PHGG, guar gum, HM pectin, or glucomannan, 5 % w/w	cellulose, 5 % w/w	↑ IgA in spleen and MLN (all fibres) ↑ IgG in spleen (glucomannan, pectin) and MLN (all fibres) ↑ serum IgA (guar gum, glucomannan, pectin) and IgM (glucomannan)
Yun *et al.* 1997	C57BL/6 mice (immunosuppressed)	Oat β -glucan, 3 mg every 48 h	Diet not specified	↑ non-specific and antigen-specific IgG in serum ↑ IFN-γ-and IL-4-secreting cells in spleen and MLN
Yun *et al.* 1998	C57BL/6 mice	Oat β-glucan, 3 mg every 48 h	diet not specified	↑ intestinal antigen-specific IgA ↓ IL-4 secreting cells in MLN
Zusman *et al.* (1998)	Rats	Cellulose, white grape or tomato peel, 15 % w/w	cellulose, 3 % w/w	↑ plasma cells in splenic red pulp (all fibres)

IEL, intraepithelial lymphocytes; PP, Peyer's patches; LP, lamina propria; MLN, mesenteric lymph nodes; Ig, immunoglobulin; IFN, interferon; IL, interleukin; PHGG, partially hydrolysed guar gum; HM pectin, highly methoxylated pectin; *Recognised prebiotic fibre; †Potential prebiotic fibre.

Table 3. Proposed mechanisms underlying the immunomodulating effects of dietary fibres that change the gut microflora

Direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine
Production of short-chain fatty acids (SCFA) from fibre fermentation
Modulation of mucin production

Direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine

It is often assumed that the consumption of prebiotics, through their effects on the colonic microflora, will have a similar effect as probiotics on immune function. In contrast to work on prebiotic fibres, many more studies have documented effects of feeding lactic acid bacteria (i.e. lactobacilli and bifidobacteria) on various parameters of immune function (Table 4). Oral administration of probiotic bacteria increased the production of immunoglobulins, especially IgA, in GALT and modulated both the number and activity of Peyer's patch immune cells (Table 4). There are also a number of studies demonstrating effects of oral probiotics on systemic immune functions, and immune parameters in the lungs, peritoneum and mesenteric lymph nodes (Table 4). The mechanism(s) by which probiotics consumed in the diet affect immune function have been largely speculative to date. One logical mechanism might be immune stimulation through direct contact of the colonic microflora with GALT. Small numbers of bacteria can cross the intestinal epithelial barrier into the Peyer's patches (Berg, 1985) inducing activation or leading to the activation of other immune cells (Berg, 1985; De Simone *et al.* 1987; Link-Amster *et al.* 1994; Schiffrin *et al.* 1995). *In vitro* studies have supported this mechanism. In a study by Park *et al.* (1999) a macrophage cell line increased its production of nitric oxide, H_2O_2, IL-6 and TNF-α after *in vitro* culture with bifidobacteria. Similarly, co-culture with bifidobacteria significantly increased the production of TNF-α and IL-6 by macrophages and the production of IL-2 and IL-5 by stimulated $CD4^+$ cells (Marin *et al.* 1997). Culturing murine Peyer's patch cells with bifidobacteria (*B. breve*) resulted in increased proliferation and antibody production by B-lymphocytes and activated macrophage-like cells (Yasui & Ohwaki, 1991).

Other authors have suggested that it is not the bacteria but microbial substances (e.g. cytoplasmic antigens, cell wall components) that penetrate the intestinal epithelia to activate GALT (De Simone *et al.* 1987; Perdigon *et al.* 1988; Solis Pereyra & Lemonnier, 1993; Takahashi *et al.* 1993; Takahashi *et al.* 1998; Tejada-Simon *et al.* 1999*a*). *In vitro*, a macrophage cell line was stimulated, similar to that produced by whole bacteria, by incubation with cell-free extracts of both *Bifidobacterium longum* and *Lactobacillus acidophilus* (Hatcher & Lambrecht, 1993). Similarly, *in vivo* administered supernatants from cultures of *L. acidophilus* and/or *L. casei* resulted in stimulated phagocytic activity of peritoneal and reticuloendothelial phagocytes and splenocyte activation similar to that produced by administration of the live bacteria (Perdigon *et al.* 1988). Cytoplasmic components of bacteria have also been demonstrated to produce some of the same immune effects (IgA production by Peyer's patch cells) as live bacteria (Takahashi *et al.* 1998). The mechanism by which cell wall components (such as peptidoglycans) or cytoplasmic antigens may activate immune cells is not well understood. It has been suggested that there may be receptor binding sites for lactic acid bacteria on lymphocytes ($CD4^+$ and $CD8^+$) (De Simone *et al.* 1988*b*). Furthermore, peptidoglycans can bind to the CD14 cell surface antigen, and can stimulate mononuclear phagocytes and endothelial cells to release cytokines (Matsuzaki, 1998). Finally, there is some speculation that the immune effects observed with the administration of probiotic bacteria may actually be due to immunogenic milk peptides generated from the bacterial hydrolysis of milk constituents present in fermented milk products used to deliver the probiotic bacteria (Perdigon *et al.* 1988; Moineau & Goulet, 1991). This hypothesis warrants further examination as many of the studies in Table 4 administered probiotic bacteria in fermented milk products. If this contributes to the immune-stimulating effects of probiotic bacteria, it is unlikely to explain the immune effects of dietary prebiotic fibres.

Production of short-chain fatty acids (SCFA) from fibre fermentation

The gut microflora may modulate immune cells through the fermentation of dietary fibres to SCFA. It is well established that the fermentation of inulin and oligofructose increases the production of SCFA, primarily acetate, butyrate and propionate in the gut (Gibson & Roberfroid, 1995), but the extent to which serum SCFA levels are increased following prebiotic consumption is not known. Nevertheless, a number of studies support direct or indirect immunomodulatory properties of short-chain fatty acids (Pratt *et al.* 1996; Bohmig *et al.* 1997). We have demonstrated in a rat model that supplementing total parenteral nutrition (TPN) with SCFA results in increased natural killer cell activity (Pratt *et al.* 1996). Other studies have demonstrated anti-inflammatory properties of SCFA. Butyrate was reported to suppress both constitutive and cytokine-induced expression of the transcription factor NFκB in the colonic cell line HT-29 (Inan *et al.* 2000). Pharmacological doses of acetate administered intravenously to both healthy subjects and cancer patients increased peripheral blood antibody production, natural killer cell activity and the allogeneic mixed lymphocyte reaction (Ishizaka *et al.* 1993). Whether these effects occur at concentrations seen after a high fermentable fibre meal is not known.

Finally, SCFA production, particularly butyrate, in the colon may reduce the requirement of epithelial cells for glutamine, thereby sparing it for other cells, such as those of the immune system (Jenkins *et al.* 1999). This hypothesis is supported by the observation that lactulose administration can increase serum glutamine levels (Jenkins *et al.* 1997), and glutamine is an essential energy source for immune lymphocytes (Wu *et al.* 1991).

Table 4. Immunomodulatory effects of orally administered lactic acid bacteria*

Immune tissue	Effect	Documented in: Humans	Mice	References
Peyer's patches	↑ B cell number		✓	De Simone *et al.* 1987
	↑ response to T-nd B-ell mitogens		✓	De Simone *et al.* 1987; Shu *et al.* 2000
	↑ proliferation of PP cells		✓	Takahashi *et al.* 1993
	↑ antibacterial activity of PP cells (via IgA)		✓	De Simone *et al.* 1988*a*
	↑ IgA production		✓	Takahashi *et al.* 1998
	↑ total Ig production		✓	Yasui *et al.* 1989
GALT†	↑ IgA in faeces/intestinal contents	✓	✓	Perdigon *et al.* 1990; Fukushima *et al.* 1998, 1999; Tejada-Simon *et al.* 1999*b*
	↑ IgA in small intestinal wall extracts		✓	Takahashi *et al.* 1998
	↑ total Ig in intestinal contents		✓	Shu *et al.* 2000
MLN	↑ response to mitogens		✓	Shu *et al.* 2000
Blood	↑ phagocytosis by blood leucocytes	✓	✓	Schiffrin *et al.* 1995; Shu *et al.* 2000; Chiang *et al.* 2000
	↑ phagocytic function of RES		✓	Perdigon *et al.* 1986*a*,*c*, 1987, 1988, 1991
	↑ NK cell tumour killing activity	✓		Chiang *et al.* 2000
	↑ B cell number	✓		De Simone *et al.* 1992
	↑ IFN-γ production by lymphocytes	✓		Halpern *et al.* 1991; Solis Pereyra & Lemonnier, 1993
	↑ IgA/IgA-secreting cells	✓	✓	Yasui *et al.* 1989; Takahashi *et al.* 1993; Link-Amster *et al.* 1994; Malin *et al.* 1996; Tejada-Simon *et al.* 1999*b*
	↓ IgE production		✓	Matsuzaki *et al.* 1998
	↑ total Ig production		✓	Perdigon *et al.* 1988, 1991; Yasui *et al.* 1989; Shu *et al.* 2000
Spleen	↑ responses to T- and B- cell mitogens		✓	De Simone *et al.* 1988*a*; Shu *et al.* 2000
	↑ IgM-secreting cells		✓	Perdigon *et al.* 1986*c*, 1987
	↓ IgE from stimulated spleen cells		✓	Matsuzaki *et al.* 1998
	↓ IL-4, IL-5, IL-6, IL-10		✓	Matsuzaki *et al.* 1998
	↑ IFN-γ, IL-2		✓	Matsuzaki *et al.* 1998
Peritoneal cells	↑ macrophage phagocytic/enzymatic activity		✓	Perdigon *et al.* 1986*a*, *b*, *c*, 1987, 1988; Shu *et al.* 2000
	↑ IL-6, IL-12, IFN-γ and nitric oxide		✓	Tejada-Simon *et al.* 1999*a*
Lung macrophages	↑ phagocytic function		✓	Moineau & Goulet, 1991

PP, Peyer's patch; Ig, immunoglobulin; GALT, gut-associated lymphoid tissue; MLN, mesenteric lymph nodes; NK, natural killer; IFN, interferon; IL, interleukin; RES, reticuloendothelial system.
*Primarily species of lactobacilli and bifidobacteria; †data insufficient to classify region of GALT.

Modulation of mucin production

The layer of mucus overlying the gastrointestinal tract prevents the adherence and subsequent translocation of bacteria across the epithelial wall (Katayama *et al.* 1997). There is some evidence to indicate that the addition of fermentable fibres to the diet can increase mucin production (Satchithanandam *et al.* 1990). Greater mucin production might contribute to the lower incidence of bacterial translocation across the gut barrier reported in studies that fed dietary fibres (Deitch *et al.* 1993; Spaeth *et al.* 1994; Frankel *et al.* 1995; Xu *et al.* 1998). The increase in mucin production may occur in response to the decreased pH accompanying the production of SCFA (Bustos-Fernandez *et al.* 1978). Further support for SCFA stimulating mucin production comes from a perfused rat colon model where the production of acetate and butyrate from the fermentation of pectin, gum arabic and cellulose stimulated mucin release, whereas the dietary fibres themselves did not (Barcelo *et al.* 2000). Whether prebiotic fibres alter mucin production is not known, but one study has reported that feeding inulin increased sulphomucin production in both germ-free and heteroxenic rats (Fontaine *et al.* 1996).

Conclusion

Although further work is needed to better define the changes, the mechanisms for immunomodulation, and the ultimate impact on immune health, there is convincing preliminary data to suggest that the consumption of prebiotic fibres can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation. Future protocols on the physiological impact of consuming prebiotics should be designed to include assessments of the gut microflora, gut physiology and the function and composition of the various regions of GALT.

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