CHAPTER 7

Diet, Immunity and Functional Foods

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Abstract

Functional foods (specific nutrient and/or food components) should beneficially affect one or more target functions in the body. The use of functional foods as a form of preventive medicine has been the subject of much research over the last two decades. It is well known that nutrition plays a vital role in chronic diseases, but it is only recently that data relating to the effects of specific nutrients or foods on the immune system have become available. This chapter aims to summarize the effects of some functional foods (e.g., prebiotics and micronutrients) on the immune system. It should be noted, however, that studies into the role of functional foods with regard to the human immune system are still in their infancy and a great deal of controversy surrounds the health claims attributed to some functional foods. Consequently, thorough studies are required in human and animal systems if we are to move towards developing a functional diet that provides maximal health benefits.

Introduction

Discoveries in the biosciences in recent years have provided evidence that, beyond nutrition, diet may also modulate various bodily (including immune) functions that are relevant to the host's health. These discoveries are shifting nutritional concepts from identifying a 'balanced' diet (ensuring an adequate intake of nutrients while avoiding excessive intake of those nutrients that can contribute to disease, e.g., fat and salt) to an 'optimized' nutrition. The outcome of 'optimized' nutrition is to maximize life expectancy and quality by identifying food ingredients that are able to improve the capacity to resist disease and enhance health when part of a 'balanced' diet and lifestyle. The latter provides a concept of functional foods, which was initiated in Japan in the late 1980s as a marketing term (linking medical and food sciences).

Functional foods can not be categorized with a single definition due to their novelty and diversity.[1] Although the scientific working definition of functional foods varies across geographical regions, all are in agreement that functional foods (specific nutrient and/or food components) should beneficially affect one or more target functions in the body. Thus, a general definition for functional foods states that 'a food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease'.[2] Quantitative evaluation of modulation of these target functions is required to scientifically substantiate the claims attributed to a particular functional food; this can be done by measuring changes in the serum or other body fluid concentration of a specific metabolite, protein or hormone, a change in physiological parameters (e.g., blood pressure or gastrointestinal transit time) and/or a change in physical or intellectual performance.[1]

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Functional foods have two main uses in terms of their action upon the immune system: (i) to overcome/prevent the effects of undernutrition and (ii) as aids in the treatment of chronic clinical conditions. Undernutrition impairs the immune system and suppresses immune functions that are essential to host protection. This state can be the result of insufficient intake of energy and macronutrients and/or deficiencies in specific micronutrients.[3] The component that makes a food 'functional' can be either an essential macronutrient with a specific physiological effect (e.g., omega-3 fatty acids) or an essential micronutrient if its intake is above the daily recommendations. Furthermore, it can be a non-essential food component (e.g., prebiotics) or a food component without nutritive value (e.g., probiotics or phytochemicals). While the beneficial effect of essential nutrients on the functioning of the immune system has been well documented, the effect on the immune function of non-essential and nonnutritive food components is a relatively recent subject of interest and thus is less well documented.[4] It is clear from studies conducted in animals, and the limited number conducted in humans, that there are a number of nutrients whose availability at an appropriate level is essential if the immune system is to operate efficiently.[3] It is also clear from recent studies that the introduction of functional foods into the diets of patients with clinical conditions can help ameliorate disease symptoms.[5,6]

The following text describes those functional foods for which data are available in relation to their effects on the immune system. Special attention is paid to the so-called 'colonic functional foods', which have been shown to affect the gut's immune response. Details are also given for some emerging functional foods that may or may not have immunomodulatory properties.

Colonic Functional Foods

The human body is host to a large number of commensal bacteria, with most residing in the gut. The large intestine is, by far, the most densely populated area of the gut and its resident microbiota plays a key role in nutrition and health as well as the proper functioning of the immune system.[7] The composition of the microbiota is influenced by various environmental and genetic factors, with dietary residues considered the most important of these. Dietary substrates reaching the large intestine are able to influence the number of bacteria (in terms of total and specific populations) present and metabolic byproducts from bacteria utilizing these dietary substrates can affect the gut-associated lymphoid tissue (GALT)—the largest component of the immune system. Consequently, dietary modulation of the intestinal microbiota is the main purpose of many current functional foods. This modulation of the intestinal microbiota by dietary means is also the basis for the pro, pre and synbiotic concepts, all of which rely upon enhancing the beneficial components of the intestinal microbiota, namely the bifidobacteria and lactobacilli. While the probiotic concept relies upon the use of live microbial supplements to modulate the microbiota, the prebiotic concept relies upon the use of nondigestible food ingredients that selectively stimulate the growth and/or activity of beneficial groups of bacteria indigenous to the colon.[8,9] The synbiotic concept is a combination of the pre and probiotic concepts.

The first records of ingestion of live bacteria by humans are over 2000 years old, but it was not until the beginning of the last century that probiotics were given a scientific basis through the work of Metchnikoff.[10,11] He hypothesized that the normal gut microbiota could exert adverse effects on the host and that consumption of 'soured milks' reversed these. Since these early observations, attempts have been made, especially in the last 20 years, to modulate the gut microbiota through the use of probiotics and these remain the most tried and tested modulators of the intestinal microbiota; their use and action have been described elsewhere in this book, so they will not be discussed further in this chapter.

Prebiotics

Any dietary material that enters the large intestine is a candidate prebiotic. This includes carbohydrates such as resistant starch and dietary fiber as well as proteins and lipids. However, current prebiotics are confined to nondigestible oligosaccharides (NDOs). These escape enzymatic digestion in the upper gut, enter the cecum without change to their structure and confer the degree of

selective fermentability that is required. Their complete fermentation by the colonic microbiota, resulting in the production of short-chain fatty acids (SCFAs), lactic acid and energy, is indicated by the fact that NDOs are not excreted in the feces.[12]

Oligosaccharides are sugars consisting of between 2 and 20 saccharide units. Some occur naturally in breast milk and certain foods such as leek, asparagus, garlic, onion, chicory, wheat, oat and soybean. However, these naturally occurring oligosaccharides can not exert a prebiotic effect in their native state, due to their low concentrations, so are produced commercially through the hydrolysis of polysaccharides (e.g., dietary fibers and starch) or through catabolic enzymatic reactions from lower molecular weight sugars. Currently, there are over 20 different types of NDOs on the world market: the most commonly used and cited prebiotics are listed in Table 1. Of these, inulin, fructooligosaccharides (FOS), transgalactooligosaccharides (TOS) and lactulose have been the most thoroughly investigated and for these a prebiotic effect has been proven.[13]

Effects of Prebiotics on Immunity

The idea that prebiotics could help the intestinal defense system originated from the observations that newborn babies, who have an underdeveloped intestinal host defense system, lack an appropriate capacity to defend themselves against intestinal infections. Furthermore, infants consuming their mother's milk were found to have a greatly reduced risk of diarrheal diseases and a lower risk of respiratory and other infections.[14] Human milk contains various protective components and active ingredients, including NDOs, which represent the third largest component of human milk and have been identified as the main factors involved in the development of an appropriate colonization process in infants, which in turn stimulates the maturation of intestinal host defenses.[15]

Although it is known that human milk oligosaccharides can exert a prebiotic effect, research into the immunomodulatory actions of prebiotics is very recent, with most data originating from animal models. In one study, mice were fed FOS or inulin for 6 weeks and then challenged with various tumor inducers and enteric and systemic pathogens.[16] It was found that prebiotic supplementation resulted in a significantly lower incidence of aberrant crypt foci in the distal colon as well as reduced pathogen-induced mortality. It was suggested that the enhanced immune functions were in response to changes in the composition and metabolism of the colonic microbiota. In another study, the same group investigated the effect of the same prebiotics on immune functions in mice. After 6 weeks' supplementation with FOS or inulin, increased natural killer (NK) cell activity and phagocytic activity of peritoneal macrophages were observed.[17]

In *Min* mice (a model for human colon cancer), FOS administration significantly reduced the incidence of colon tumors.[18] Furthermore, *Min* mice depleted of CD^{4+} and CD^{8+} lymphocytes developed twice as many tumors as immunocompetent mice, suggesting that the reduced incidence of colonic tumors after FOS supplementation was due to an appropriate functioning of the immune system.[19]

Increased IgA secretion and production of IFN-γ, IL-10, IL-5 and IL-6 from Peyer's patch (PP) cells and increased size of PPs in the small intestine were observed in mice after 6 weeks of FOS supplementation.[20] The IgA response and polymeric immunoglobulin receptor expression in the small intestine and the colon were observed in another study which examined the effect of FOS in infant mice.[21] Increased IgA secretion and phagocytic activity of macrophages were also observed in rats fed lactulose.[22,23] In addition, several studies with mice observed a change in PP cellularity in different regions of the gastrointestinal tract after administration of FOS.[18,20,24] These studies suggest that prebiotic fermentation in the colon can induce changes in several regions of the GALT.

Evidence for a direct effect of prebiotics on the human immune system is documented only in one trial, in which frail elderly individuals in a nursing home received 8 g of FOS daily for 3 weeks.[25] Significant increases in the total lymphocyte count and the number of CD^{4+} and CD^{8+} cells were observed, along with increased numbers of bifidobacteria. Reduced phagocytic activity of polymorphs and monocytes and the expression of IL-6 mRNA in peripheral blood monocytes were also observed and attributed to a general decrease in inflammation. However, another study showed that a nutritional supplement containing either placebo or inulin and FOS did not augment

Table 1. Prebiotics available on the world market

Name	Structure	Production Process
Inulin	α-D-Glc-(1→2)-[β-D-Fru-(1→2)-]n; n > 20	Extracted from chicory
Fructooligosaccharides	β-D-Fru-(1→2)-[β-D-Fru-(1→2)-]n; n = 1 – 9	Controlled enzymatic hydrolysis of inulin by inulinase
	α-D-Glc-(1→2)-[β-D-Fru-(1→2)-]n; n = 2 – 9	Transfructosylation of sucrose by β-fructofuranosidase
Transgalactooligosaccharides	α-D-Glc(1→4)-[β-D-Gal-(1→6)-]n; n = 2 – 5	Transgalactosylation of lactose by β-galactosidase
Lactosucrose*	β-D-Gal-(1→4)-α-D-Glc-(1→2)-β-D-Fru	Transfructosylation of lactose and sucrose by β-fructofuranosidase
Lactulose*	β-D-Gal(1→4)-β-D-Fru	Alkali isomerization of lactose
Isomaltooligosaccharides*	[α-D-Glc-(1→6)-]n; n = 2 – 5	Degradation of starch by α- and β-amylase to maltose and transglucosylation by α-glucosidase
Soyabeanoligosaccharides*	[α-D-Gal(1→6)-]n-α-D-Glc(1→2)-β-D-Fru; n = 1 – 3	Extracted from soya beans
Xylooligosaccharides*	[β-Xyl-(1→4)-]n; n = 2 – 9	Controlled enzymatic hydrolysis of xylan by endo-1, 4-β-xylanase

Abbreviations: Glc, glucose; Gal, galactose; Fru, fructose; Xyl, xylose.
*Available only in Japan.

the results of vaccination with influenzal and pneumococcal antigens in the elderly.[26] An indirect indication of improved immune status after consumption of milk fortified with *Bifidobacterium lactis* (DR-10TM) and TOS was documented in one trial where a reduced incidence of diarrhea and improved nutritional status of children were observed.[27]

It is clear from the limited number of studies done to date that more human studies with prebiotics are required to demonstrate the effect of these compounds on the immune system, especially since animal models suggest they have a beneficial effect. More studies into the effects of an altered intestinal microbiota on immune function are also needed, as this is also expected to modulate GALT activity.

Mechanisms for the Effects of Prebiotics on the Immune System

The underlying mechanisms of how prebiotics modulate the immune system are not known at present. Experimental data, however, suggest that these compounds exert effects in the GALT and also point to a few different mechanisms that might explain these effects:

- Selective changes in bacterial composition and bacterial products which modulate cytokine and antibody production;
- Production of SCFAs and their interactions with leukocytes;
- Modulated mucin production;
- Interaction with carbohydrate receptors of pathogens inhibiting their attachment to epithelial cells as well as receptors on immune cells.

Selective Changes in Bacterial Composition and Bacterial Products

It is well known that prebiotics increase the number of beneficial bacteria (i.e., bifidobacteria and lactobacilli).[28-31] Probiotics (usually bifidobacteria or lactobacilli), when administered orally, are known to increase the secretion of IgA in the small intestine and the feces and to stimulate PP B lymphocyte IgA production.[32,24] They are also known to exert effects on systemic immune functions and various immune parameters in the lungs, spleen and peritoneal cells.[35-37]

Intestinal epithelial cells are involved in both innate and adaptive immune responses and act by transducing signals from luminal pathogens to adjacent immune cells of the intestinal immune system, via specific germline-encoded pattern-recognition receptors, such as Toll-like receptors (TLRs) and cytoplasmic receptors.[38] TLRs are able to discriminate between the normal commensal biota and pathogens and induce the transcriptional activation of a number of genes mediating immune and inflammatory responses.[39] Pathogen-associated molecular patterns (PAMPs) [e.g., endotoxin (lipopolysaccharide), lipoproteins, lipopeptides and imidazoquinolines] present on diverse microbes are initially recognized by TLRs and their interaction results in the activation of intracellular signaling pathways, nuclear translocation of transcription factor NF-κB and the transcription of pro-inflammatory cytokines.[39] The changes that occur in the composition of the intestinal microbiota due to prebiotic fermentation could potentially reduce the presence of PAMPs and thereby exert a positive effect on the immune system.

Prebiotics also promote an increase in bacterial cell-wall components that are recognized by TLRs and in DNA derived from luminal bacteria that, in turn, stimulate the intestinal immune system.[40] Cytoplasmic components and cell-free extracts of probiotics have also been demonstrated to produce some of the same immune effects (e.g., IgA production by PP and macrophage stimulation) as live bacteria.[32,41]

Production of SCFAs

The major end-products of carbohydrate fermentation are SCFAs, of which acetate, propionate and butyrate are quantitatively the most important in the human colon. The production of SCFAs in the colon averages 400 mmol day^{-1}, with a range of 150-600 mmol day^{-1}.[42] All SCFAs are rapidly absorbed from the large intestine and stimulate salt and water absorption: principally, the gut epithelium, liver and muscle metabolize them, with virtually none appearing in the urine and only small amounts appearing in the feces. The three major SCFAs are trophic when infused into the colon and these trophic properties have important physiological implications in addition

to maintaining the mucosal defense barrier against invading organisms.[43] However, butyrate appears to be the most effective in this regard as it is a principal energy source for epithelial cells.[44] Furthermore, butyrate is known to suppress lymphocyte proliferation, inhibit cytokine production of Th1-lymphocytes and upregulate IL-10 production; it also suppresses expression of the transcription factor NF-κB and upregulates TLR expression.[45,46] Butyrate is also believed to protect against colon cancer as it inhibits DNA synthesis and induces cell differentiation.[47,48]

Increased SCFA production during prebiotic fermentation has been confirmed in a number of studies, although the extent to which serum SCFA levels are affected following prebiotic consumption is not known.[28,29,31] However, it has been demonstrated in a rat model that supplementing total parenteral nutrition with a SCFA mixture results in increased NK cell activity.[49] Pharmacological doses of acetate administered intravenously to both healthy individuals and cancer patients also increased NK cell activity and peripheral blood antibody production.[50] In addition, it has been shown that serum glutamine levels are raised following lactulose administration and suggested that increased SCFA levels were responsible for this (glutamine is a preferred substrate for lymphatic tissue).[51,52] Therefore, SCFA production in the large intestine could potentially reduce the requirement of epithelial cells for glutamine, making it available to the cells of the immune system.[53]

Mucin Production

The first line of defense of the mucosa against luminal contents is the mucous layer, which is mainly composed of high-molecular-weight glycoproteins (mucins) that are secreted by goblet cells.[54] The thickness of the mucous layer and the number of goblet cells varies throughout the gastrointestinal tract, and in the colon it increases distally, where the number of bacteria is also the highest.[55] In addition, mucin in the colon is more sulfated than in other regions, giving it a strong negative charge and making it less sensitive to degradation by bacterial enzymes (only about 1% of the total intestinal microbiota is able to degrade mucin).[56]

Thus far, the effect of prebiotics on mucin production has been reported in only one study, where it was shown that inulin administration resulted in increased mucin production in rats.[57] Greater mucin production was found to be associated with a lower incidence of bacterial translocation across the mucosa following dietary fiber supplementation.[58,59] Furthermore, SCFA production, especially butyrate, is known to modulate mucin synthesis, release and gene expression.[60-62] It has been shown in a perfused rat colon model that the production of acetate and butyrate from the fermentation of dietary fiber stimulates mucin secretion, but fibers do not have the same effect on their own.[63]

However, the mucous layer is a dynamic environment and there is still a lack of understanding as to what mucin-associated bacteria do and whether increased mucin production is a positive or a negative outcome. Pathogens and beneficial commensal bacteria are able to modulate mucin synthesis by regulating some of the mucin genes. Currently, there are 16 identified mucin genes, but further work is needed to fully explain the function of each of them and to identify new genes.

Carbohydrate Receptors

Studies suggest that some prebiotics are directly involved in protecting the gut from infection and inflammation by inhibiting the attachment of pathogenic bacteria or their toxins to the colonic epithelium.[64] This attachment is necessary before pathogens can colonize and cause disease and it is mediated by glycoconjugates on glycoproteins and lipids present on the microvillus membrane.[65] Certain prebiotic oligosaccharides contain structures, similar to those found on the microvillus membrane, that interfere with the bacterial receptors by binding to them and thus preventing bacterial attachment to the same sugar on microvillus glycoconjugates. For example, α-linked TOS, present in human milk, are known to have anti-adhesive properties and be capable of toxin neutralization.[66,67] Recently, a novel TOS mixture, which contains an oligosaccharide in alpha anomeric configuration, was shown to significantly decrease the attachment of enteropathogenic *Escherichia coli* (EPEC) and *Salmonella enterica* serovar Thyphimurium in vitro.[68]

In addition, immune cells also express specific carbohydrate receptors which mediate various cellular reactions when activated. For example, C-type receptors expressed on phagocytic cells, minor

subsets of T- and B-lymphocytes and NK cells as well as the dectin-1 receptor expressed on neutrophils and macrophages, are known to be activated by β-glucans from fungi, plants and yeast.[69,70] Recently, nigerooligosaccharide (an α-glucan-derived NDO) was found to stimulate NK cell activity in vitro, suggesting a direct effect of this NDO on NK cells via a specific lectin-type receptor.[71]

More studies, however, are required to determine whether these approaches will be successful for other prebiotics.

Dietary Fibers

There are many different types of dietary fiber (e.g., gum arabic, pectin, celfur, glucomannan, curdlan, guar gum and sugar beet) derived from plant material. After ingestion, these compounds pass into the large intestine intact (i.e., are neither fermented nor hydrolyzed) and are metabolized by intestinal micro-organisms. [It should be noted that dietary fibers differ from prebiotics in that they are not selectively fermented by the perceived beneficial bacteria (i.e., lactobacilli and bifidobacteria) of the large intestine.] A number of studies have shown that the fermentation of these fibers leads to changes in the function and structure of the gut and the production of gut-derived hormones.[72] Several studies have also demonstrated that dietary fibers enhance immunity. However, too few data are available to draw conclusions about the immunomodulatory properties of specific dietary fibers.

A feeding study involving adult dogs showed that adding fermentable fiber (in the form of a mixture of beet pulp, oligofructose powder and gum arabic) to the diet led to changes in the type and function of cells from different parts of the GALT. The fermentable fiber content of the diet (either 8.3 g/kg or 8.7 g/kg per day for 2 weeks) significantly altered the proportion of CD^{4+} and CD^{8+} cells and their in vitro response to mitogens.[72] Switching from the low- to the high-fiber diet led to increased mitogen responses in T-cell tissues (mesenteric lymph nodes and intraepithelial lymphocytes), but decreased responses in B-cell tissues (lamina propria and PPs): these effects were not observed when switching from the high- to the low-fiber diet. Switching to the high-fiber diet also led to increased NK cell activity. Studies in which rats were fed pectin and sugar beet, respectively, have also demonstrated an increase in CD^{4+} T-cell numbers in the mesenteric lymph nodes and in CD^{8+} cell numbers.[72] Studies in which the dietary fiber and/or its dose were changed have demonstrated a number of effects on the immune response: an increase in immunoglobulin production (mesenteric lymph node, serum and mucosal), an increase in the number of PPs, altered cytokine production in the mesenteric lymph nodes and altered leukocyte and lymphocyte numbers in the spleen, blood and intestinal mucosa.[72] Clearly, more work is needed to determine the doses and types of dietary fibers that are most beneficial to the immune system.

Other Functional Foods

Micronutrients

Numerous studies have shown that micronutrients such as zinc, selenium, iron, copper, β-carotene, vitamins A, C and E, and folic acid can influence several components of the immune system and have roles to play in disease prevention and the promotion of health.[73,74] Consequently, many of these nutrients are routinely included in, for example, breakfast cereals, juices and dairy products. The following is a brief overview of the effects that deficiencies of some of these micronutrients have on the body. Details for the beneficial effects of these nutrients are given in Table 2.

Zinc

Zinc deficiency has a marked effect on the bone marrow, decreasing the production of nucleated cells and of those that are lymphoid precursors.[3] In man, experimental or mild zinc deficiency results in decreased thymulin activity, NK cell activity, lymphocyte proliferation, IL-2, IFN-γ and TNF-α production and delayed-type hypersensitivity response and a lowered CD^{4+}-to-CD^{8+} ratio. Zinc deficiency is also associated with diseases such as sickle cell anemia and acrodermatitis enteropathica, where NK cell activity is decreased in the former and thymic atrophy, impaired leukocyte development, fewer CD^{4+} cells and reduced responsiveness and delayed-type hypersensitivity are observed

Table 2. Some functional foods and their properties. As well as giving details of the beneficial properties of the micronutrients discussed in this chapter, this table also contains details for foods/food components that are currently under investigation as potential functional foods.

Functional Food	Role(s)	Reported Effects on Immune System
Glutamine[77]	Indispensable metabolic fuel that is fully oxidized by the epithelial layer of the mucosa of the small intestine Maintains intestinal structure and function by providing precursors for anabolic pathways Supplies hepatocytes with an optimal substrate mix Provides citrulline/arginine for the whole organism	Increases numbers of mucosal macrophages and intra-epithelial lymphocytes in piglets; therefore, may be suitable in humans when pathologies develop in them linked to a loss of intestinal mucosa
Glutamate[77]	Suggested metabolic substrate for epithelial cells of the small intestine Specific precursor for the biosynthesis of glutathione, arginine and proline by the mucosa of the small intestine	No information with regard to role in gut inflammation, but strong suspicion that it plays a role in this process
Arginine[77]	Known to be conditionally essential in the small intestine of the neonate and for promoting intestinal repair Hypothesized to have a role in promoting intestinal cell migration (as a nitric oxide donator)	In a swine animal model challenged with *Escherichia coli* endotoxin, increased production of protective nitric oxide was observed in the alimentary canal (and several other organs) when the animals were given an intravenous arginine supplementation
Vitamin A[74]	Required for protection of membrane lipids from peroxidation Required for proper functioning of many different immune cells	Improved immune function has been observed in vitamin-A deficient hosts after supplementation
Vitamin C[74]	Required for protection of membrane lipids from peroxidation Plays a major and beneficial role in the prevention of cardiovascular disease, cancer and cataract	Dietary supplementation with ascorbic acid has been shown to enhance a number of lymphocyte functions, most notably in the elderly
Vitamin E[3,75]	Required for protection of membrane lipids from peroxidation Optimizes or even 'enhances' the immune system	Increased lymphocyte production, IL-2 production and delayed-type hypersensitivity in mice fed a diet supplemented with vitamin E Reduction of cardiovascular disease in humans associated with high vitamin E intake: may modulate atherogenesis through a number of mechanisms (e.g., reduction of the interaction of the endothelium with immune and inflammatory cells) Enhances cell-mediated immunity in the elderly Decreases incidence of diarrhea in malnourished infants

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Table 2. Continued

Functional Food	Role(s)	Reported Effects on Immune System
Vitamin D[78-80]	Essential for bone health (regulation of calcium homeostasis) and immune function	Increased intake decreased prostate-specific antigen levels in men with metastatic prostrate cancer In vivo treatment of IL-10 knockout mice with active vitamin D blocked the progression of IBD and prevented their death Dietary functional form of vitamin D3 (1α,25-dihydroxyvitamin D_3) prevented onset of EAE, a multiple sclerosis-like disease whose progression is driven by T-cells, in mice
Nucleotides[77,81]	Serve as nucleic acid precursors, physiological mediators, constituents of coenzymes and sources of cellular energy via respiratory pathways Play a role in the growth, differentiation and repair of the gastrointestinal tract in infants	Enhance immunity in infants: mechanism of action is unknown, but is thought to be due to lymphoid cells requiring an exogenous supply of nucleotides for optimal metabolism and function Reduce risk of sepsis in infants
Garlic[82]	Consumption reduces the incidence of stomach, colon, mammary and cervical cancer	Preparations of garlic have been shown to stimulate natural killer cell activity and increase the proliferation of lymphocytes Has been shown to be a scavenger of free radicals (OH•)
Herbs[82]	Those rich in flavonoids, vitamin C or the carotenoids may enhance immune function	*Echinacea* promotes the activity of lymphocytes, increases phagocytosis and induces interferon production Glycyrrhizin, a major component of licorice root, induces interferon activity and augments natural killer cell activity
Mushrooms[83]	Whole mushrooms and components, in particular (1→3)-β-D-glucans, potentially exert tumor-inhibitory effects	Thought that mechanisms of immune stimulation involve T-cells and macrophages, but further work is required to confirm this
Selenium[74,84]	Involved in bone health and immune function Deficiency of selenium leads to immunocompetence	Selenium supplementation studies in man have shown increased lymphocyte proliferation in response to mitogen and increased expression of high-affinity IL-2 receptor Selenium supplementation of healthy human adults with marginal selenium deficiency improved polio virus clearance in these individuals; the same study also demonstrated increases in T-cell and CD4+ cell numbers

in the latter.[3] While zinc deficiency can affect the immune system, it should be remembered that excessive zinc intake also impairs immune responses: high zinc intakes can decrease lymphocyte and phagocyte functions and can result in copper depletion (copper also being necessary for proper immune function).

Dietary Antioxidants—Vitamins A and E

Reactive oxygen species (free radicals) are produced by phagocytes as part of the body's defense against infection. These species can cause injury to immune cells, impairing cell-cell communication and, consequently, immune responsiveness. In addition to endogenous oxidative stress, exposure to oxidants and free radicals in the environment (e.g., cigarette smoke, ultraviolet light and ozone) can contribute to the level of oxidants in the body.[75] Many anti-oxidants are obtained from the diet, but adequate amounts of neutralizing anti-oxidants are required to prevent damage to immune cells by phagocyte-produced reactive oxygen species. It has long been known that there is a link between diets rich in anti-oxidants and a reduced incidence of cancer and it is thought that this is due, at least in part, to anti-oxidants boosting the body's immune system and helping to protect it from the toxic products (i.e., reactive oxygen species) produced by the action of phagocytes.[74]

Vitamin A affects many different types of immune cell. A deficiency of this vitamin can cause defects in phagocytic activity (i.e., defective chemotaxis, adhesion and ability to generate reactive oxygen metabolites in neutrophils), impairment of T- and B-cell function and reduced NK activity, production of IFN, effectiveness of fixed fat macrophage activity and lymphocyte response to stimulation by mitogens.[74] It can also change the integrity of the intestinal epithelium, which may lead to an altered immune response that allows translocation of bacteria (i.e., the movement of intestinal bacteria to extraintestinal organs) and, possibly, systemic infection.[76]

Vitamin E is the major lipid-soluble anti-oxidant in the body and is required for protection of membrane lipids from peroxidation.[3] Vitamin E deficiency has been shown to decrease spleen lymphocyte proliferation, NK cell activity and phagocytosis by neutrophils in animals.[3] Vitamin E deficiency is also known to increase susceptibility of animals to infectious pathogens; indeed, studies in chickens, turkeys, mice, sheep, pigs and cattle have shown that an increased intake of vitamin E promotes resistance to pathogens.[3] It should be noted that the effects of vitamin E deficiency are more marked if animals are fed a diet containing a high level of polyunsaturated fatty acids. In addition, the amount of vitamin E required for maximal effect is age-dependent (i.e., increases with age due, in part, to prolonged exposure to free radicals). As noted for zinc, excessive vitamin E in the diet can impair immune functions: some studies have reported that ≥300 mg vitamin E per day can decrease the ability of neutrophils to undergo phagocytosis and to kill bacteria and decrease monocyte respiratory burst and IL-1β production.[3]

Glutamine

Glutamine is defined as a conditionally essential amino acid. Studies have demonstrated that the ability to synthesize and store glutamine may be impaired in some individuals and may affect optimal growth and renewal of cells during long-term stress, hypercatabolic and hypermetabolic states or prolonged starvation.[77] Glutamine has been shown to change the cellular structure of the piglet small intestine (particularly the ileum and jejunum) and to possibly restore the intestinal mucosa after thinning (e.g., after weaning).[77] Therefore, there may be a future role for glutamine supplementation (either via enriched foods or production by probiotic bacteria) in the treatment of inflammatory conditions in the human small intestine.[77]

Vitamin D

Very few foods naturally contain vitamin D, which is why at the turn of the 20th century more than 80% of European and American children suffered from rickets.[78] Nowadays, many foods (e.g., dairy products, orange juice, cereals and bread) are fortified with vitamin D in the US. However, only margarine and some cereals are allowed to be fortified in most European countries due to an outbreak of vitamin D poisoning in the 1940s.[78] The majority of people's vitamin D (in the form of vitamin D_3) requirement is obtained by exposure to sunlight. Circulating levels of active

vitamin D in the body mean that it can interact with tissues that have a vitamin D receptor (i.e., skin, colon, prostate, breast, heart, skeletal muscle, brain, monocytes and activated T-cells), which helps maintain cellular growth and prevents the cells from becoming malignant; therefore, there is a strong link between vitamin D deficiency and the development of cancer.[78] Deficiency of this vitamin has also been observed in patients with irritable bowel disease (IBD) and accelerates the development of experimental IBD in IL-10 knockout mice.[79]

Nucleotides

Nucleotides, like many amino acids, are considered conditionally essential. They have been added to infant formulae for many years in an effort to improve immune function. During periods of rapid growth or limited nutrient intake, or in certain disease states in which a loss of gastrointestinal mass occurs, intake of nucleotides spares the organism from de novo synthesis and may bring tissue metabolic levels to full working conditions.[77]

Summary

The examples given in this chapter demonstrate clearly that a number of foods and food components beneficially stimulate the immune system and confer health benefits upon the consumer. Although studies into functional foods and their action on the immune system are still in their infancy, it is an exciting area of research that may allow the development of foods that will obviate the need for resorting to medicines for the treatment of certain conditions. The ultimate aim of these studies would be the development of a 'functional diet' that confers maximal health benefits upon the individual.[74] However, there may be a need to develop analytical methods (biochemical and/or molecular) that allow tailoring of functional foods to a particular individual's needs. It is important, also, to consider cultural and regional aspects when developing functional diets.[74]

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