Immunity

Beneficial Roles of Citrus

Executive Summary

Gail C. Rampersaud, Filomena Valim and Sandy Barros

The immune system is a complex system of cells, proteins, tissues, and organs that work together to help protect the body against infections or diseases. Nutrition plays an important role in immunity because nutrients are needed to keep all of these cells and processes running properly. Virtually every vitamin and mineral plays some role, however small, to support the immune system.

Several nutrients play especially important roles, including vitamins A, B6, C, E, and folate, as well as the minerals iron, zinc, selenium, and copper. Several of these key nutrients are provided in substantial amounts in citrus fruits and juices. For example, an 8-ounce glass of 100% orange juice is an excellent source of vitamin C, providing at least 100% of the Daily Value for that nutrient, and is a good source of folate. Orange juice supplies vitamin B6 as well as carotenoids that can be converted to vitamin A in the body.

To keep the immune system in top shape, consume a healthy diet that includes a variety of foods so that the body gets a wide range of nutrients needed to keep the immune system functioning properly. Citrus fruit and juices can be beneficial by providing substantial amounts of key nutrients to help support the immune system.

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Literature Review

Gail C. Rampersaud, Filomena Valim and Sandy Barros

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Executive Summary

The immune system is a complex network of cells, proteins, tissues, and organs that work together to protect the body against infectious diseases or other insults. An essential characteristic of the immune system is that it is composed of a number of cell types that function in different ways, and other blood-borne factors to provide a large variety of defense mechanisms. The immune system can be divided into two major components: nonspecific immunity which is present at birth and provides a general response against invasion by a wide range of antigens, and specific immunity which develops through the body's exposure to antigens. Cells associated with the immune system (Table 1) are generally termed leukocytes, or white blood cells. Leukocytes are divided into two main categories: phagocytes (granulocytes, monocytes, and macrophages) and lymphocytes. Macrophages participate in phagocytosis (engulfing and killing invading microbes). Lymphocytes are further categorized as T lymphocytes also referred to as T cells (including helper, cytotoxic, and suppressor T cells), B lymphocytes also referred to as B cells, and natural killer cells. T lymphocytes kill foreign cells through chemical lysis and help B lymphocytes participate in specific immune functions. B cells secrete antibodies that mark foreign cells for destruction. Natural killer cells target viruses and tumors. Many cells secrete cytokines, which are chemical messengers that allow the various immune cells to communicate with each other and elicit certain responses (Table 2).

It is widely accepted and understood that nutrition plays an important role in immunity. The immune system is a complex and integrated system involving a host of cellular and metabolic processes that require a readily available supply of nutrients in order to function properly. Several nutrients have been singled out as being most important for a healthy immune system. These nutrients include the vitamins A, C, E, B6, and folate (folic acid), and

the minerals iron, zinc, selenium, and copper. Several of these nutrients (e.g., vitamin C, folate, vitamin B6, and vitamin A via provitamin A carotenoids) are provided in substantial amounts in citrus fruits and juices.

Some micronutrients found in citrus, specifically vitamin C, folate, vitamin B6, and various carotenoids, appear to be required at least in some threshold amount for proper functioning of the immune system. This is clearly supported by studies where animals that are severely deficient in these nutrients have significant aberrations in immune function. Although the data are equivocal, studies show that supplementation of these nutrients, often at supraphysiological doses (i.e., doses significantly higher than dietary reference intakes), appears to improve the function of some components of the immune system, even in healthy individuals. Supplementation with individual nutrients at lower amounts (i.e., at or near the Recommended Dietary Allowances [RDA]) may be less effective or have no effect in modulating immune response; however more research is needed in this area. Several studies report that the inclusion of a nutritional supplement providing a variety of vitamins and minerals at physiological doses into the diet may enhance certain aspects of immune function, particularly in older individuals. However, more research is needed in healthy, younger populations.

It is important to note that no human intervention studies have been conducted to evaluate the effect of citrus fruits or juices on immune parameters. This represents a gap in the research that needs to be filled and limits the way that health messages regarding citrus and immunity can be presented to consumers. Nutrition-based long-term immune enhancement that directly relates to a reduced risk for diseases and infection is generally unproven at this time. In developed countries, frank nutrient deficiency is becoming more infrequent and individuals are more likely to have a moderate nutrient deficiency. It is not clear how a moderate deficiency would, if at all, negatively impact immune function. It also is unclear whether modest increases in nutrient intake, such as those expected from the normal consumption of citrus fruits and juices, would enhance immune response in healthy individuals and whether this would translate into clinically meaningful outcomes (i.e., reduced rates of infection or disease). These issues make it challenging to interpret and apply the results of studies to populations where individuals may be moderately or seldom

deficient in one or more nutrients (e.g., the United States). However, it is clear that citrus fruits and juices provide key nutrients that the immune system needs to function properly.

Based on this review, the following conclusions can be made:

- Several micronutrients provided in substantial amounts by citrus fruits and juices, specifically vitamin C, folate, vitamin B6, and some carotenoids, are required for proper functioning of the immune system. This is clearly supported by studies where severely deficient animals have overt aberrations in immune function. Many times the aberrations are reversed with nutrient restoration.
- Supplementation with single nutrients, some of which are found in citrus, often at supraphysiological doses, appears to improve the function of some components of the immune system, although the data are equivocal. Supplementation with individual nutrients at lower amounts (i.e., at or near the RDA) may be less or not effective in modulating immune response.
- Although they may play a role in modulating some aspects of immune function, there are no data to support that citrus flavonoids are required for adequate immune function.
- Several studies report that the consumption of a nutritional supplement providing a variety of vitamins and minerals at physiological doses into the diet over an extended period of time (i.e., greater than 6 months) may enhance certain aspects of immune function, particularly in older individuals. These data help support the concept that consumption of a varied diet that includes nutrient-rich foods, such as citrus fruits and juices, can contribute to maintaining a healthy immune system.
- Studies show that the inclusion of foods that provide particular nutrients in the diet may help enhance immune response. However, no studies have been conducted regarding citrus fruits or juices.
- From a research standpoint, it appears to be very difficult to consistently demonstrate immune enhancement in healthy individuals through the supplementation of single or multiple nutrients. Despite a vast body of research on various aspects of nutrition and immune function, it is unclear at this time which immune markers or how many markers might signal or present significant enhancements to immune function that may translate into lower disease rates.

The Immune System: Its Components and Function

Note: the information presented in this section was adapted from a United States Department of Health and Human Services publication (USDHHS 2003).

Introduction

The immune system is a complex network of cells, proteins, tissues, and organs that work together to protect the body against infectious diseases or other insults. The first line of immune defense is to keep infectious agents out of the body. Failing that, the job of the immune system is to seek out and destroy the intruder. An agent or substance that can trigger an immune response is called an antigen. The most common antigens and disease-causing microbes are bacteria, viruses, and parasites. Tissues or cells from another person (except an identical twin) are not recognized as the body's own cells and also can act as antigens, which explains why tissue or organ transplants may be rejected. An essential characteristic of the immune system is that it is composed of a number of cells types that function in different ways, and other blood-borne factors to provide a large variety of defense mechanisms.

The immune system can be divided into two major components: **nonspecific** and **specific** immunity. **Nonspecific immunity**, also known as innate or natural immunity, is present at birth and comprises defense mechanisms that provide a general response against invasion by a wide range of antigens. Nonspecific immune components include structural or mechanical barriers (e.g., skin, mucus membranes, and tears) that provide an imposing barrier to invading organisms. If antigens survive the body's front-line defenses, they still have to find a way through the walls of the digestive, respiratory, or urogenital passageways to the underlying cells. These passageways are lined with tightly packed cells covered in a layer of mucus, effectively blocking the transport of many organisms. Other physiological properties of the body, such as temperature, pH (acid/base), and oxygen levels, also act to destroy or keep out potential antigens.

The other component of the immune system is called **specific immunity**, also known as humoral or acquired immunity. This part of the immune system develops based on the exposure to antigens and i nvolves the production of specific antibodies or activation of specific cells against a particular pathogen or other foreign substance. After exposure to an antigen, some immune cells become memory cells and can respond more readily when the immune system encounters that antigen again. An immune response can be sparked not only by infection but also by immunization with vaccines. Vaccines are basically low concentrations of a particular antigen, which makes the body produce memory cells that can act quickly if the body is exposed to the antigen again at a later time.

The Structure of the Immune System

The primary organs of the immune system are positioned throughout the body as depicted in Figure 1. The cells associated with the immune system are generally termed **leukocytes, or white blood cells**. Leukocytes are divided into two main categories: **phagocytes** (granulocytes, monocytes, and macrophages) and **lymphocytes.** Lymphocytes are further categorized as **T lymphocytes, B lymphocytes,** and **natural killer cells** and are the major players in the immune response. T lymphocytes are further categorized into **helper T cells** (also called CD4 cells because they carry the CD4 protein surface cell marker) and **cytotoxic T cells** (also known as killer T cells). There is also a

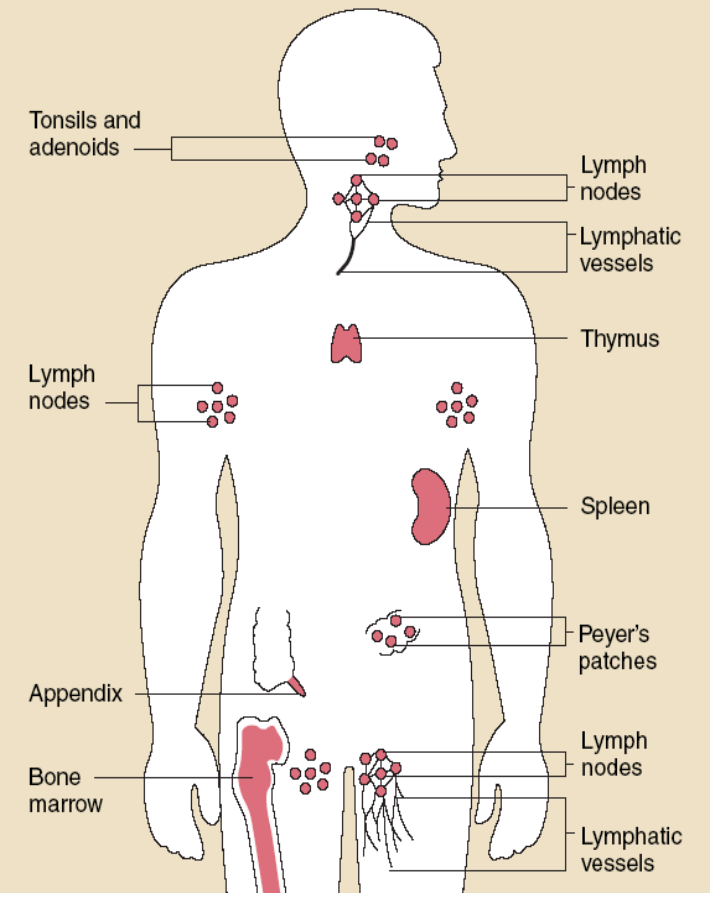


Figure 1. Organs of the immune system (USDHHS 2003).

class of T cells called **T suppressor cells**, which regulate the actions of T cells and help prevent the immune system from overreacting. All cells associated with the immune system originate from the bone marrow. They respond to different **cytokines**,
powerful chemical substances secreted by cells that enable the body's cells to communicate with one another (see below for more explanation), and other signals to grow into specific immune cell types. Once released, the immune cells are sequestered into the lymphoid organs (Figure 1), found circulating in the blood stream, or dispersed in other tissues. Table 1 presents a summary of the cells that participate in the immune response. In non

Table 1. Immune Cells that participate in the immune response (Tortora et al. 1993)

Cell Type	Function(s)
Macrophage	▪ Phagocytosis ▪ Processes and presents antigens to T cells ▪ Secretion of cytokines, interleukin-1 (IL-1) to induce proliferation of B cells ▪ Secretion of interferon that stimulates T cell growth
B cell	▪ Differentiates into antibody-producing plasma cells ▪ May process and present antigen to helper T cells
Cytotoxic (killer) T cell	▪ Kills foreign cells through chemical lysis, etc. ▪ Releases cytokines, interferon-gamma (IFN-γ) that attract macrophages and increases their phagocytic activity ▪ Prevents macrophage migration from site of action
Helper T cell	▪ Cooperates with B cells to amplify antibody production by plasma cells ▪ Secretes interleukin-2 (IL-2) to help stimulate proliferation of T and B cells ▪ May secrete IFN-γ and tumor necrosis factor (TNF), which stimulate the inflammatory response
Suppressor T cell	▪ Thought to downregulate immune responses by producing cytokines which inhibit B and T cells ▪ May directly destroy activated lymphocytes
Memory T cell	▪ Remains in lymphoid tissue and recognizes original invading antigens, even years after exposure
Plasma cell	▪ Descendent of B cells ▪ Produces antibodies
Memory B cell	▪ Responds more rapidly and forcefully should the same antigen enter the body in the future

specific (innate) immunity, the goal is to either block infectious agents from entering the body or quickly destroy them after entry. Antigen destruction can be accomplished through the direct destruction of pathogens by complement, toxic chemicals (superoxide radicals, hydrogen peroxide) secreted by phagocytes, or by toxic proteins secreted by special cells called natural killer cells. The **complement** system is made up of about 25 proteins that complement or enhance other immune responses. For instance, complement can cause blood vessels to become dilated and then leaky, contributing to the redness, warmth, swelling, pain, and loss of function that characterize an inflammatory response. Antigens also can be eliminated through the action of **phagocytes**, large white cells that can swallow and digest microbes and other foreign particles. **Monocytes** are phagocytes that circulate in the blood. When monocytes migrate into tissue, they develop into **macrophages**, a large and versatile immune cell that devours invading pathogens and other intruders. Macrophages play many roles. As scavengers, they rid the body of worn-out cells and other debris. The process of phagocytosis is

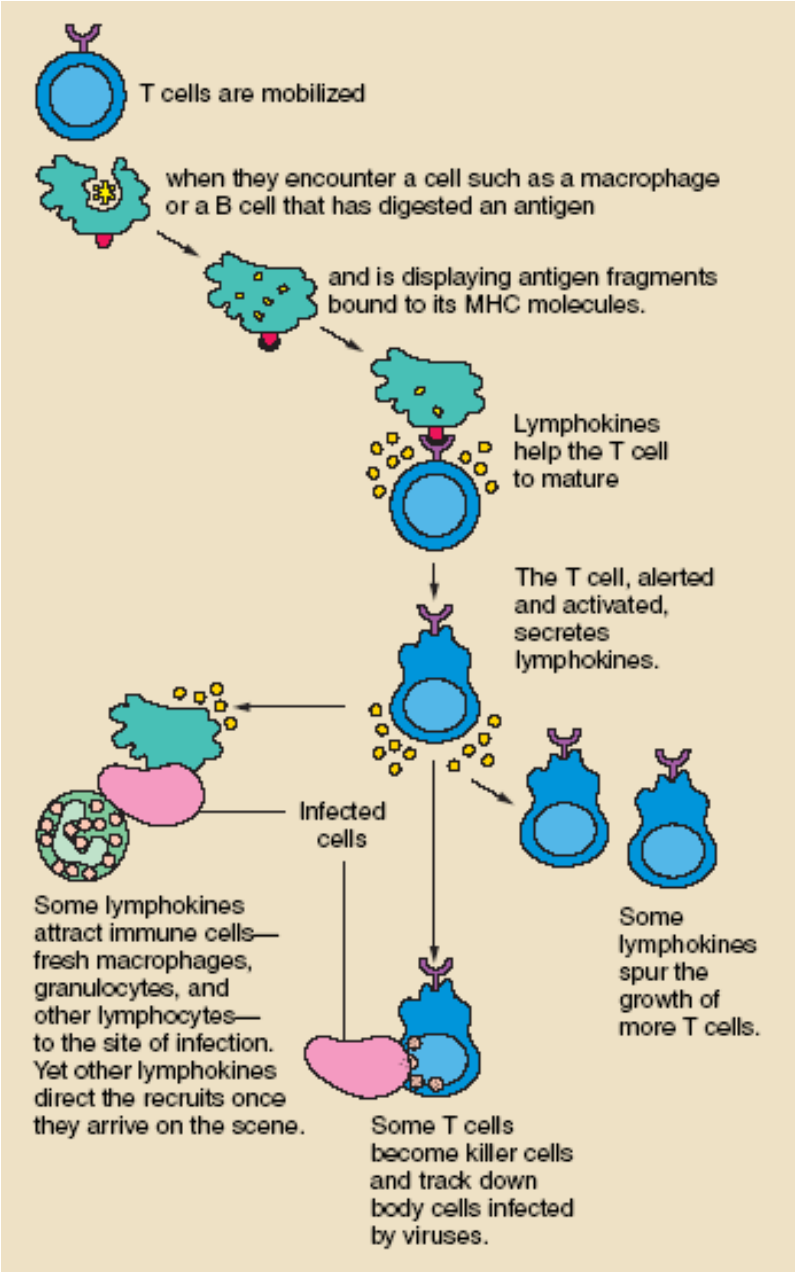


Figure 2. T cell function (USDHHS 2003).

enhanced when the invader is coated with proteins such as complement or antibodies.

Specific (acquired) immunity involves the recognition of an antigen by antibodies, which are **immunoglobulins** (Ig) produced by B cells, and by T cells. T cells recognize antigen infected cells through proteins, termed **major histocompatibility complex** (MHC), displayed on the surface of an infected cell. Cytotoxic T cells can then recognize and destroy the infected cells. Extracellular pathogens stimulate a response from helper T cells. Helper T cells coordinate immune responses by communicating with other cells that can destroy the infected cell (Figure 2).

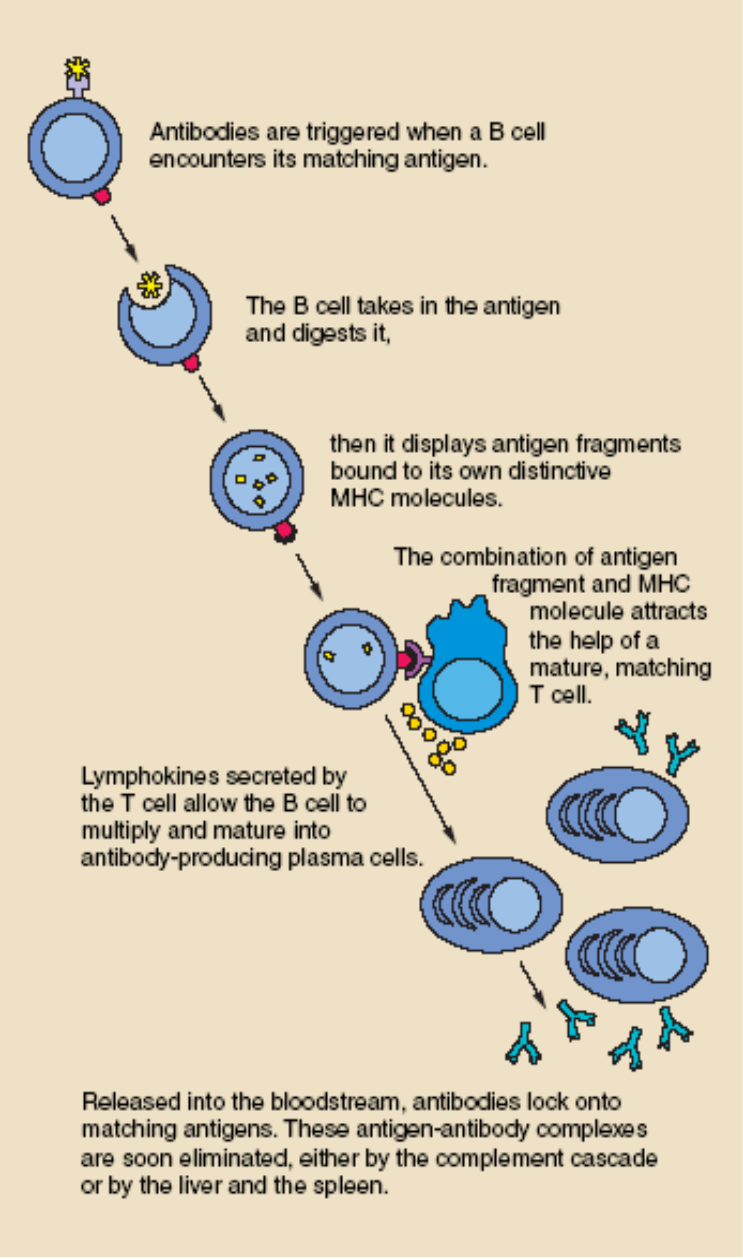


Figure 3. B cell function (USDHHS 2003).

B cells work chiefly by secreting substances called antibodies into the body's fluids. Antibodies recognize antigens circulating in the bloodstream. They are powerless, however, to penetrate cells. Each B cell is programmed to make one s pecific antibody. When a B cell encounters its triggering antigen, it gives rise to many large cells known as **plasma cells**. Every plasma cell is essentially a f actory for producing an antibody. Whenever antigen and antibody interlock, the antibody marks the antigen for destruction (Figure 3). Antibodies belong to a family of large molecules known as immunoglobulins. Different types play different roles in the immune defense strategy. Immunoglobulin E or IgE, whose natural job probably is to protect against

parasitic infections, is responsible for the symptoms of allergy. Components of the immune system communicate with one an other by exchanging chemical messengers called **cytokines**. Some cytokines function as chemical "switches" that turn certain immune cell types on and off. Examples of cytokines include interleukins 1, 2, 4 and 5 (IL-1, IL-2, IL-4, IL-5), tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ). Selected cytokines and their activities are presented
in Table 2.

Table 2. Selected Immune Cytokines and Their Activities (Decker 2006)

Cytokine	Producing Cell	Target Cell	Function**
IL-1α IL-1β	monocytes macrophages B cells DC	Th cells	co-stimulation
		B cells	maturation and proliferation
		NK cells	activation
		various	inflammation, acute phase response, fever
IL-2	Th1 cells	activated T and B cells, NK cells	growth, proliferation, activation
IL-3	Th cells NK cells	stem cells	growth and differentiation
		mast cells	growth and histamine release
IL-4	Th2 cells	activated B cells	proliferation and differentiation IgG_1 and IgE synthesis
		macrophages	MHC Class II
		T cells	proliferation
IL-5	Th2 cells	activated B cells	proliferation and differentiation IgA synthesis
IL-6	monocytes macrophages Th2 cells stromal cells	activated B cells	differentiation into plasma cells
		plasma cells	antibody secretion
		stem cells	differentiation
		various	acute phase response
IL-7	marrow stroma thymus stroma	stem cells	differentiation into progenitor B and T cells
IL-8	macrophages endothelial cells	neutrophils	chemotaxis
IL-10	Th2 cells	macrophages	*cytokine production*
		B cells	activation
IL-12	macrophages B cells	activated Tc cells	differentiation into CTL (with IL-2)
		NK cells	activation
IFN-γ	Th1 cells, Tc cells, NK cells	various	*Viral replication*
		macrophages	MHC expression
		activated B cells	Ig class switch to IgG_{2a}
		Th2 cells	*proliferation*
		macrophages	pathogen elimination
TNF-α	macrophages, mast cells, NK cells	macrophages	CAM and cytokine expression
		tumor cells	cell death
TNF-β	Th1 and Tc cells	phagocytes	phagocytosis, NO production
		tumor cells	cell death

Abbreviations: CTL: cytotoxic T lymphocytes; DC: dendritic cells; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; IL: interleukin; IFN: Interferon; TGF: Tumor Growth Factor; TNF: Tumor Necrosis Factor.
** Italicized activities are inhibited.

Disorders of the Immune System

The most common types of allergic diseases occur when the immune system responds to a false alarm. In persons with allergies, a normally harmless material such as grass pollen or house dust is mistaken as a threat (antigen) and attacked. Sometimes the body begins to manufacture T cells and antibodies directed against its own cells and organs. T cells that attack pancreas cells contribute to diabetes, while an autoantibody known as rheumatoid factor is common in people with rheumatoid arthritis. No one knows exactly what causes an autoimmune disease, but likely candidates are the environment, hormones, or heredity.

When the immune system is missing one or more of its components, the result is an immunodeficiency disorder. Immunodeficiency disorders can be inherited, acquired through infection, or produced unintentionally by drugs such as those used to treat people with cancer or those who have received transplants. Acquired immunodeficiency syndrome (AIDS) is an immunodeficiency disorder caused by the human immunodeficiency virus (HIV) that infects immune cells. HIV can destroy or disable vital T cells. A contagious disease, HIV is spread by intimate sexual contact, transfer of the virus from mother to infant during pregnancy, or direct blood contamination.

Chronic Inflammation: A Persistent Immune Response

Some diseases and conditions are thought to have a strong immune component, including asthma, atherosclerosis, cancer, Crohn's Disease, myasthenia gravis (a neuromuscular disorder), multiple sclerosis, rheumatoid arthritis, and lupus (Albers et al. 2005). The initiation or progression of some of these conditions can be exacerbated by an inappropriate immune response. Inflammation is the body's response to injury or insult and is part of the normal immune response. However, prolonged and persistent inflammation can result from chronic stimulation and response of the immune system, and is now thought to be the underlying mechanism in a variety of chronic diseases including cancer and cardiovascular disease. The subject of chronic inflammation as it relates to immunity and disease processes has been recently reviewed and reported (Zhang 2005) and will therefore not be covered in this report except where deemed pertinent to the topic.

Role of Nutrition in Immune Function

Introduction

There is a strong consensus that nutrition plays a role in modulating immune function and that immune function relies on an adequate supply of nutrients to function properly (Albers et al. 2005). The complexity and range of function of the immune system supports this idea because optimal functioning of the immune system involves a variety of biological activities including cell division and proliferation, energy metabolism, and production of proteins. Calder and Kew (Calder 2002) observed that nutrient status is an important factor contributing to immune competence. Nutrients that have been demonstrated (in animal or human studies) to be required for the immune system to function efficiently include amino acids, the essential fatty acid linoleic acid, vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, Zn, Cu, Fe and Se. Studies indicate that undernutrition impairs the immune system, thereby suppressing immune functions that are fundamental to host protection. This is observed in developing countries where malnutrition, especially in preschool children, often coexists with infectious diseases (Bhaskaram 2002). Animal and human studies have demonstrated that adding deficient nutrients back to the diet can restore immune function and resistance to infection (Calder 2002).

Studies clearly indicate that single or multiple deficits in macro- or micro-nutrients can result in immune dysfunction (Amati et al. 2003). Some but not all studies have suggested that supplementation with single or multiple micronutrients may enhance some markers of immune function even in healthy individuals. However, Calder and Kew (Calder 2002) warn that excess amounts of some nutrients also may impair immune function (e.g., vitamin E, zinc). Additionally, over-activation of the immune system can lead to detrimental effects such as chronic inflammation or autoimmune diseases as described earlier.

Immune function can be influenced by genetic as well as environmental factors. Factors that may affect immune function include age, gender, nutritional status, psychological stress, prior exposure to pathogens, and clinical disorders such as human immunodeficiency virus (HIV) infection and autoimmune diseases such as lupus (Albers et al. 2005). The aging process has been associated with an increase in disease and infection. Abluwalia

(Ahluwalia 2004) states that some of the changes in an aging person's immune system may be due to deficiencies of macronutrients and micronutrients, notably, vitamins B6, B12, and folic acid as well as iron and zinc.

Antioxidants also may play an important role in immune function, primarily because the immune system may be particularly vulnerable to the effects of oxidative stress and damage (Hughes 2001). Immune cells are highly dependent on cell-to-cell signaling and communication, much of which transpires through cell surface membrane receptors. Reactive species may affect membrane integrity of immune cells which could negatively impact immune function. Immune cells also contain a high percentage of polyunsaturated fatty acids in their cells membranes, making them especially susceptible to lipid peroxidative damage. Phagocytes produce reactive oxygen species as part of their normal function and antioxidants can protect these cells from self-damage.

The micronutrients most often cited as being important to immune function include vitamins A, C, E, and B6, folate (folic acid), iron, zinc, selenium, and copper (Scrimshaw et al. 1997; Meydani et al. 2001; Calder 2002; Chandra 2002). Other nutrients mentioned as playing a roll in immune function include beta-carotene (a precursor to vitamin A) (Scrimshaw et al. 1997; Meydani et al. 2001), vitamin B12 (Scrimshaw et al. 1997; Meydani et al. 2001; Calder 2002), and vitamin D (Scrimshaw et al. 1997; Meydani et al. 2001). Several of these nutrients are provided in significant amounts in citrus fruits and juices, particularly vitamin C, folate, vitamin B6, and various provitamin A carotenoids (e.g., beta-cryptoxanthin in oranges and beta-carotene in pink grapefruit). Along with select citrus phytochemicals (i.e., flavonoids), these nutrients will be the primary focus of this review.

Table 3. Common immune system markers

Immune Response/Function	Parameter/Marker	Biological Function and Clinical Significance of Marker
In vivo integrated response	Response to vaccination	Reflects immune function (B and/or T cell responses) and vaccination status
	Delayed-type hypersensitivity (intradermal test)	Measures in vivo cell-mediated immune response
Ex vivo: innate immune functions	Phagocyte function (phagocytosis, oxidative burst)	Neutrophil and monocyte function(s) Ability to combat bacterial infection
	Natural killer (NK) cell function (NK cell-mediated cytosis)	Reflects defense against virus-infected and malignant cells
	Antigen presenting cell (APC) function	Cell activation and capability of cells to produce cytokine profile inflammatory mediators
Ex vivo: acquired immune functions	Lymphocyte proliferation	Measure of lymphocyte replication
	Lymphocyte activation	Quantification of activated cells
	Lymphocyte derived mediator production	Response of T helper cells (interleukin and interferon production)
Basal markers of immune function	Complement activity	Opsonization (coating of pathogen for recognition), bacterial lysis
	Circulating levels of immunoglobulins	Detection of B cell defects or unspecific polyclonal activation
	Differential leukocyte count	Indicates circulating leukocyte pool
	Lymphocyte subpopulations	Marker of current and previous activation
	Circulating cytokine and cytokine receptor concentrations	Reflect in vivo pro- and anti-inflammatory state
Gut-associated immune function	Integrity of mucosal barrier	Defense against microbial and antigen invasion
	GALT plasma cell function	GALT B-cell activity
	Intestinal inflammation	Gut defense
	Mucosal histology	T cell function and mucosal architecture
	Stool cytokine concentration	Intestinal cytokine production

It should be noted that despite a vast body of research on various aspects of nutrition and immune function, it is unclear at this time which immune markers or how many markers might signal or present significant enhancements to immune function that may translate into lower disease rates. Studies do indicate that nutrition can affect immune markers even in well-nourished individuals (Albers et al. 2005). However, nutrition-based long-term immune enhancement that directly relates to a reduced risk for diseases and infection is generally unproven at this time (Calder 2002).

Measurement of Immune Parameters in Nutritional or Other Studies

The immune system involves a wide array of functionally differing cell types and other processes including the production of proteins and other chemical messengers. Therefore, adequately addressing immune status and function involves an assessment of a wide array of parameters. Table 3 presents a general overview of immune system markers that are commonly measured in research studies, including the biological function and clinical significance of the parameter/marker (Albers et al. 2005). This information may be useful when evaluating the methodology and results of research studies concerning nutrition and immunity.

Role of Citrus Nutrients in Immune Function

Although several micronutrients have been identified as important to the functioning of the immune system, this report will focus on those nutrients provided in substantial quantities in citrus or citrus juices, particularly vitamin C, folate, vitamin B6, carotenoids (beta-carotene, beta-cryptoxanthin and lycopene), and thiamin. This report also will address the potential role of several citrus flavonoids in immune function.

Vitamin C

Background

Vitamin C is a water-soluble vitamin that has high reducing power and therefore functions as a powerful antioxidant. Vitamin C is a cofactor for enzymes involved in the biosynthesis of collagen, carnitine (which transports fatty acids into the part of the cell where they can be oxidized), neurotransmitters, hormones, and amino acids. Vitamin C also helps facilitate iron transport. As an antioxidant vitamin C can quench a variety of reactive oxygen and nitrogen species. Vitamin C is a term that describes two forms of the vitamin: ascorbic acid, which is the functional and primary form of the vitamin in the body, and dehydroascorbic acid, an oxidized form of the vitamin which also has antiscorbutic action. Animals such as guinea pigs, primates, and humans lack the enzyme needed to produce vitamin C and therefore must obtain vitamin C from exogenous sources. The Recommended Dietary Allowance (RDA) for vitamin C is 75 mg/day for adult women and 90 mg/day for adult men (Institute of Medicine 2000). Because smokers have higher oxidative stress and a higher rate of vitamin

C turnover, it is recommended that adult men and women who smoke obtain an extra 35 mg/day vitamin C above the RDA. The Tolerable Upper Intake Level (UL) is 2 g/day, an amount associated with diarrhea and other GI disturbances. Median intake of vitamin C for men and women in the US population age 20 years and older ranges from 60 to 85 mg/day and 52 to 81 mg/day, respectively (Ervin et al. 2004). Median intakes for men are below the RDA of 90 mg/day, and median intakes for women age 20 through 59 years are below the RDA of 75 mg/day. These data indicate that significant numbers of the population may not consume the RDA for vitamin C on any given day. Data from the population-based Third National Health and Nutrition Examination Survey (NHANES III) (Hampl et al. 2004) indicate that vitamin C deficiency and depletion occurred in 5 percent to 17 percent of respondents, and 13 percent to 23 percent of respondents, respectively, based on serum values. Smokers were 3 to 4 times more likely to be deficient in vitamin C. Data suggest that significant number of Americans do not consume enough vitamin C (Ervin et al. 2004) or may be considered vitamin C deficient or depleted based on serum vitamin C concentrations (Hampl et al. 2004), thereby supporting the need to encourage the intake of vitamin C-rich foods in the general population. In addition, vitamin C is listed as a "nutrient of concern" for adults in the 2005 Dietary Guidelines for Americans (USDHHS 2005). The vitamin C content of citrus fruit and juices is presented in Table 4.

Table 4. Vitamin C content of citrus fruits and juices

Product	Portion	% Daily Value*	Amount (approximate) (mg)
Orange juice	8 oz	120**	72
Grapefruit juice	8 oz	100	60
Orange	154 g	130	78
Grapefruit	154 g	110	66
Tangerine	109 g	50	30

* The Daily Value for vitamin C is 60 mg.
** Represents end-of-shelf-life amount for not-from-concentrate juice.
Source: www.floridajuice.com.

Vitamin C and Immunity

Vitamin C may play an important role in the immune system. It is well known that vitamin C is highly concentrated in leukocytes (Field et al. 2002). In fact, along with the pituitary gland,

adrenal gland and eye lens, leukocytes have the highest vitamin C concentration of all body tissues (Institute 2000). In neutrophils, it is thought that the antioxidant properties of vitamin C protect against reactive oxygen species produced during the process of phagocytosis. Neutrophils take up vitamin C during oxidative burst (the release of reactive oxygen species to kill bacteria or other invaders) and display decreased vitamin C levels following activation. Because vitamin C is needed for the biosynthesis of collagen, an integral component of bone and skin, it also can affect the body's first lines of immune defense. The immune modulating functions of vitamin C are thought to fall into two main categories: vitamin C's role in neutralizing reactive oxygen species produced by phagocytes during destruction of antigens, and the effects of vitamin C on various immune system components (Jacob et al. 2002).

Immune function is negatively impacted in vitamin C deficient animals (Leibovitz et al. 1981; Bendich 1990). Neutrophils from vitamin C deficient guinea pigs display reduced chemotaxis and bactericidal activities (Shilotri 1977; Goldschmidt et al. 1988), and vitamin C deficient guinea pigs had depressed cytotoxic T lymphocyte killing of tumor cells (Anthony et al. 1979) and lower antibody production (Thurman et al. 1979). However, lymphocyte proliferation was found to be unaffected in vitamin C deficient guinea pigs (Zweiman et al. 1966; Bendich et al. 1984).

Some but not all studies in humans have reported beneficial associations between vitamin C intake and immune parameters including cytokine production, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity response to skin antigens (cell-mediated response). Two studies evaluated the association between vitamin C and the production of cytokines. An in vitro assay on human whole blood cells reports that stimulated monocytes cultured in various concentrations of vitamin C produced significantly lower levels of the proinflammatory cytokines IL-6 and TNF-α in a dose dependent manner (Hartel et al. 2004). Other proinflammatory cytokines IL-1α and IL-8 were not affected. In this same study, a dose-dependent inhibition of IL-2, but not TNF-α or IFN-γ, was observed in lymphocytes cultured in higher vitamin C concentrations. However, in 40 healthy male and female volunteers (22-55 years of age), vitamin C supplementation of 1 g/day for 28 days resulted in no differences in the production of IL-1β and TNF-α in isolated peripheral blood

mononuclear cells compared to the consumption of a placebo (Jeng et al. 1996). These data suggest that vitamin C may play a role in the inflammatory response through regulation of cytokine production in particular cell types.

In vitro studies suggest that vitamin C modulates some immune parameters. Using the blood of elderly subjects over the age of 65 years and many with diagnosed cardiovascular disease, lymphocytes incubated with vitamin C had increased proliferation in vitro in a dose response manner (Delafuente et al. 1986). In vitro incubation of peripheral blood cells with vitamin C increased phagocytosis potential in polymorphornuclear cells, but not monocytes, and increased the number of apoptic cells (Bergman et al. 2004).

Several short-term human intervention studies suggest that vitamin C supplementation may enhance aspects of immunity. A placebo-controlled trial in healthy older persons (n=20, over 70 years of age) evaluated daily intramuscular injections of vitamin C (500 mg/day) for 1 month on various immune markers. Individuals in the vitamin C treatment group had no changes in the blood immunoglobulins IgA, IgG and IgM, nor the proportion of T cells in blood; however, lymphocyte proliferation to mitogens increased in the treatment group but not in the placebo group (Kennes et al. 1983). In another study, five healthy adults were administered an increasing dose of ascorbate over 3 weeks (i.e., 1 g daily for week one, 2 g daily for week two, and 3 g daily for week three) (Anderson et al. 1980). In vitro testing of immune function was performed after each week. Neutrophil chemotaxis (the chemical attraction of phagocytes to a particular area) was significantly enhanced following the 2 and 3 g doses of vitamin C and lymphocyte DNA and protein synthesis was significantly enhanced following ingestion at all dose levels (i.e., 1, 2, and 3 g/day ascorbate). However, ascorbate did not affect phagocytic metabolic activity or immunoglobulin or complement concentrations. In another study, fifteen subjects were randomized to consume 2 g/day vitamin C or placebo 3 weeks. Vitamin C supplementation did not affect in vitro lymphocyte proliferation from blood samples (Delafuente et al. 1986). There also were no differences in white blood cell counts or lymphocyte counts in those receiving vitamin C compared to placebo. In young healthy men, vitamin C intake of 200 mg/day did not affect bactericidal activity of leukocytes. However, 2 g/day impaired bacterial killing by leukocytes (Shilotri et

al. 1977), suggesting that vitamin C had a negative effect on immune response at higher intakes.

In a depletion-repletion study in 8 healthy men, moderate vitamin C deficiency caused by intake of 5 to 20 mg/day vitamin C for 60 days resulted in a significantly reduced level of vitamin C concentration in mononuclear leukocytes and semen, and markedly reduced induration and antigen score in the delayed-type hypersensitivity skin response, but did not alter the proliferation capability of peripheral blood mononuclear cells to T and B cell antigens (Jacob et al. 1991). Repletion with vitamin C at 60 or 250 mg/day for 28 days did not restore antigen response to pre-depletion levels, although induration increased in 3 subjects. These data suggest that moderate depletion of vitamin C impacts some but not all aspects of cell-mediated immunity and repletion at levels near or moderately above the RDA was not effective in restoring immune function in this study. Several studies in humans report no effect of vitamin C on leukocyte function (Ludvigsson et al. 1979), leukocyte levels (Ludvigsson et al. 1979), phagocytic activity (Shilotri et al. 1977), or components of the complement system (Johnston 1991).

In humans, the role of vitamin C in immune function is often discussed in the context of its potential effects on the common cold. In 1970, Linus Pauling published his book titled "Vitamin C and the Common Cold", which generated an extensive amount of public interest regarding vitamin C and the common cold. Since that time, the public perception has been that vitamin C could have an effect on helping to prevent colds. A recent meta-analysis evaluated the research regarding vitamin C and the common cold (Douglas et al. 2004). The analysis included research regarding vitamin C supplementation for both prevention and treatment, and when vitamin C was used as a prophylaxis or as treatment after the onset of illness. After evaluating 29 trials that included more than 11,000 participants, there was no evidence to suggest that vitamin C supplementation could reduce the risk of developing a cold. However, a subset of six studies that included marathon runners, skiers, and soldiers on sub-arctic exercises did find a very significant benefit of vitamin C in reducing the risk of getting a cold by 50 percent. The meta-analysis concluded that vitamin C may help modestly reduce the duration (by 8 per cent in adults and 13.5 percent in children; 30 trials) and severity (15 trials) of a cold if vitamin C is taken prophylactically,

although doses ranged from 200 to 2000 mg/day in these studies. There was no benefit to colds if vitamin C was used as therapy following the onset of cold symptoms.

Vitamin C may indirectly affect immune response by helping to maintain circulating concentrations of vitamin E (Bendich 1990). Vitamin E is important in maintaining the integrity of cell membranes, including those of lymphocytes. Although vitamin C did not effect T and B cell responses in guinea pigs, it did help enhance vitamin E levels in the lungs of guinea pigs (Bendich et al. 1984). In human subjects, the production of cytokines was significantly increased in those receiving both vitamin C and vitamin E supplementation, but not vitamin C or vitamin E alone (Jeng et al. 1996).

Vitamin C and Histamine

Histamine is a chemical that acts as a messenger in times of stress. Histamine is released from two types of specialized cells, basophils and mast cells, which are concentrated in the lungs, airways, skin, and gastrointestinal tract (Johnston 1996). Histamine is released based on various stimuli, including cytokines, and acts on a variety of cell types that have histamine receptors. The result includes a variety of physiologic responses including increased gastric secretions, vasodilation of cerebral arteries, relaxation of airway smooth muscle, increased mucus secretion, and rise in heart rate and contraction force (Johnston 1996). In these ways, the role of histamine is to promote alertness and preparedness (for “fight or flight” responses) as well as healing in localized tissues. Antihistamine drugs are commonly effective in reducing adverse symptoms caused by histamine, including sneezing and nasal secretions.

Excess histamine can have adverse impacts by causing unwanted responses in circulatory, respiratory, and immunologic processes, including hypotension, breathing difficulty, and allergic reactions (Johnston 1996). It has been postulated that vitamin C deficiency can lead to histinemia (excess histamine in the blood) and subsequently an increased risk for atherosclerosis and coronary heard disease (Clemetson 1999).
Studies suggest that vitamin C can act to reduce excess histamine through degradation (Johnston 1996), thereby potentially reducing the risk for adverse responses caused by excess histamine. However, it appears that vitamin C intake and status must be quite high

for this to take place. One study reports that after graded vitamin C doses of 500 mg for 2 weeks and 2000 mg for 2 weeks, 2000 mg of vitamin C was required to significantly reduce blood histamine from baseline in a small group of men and women (Johnston et al. 1992). Johnston (Johnston 1996) concludes that based on the results of this study, individuals would need to consume at least two vitamin C-rich fruits or vegetables on a daily basis or take a daily vitamin C supplement to benefit from vitamin C-induced antihistamine effects. In another small study, depletion and subsequent repletion of individuals with vitamin C (125 mg for one week followed by 250 mg for one week) resulted in significant drops in blood histamine concentrations compared to histamine concentrations following vitamin C depletion (Johnston et al. 1996). However, supplementation with 30 and 60 mg/day for one week each was not effective in reducing histamine levels following vitamin C depletion.

The clinical benefit of vitamin C remains unclear. Several studies have found a significant benefit of vitamin C administration on bronchial response to histamine (Bucca et al. 1989, 1990), while no benefit was observed in bronchial response (Malo et al. 1986) or skin response to histamine (Fortner et al. 1982). Hemila (Hemila 1992) notes that the antihistamine effect of vitamin C is probably not the main mechanism accounting for the association between vitamin C and reduced severity of a cold. Therefore, it remains unclear as to whether vitamin C would provide a clinically relevant benefit with regard to histamine-mediated processes.

Vitamin C and Cancer

Vitamin C has been associated with reducing the risk of several chronic diseases, including certain cancers. A World Cancer Research Fund/American Institute for Cancer Research report (World Cancer Research Fund 1997) states that the scientific evidence suggests that it is “probable” that vitamin C may reduce the risk of stomach cancer and “possible” that vitamin C may reduce the risk of cancers of the mouth and pharynx, esophagus, lung, pancreas and cervix. The report suggests several mechanisms by which vitamin C may reduce cancer risk, including acting as an antioxidant to detoxify carcinogens before they can damage DNA; helping maintain collagen for healthy membranes and other tissues to

reduce the chance of a conducive environment for tumor formation or progression; and enhancing the immune system and tumor surveillance. However, no current human clinical trials have directly linked immune enhancement by vitamin C with a reduced risk for cancer.

Summary of Vitamin C and Immune Function

Animal studies suggest that vitamin C can modulate several markers of immune function, including antibody production and neutrophil (phagocyte) and cytotoxic T cell function. In humans, studies support a role for vitamin C in cytokine production, lymphocyte proliferation, chemotaxis, and cell-mediated immune responses. Based on the results of a meta-analysis, it appears that vitamin C may play some role in respiratory defense mechanisms, particularly subsequent to onset of illness. Studies evaluating prophylactic treatment and cold duration/severity used vitamin C supplements in amounts ranging from 200 to 3000 mg per day, with an average intake of approximately 1000 mg/day considering all studies. This average intake represents an amount more than 10-fold greater than the vitamin C content of an 8-ounce glass of orange juice.

There is evidence that vitamin C plays a role in immune response. However, the mechanisms have not been completely elucidated but likely involve vitamin C's role as a potent antioxidant (Jacob et al. 2002). Methodological differences in studies likely account for the inconsistencies in study results regarding vitamin C and immunity. Also, it appears that vitamin C may exert different or no effects on immune response in well-nourished individuals compared to those that may be more deficient in vitamin C. Many of the intervention studies used vitamin C amounts of at least 1 g/day, which is over 10 times the RDA for vitamin C and more than most (unsupplemented) individuals would obtain from the diet. The repletion phase of a depletion-repletion study (Jacob et al. 1991) used vitamin C amounts that were more representative of normal intake (i.e., 60 and 250 mg) and found that these amounts consumed over a 4-week period were unable to restore immune functions following vitamin C depletion. It may be that higher vitamin C amounts or a longer repletion period would be required to reverse the immune abnormalities, but also calls into question whether modest supplementation is effective for improving immune response. Although there appears to be an inverse relationship between plasma vitamin C and

histamine concentrations, the clinical significance of this association has not been firmly established at this time.

Folate

Background

Folate is a water-soluble B vitamin that includes the form of the vitamin found naturally in certain foods (including citrus fruits and juices), called food folate, and the synthetic form of the vitamin that is added to fortified foods and supplements, called folic acid. Folate has various biological functions, including functioning as a coenzyme in the transfer of one-carbon units, synthesis of pyrimidine nucleotides that are essential for DNA, synthesis of purines, methylation reactions including DNA methylation, and amino acid interconversions. Clinical or other manifestations of low folate status include megaloblastic anemia, a type of anemia characterized by large red blood cells with abnormal nucleations. Low folate status also has been associated with elevated blood homocysteine concentrations (Selhub et al. 1993; Verhoef et al. 1996; Riddell et al. 2000), an identified independent risk factor for cardiovascular disease (Refsum et al. 1998). Controlled intervention trials definitively link folic acid supplementation with significantly reducing the risk of a woman having a baby with a neural tube defect (Medical Research Council 1991; Berry et al. 1999), leading to public health recommendations that all women of childbearing age consume 400 µg of synthetic folic acid and ample amounts of food folate on a daily basis. The Recommended Dietary Allowance (RDA) for adults for folate is 400 µg dietary folate equivalents (Institute of Medicine 1998). The Tolerable Upper Intake Level (UL) is 1,000 µg per day for adults, consumed as synthetic folic acid. The Institute of Medicine has identified special folic acid needs for women of childbearing age as indicated above. Median intake of folate for men and women in the US population age 20 years and older ranges from 351 to 394 µg/day and 275 to 291 µg/day, respectively (Ervin et al. 2004). Median intakes for men and women in all age groups fall below the RDA of 400 µg/day, suggesting that significant numbers of individuals, particularly women, may not meet their folate intake recommendations on any given day. The folate content of citrus fruits and juices are presented in Table 5.

Table 5. Folate content of citrus fruits and juices			
Product	**Portion**	**% Daily Value***	**Amount (approximate) (µg)**
Orange juice	8 oz	15	60
Grapefruit juice	8 oz	6	24
Orange	154 g	10	40
Grapefruit	154 g	4	16
Tangerine	109 g	2	8
* The Daily Value for folate is 400 µg. Source: www.floridajuice.com.			

Folate and Immunity

Because folate is essential for DNA synthesis, which is necessary for cell division and replication, folate status may significantly impact immune cell proliferation and function. Insufficient folate may predispose DNA to instability and damage such as strand breaks (Duthie et al. 2002). Folate also may affect immune function based on its role in RNA and protein synthesis.

A comprehensive review by Dhur et al. (Dhur et al. 1991) evaluated earlier (i.e., pre-1991) research regarding folic acid and immune function. Based on the results of animal studies, folate deficiency has been associated with increased susceptibility to infection, and alterations in cell-mediated immunity (decreases in circulating white blood cells and T lymphocytes, delayed-type hypersensitivity and lymphocyte-mediated cytotoxicity). In several studies, even moderate folate deficiency resulted in alterations in cell-mediated immunity. Antibody responses also have been reported to be impaired in folate-deficient animals. In humans, studies conducted prior to 1991 report associations between folate deficiency and an increased susceptibility to various diseases and infections including malaria, respiratory infections, influenza, mononucleosis, gastroenteritis and diarrheas. Some studies indicate that several parameters of cell-mediated immunity may be impacted by folate deficiency, including blastogenic response of T lymphocytes to mitogens, and T lymphocyte proliferation and response to mitogens. Some studies have reported decreased

serum immunoglobulin levels due to folate deficiency caused by anti-folate medications such as antiepileptic drugs. However, these studies make it difficult to differentiate between drug-induced immunomodulatory effects and those potentially caused by folate deficiency. Folate deficiency also has been associated with reduced phagocyte function. In many cases, observed aberrations were reversed with folic acid administration or treatment. In summary, studies conducted prior to 1991 in animals and humans support a role for folate in various aspects of immune system function (Dhur et al. 1991).

Animal studies conducted since the Dhur et al. review continue to support a role for folate in immune function. A study conducted in rats evaluated the effects of both severe and moderate folate-deficient diets for 5 and 24 weeks, respectively, on natural killer (NK) cell function (Kim et al. 2002). A severe folate deficiency was associated with a 60% reduction in lymphocyte counts and significantly reduced levels of NK cytotoxicity in splenic tissue compared with folate supplemented animals. However, compared to folate supplemented rats, no differences in NK cytotoxicity were observed in rats fed a diet that was moderately deficient in folate. In addition, modest folate supplementation beyond the basal diet did not enhance NK function. Another study in rats evaluated the impact of folate supplementation on age-related changes in immune function (Field et al. 2006). Older rats displayed some reductions in immune cell counts, proliferation rate and cytokine production compared to younger rats. Supplementation of the older rats with folic acid at 10 times the amount found in the basal diet resulted in improved distribution of T cells, improved mitogen response, and increased cytokine production. These improvements were observed in the spleen, although not in mesenteric lymph nodes, suggesting that the effects of folic acid in this study were site or organ specific. These animal studies support the hypothesis that overt folate deficiency, but not modest folate deficiency, may unfavorably affect some markers of immune function. In some cases, folate supplementation above basal requirements was able to enhance immune function.

Since the Dhur et al. review, relatively few studies have been conducted evaluating the effect of folate on immune function in humans. An in vitro study measured the mitogen-stimulating activities of human T lymphocytes (Courtemanche et al. 2004). Cells cultured in low folate concentrations had reduced lymphocyte cell numbers and proliferation rates and

more readily displayed S-phase (the phase of the cell cycle when chromosomal replication occurs just prior to cell division) arrest. These aberrations were reversed when folate was added to the culture medium. A cross-sectional study in 153 elderly Japanese nursing home residents found that lower age and higher serum folate concentrations were associated with an intact (non-diminished) immune response (based on postvaccination antibody titers) to the influenza vaccine compared to healthy, younger individuals (Hara et al. 2005). However, after adjusting for age, the association between serum folate and immune response was not statistically significant. A cross-sectional study in 62 healthy, free-living elderly (aged 90 to 106 years) found that folate deficiency was not significantly associated with reduced NK cell cytotoxicity (Ravaglia et al. 2000). Less than 10 percent of men or women in this study had a folate deficiency. In such a study with a small number of subjects, the statistical power may not have been high enough to detect an association. A cross-sectional study in 105 post-menopausal overweight or obese women reported that women with higher dietary folate intake (≥ 233 µg/day) and who were taking > 400 µg/day folic acid supplements had significantly lower NK cytotoxicity compared to women not using supplements (Troen et al. 2006). In this study, higher blood concentrations of unmetabolized synthetic folic acid were significantly associated with decreases in NK cytotoxicity, particularly in older women aged 60 to 75 years. This study suggests that higher folate intake may be detrimental to NK cell function. However, synthetic folic acid intake (from supplements) as opposed to folate intake from foods may have been responsible for the observed results.

It is well documented that folate status is inversely associated with plasma homocysteine concentrations and that folate can lower homocysteine concentrations to some degree (Homocysteine Lowering Trialists' Collaboration 1998). Elevated blood homocysteine concentrations have been associated with an increased risk for cardiovascular diseases in observational studies (Refsum et al. 1998). There are limited data regarding the possible effect of homocysteine on immune function. Several studies regarding homocysteine and immune function relate to how immune activation, observed with some chronic diseases, contributes to hyperhomocysteinemia (Widner et al. 2002; Schroecksnadel et al. 2003; Schroecksnadel et al. 2003). One in vitro study reports that homocysteine, in a range of physiologic concentrations reflective of several disease states, increased T lymphocyte apopoptic (programmed) cell death and increased production of the cytokines IL-2, IFN-γ,

TNF-α and IL-10, thereby reducing immune cell response or influencing proinflammatory processes (Dawson et al. 2004). Indeed, homocysteine has been identified as having inflammatory properties and may therefore have modulatory effects on the inflammatory functions of the immune system (Grimble 2006).

Folate and Cancer

A relationship between folate status or intake and several types of cancer, including cancers of the colon (Giovannucci et al. 1993; Giovannucci et al. 1998; Larsson et al. 2005), breast (Zhang et al. 1999; Rohan et al. 2000; Sellers et al. 2004), and cervix (Butterworth et al. 1992; Kwasniewska et al. 1997), has been reported. The mechanisms by which folate influences cancer risk have not been elucidated. However, candidate mechanisms include folate's role in DNA production and cell division (lack of folate may result in DNA damage and therefore predispose cells to carcinogenesis), and DNA methylation, which may effect gene expression. It appears unlikely that the mechanism involves enhancement of NK cell activity (Ravaglia et al. 2000; Troen et al. 2006). There is some controversy in this area due to the fact that emerging evidence suggests that folate may enhance the development and progression of some existing, undiagnosed premalignant or malignant lesions (Kim 2004).

Summary of Folate and Immune Function

Because of folate's physiologic role in cell division, cell proliferation, and protein synthesis, there is little doubt that folate is essential for helping maintain a healthy and operational immune system. Dhur et al. (Dhur et al. 1991) conclude that some of the deleterious effects of folate deficiency on immune function are likely caused by defects in DNA and RNA synthesis or methyl metabolism, both of which may be significantly influenced by folate availability. Based on the results of human, in vitro and animal studies, it can be hypothesized that folate is associated with a decreased rate of infection, positive effects on blastogenic response and proliferation of T lymphocytes, enhanced delayed-type hypersensitivity response, enhanced phagocytosis, and immunoglobulin production. Folate appears to have no effect on NK cell function. However, more recent human studies are not as supportive of a role for folate in enhancing immune function, although limited studies

have been conducted. Of particular concern, one study suggests that higher dietary folate intake coupled with increased supplemental (synthetic) folic acid may actually be detrimental to certain components of the immune system (Troen et al. 2006). This potential effect needs to be confirmed in intervention studies, particularly since a wide variety of commonly consumed foods such as ready-to-eat breakfast cereals and other cereal grain foods are fortified with synthetic folic acid.

Animal studies do not support the idea that moderate folate deficiency significantly impairs immune function. However, it is not clear whether this would be the case in humans. Since mandatory grain fortification with folic acid in 1998, folate status in the US has dramatically improved (Jacques et al. 1999; Lawrence et al. 1999; CDC 2000; Choumenkovitch et al. 2001), resulting in considerably fewer individuals with severe folate deficiency. This improvement in folate status has coincided with a decrease in plasma homocysteine concentrations (Jacques et al. 1999; Anderson et al. 2004). Severe folate deficiency in the US population has now become increasingly rare and any observed folate deficiency is likely to be in the moderate range. Intervention studies need to be conducted to determine whether enhancing folate intake can benefit immune function in individuals with moderate folate deficiency, or whether additional dietary (or supplemental) folate above normal folate intake or status can significantly enhance immune function.

Vitamin B6

Background

Vitamin B6 is a water-soluble vitamin and is a collective term for pyridoxal and related compounds, including pyridoxine, pyridoxal, and pyridoxamine and their related 5'-phosphate compounds. The compound pyridoxal 5'-phosphate, or PLP, is the functional coenzyme for more than 100 enzymes involved in various metabolic activities. The physiologic functions of vitamin B6 include amino acid metabolism, formation of neurotransmitters, carbohydrate and lipid metabolism, heme synthesis, and biosynthesis of nucleic acids and proteins (Institute of Medicine 1998). Because immune cells must differentiate, proliferate, and carry out normal cellular metabolic activities, vitamin B6 is clearly necessary for normal immune function. Vitamin B6 also is essential in the

biochemistry of amino acids which are essential components of antibodies. Low vitamin B6 concentrations could result in a reduced number of one-carbon compounds, which may impair DNA and RNA synthesis and therefore cell proliferation and protein synthesis. Vitamin B6 also may affect immune function because it is necessary for the formation of the amino acid cysteine, an important precursor in glutathione, which is closely associated with lymphocyte proliferation (Grimble 1997). The Recommended Dietary Allowance for vitamin B6 ranges from 1.3 to 1.7 mg/day for adult males and 1.3 to 1.5 mg/day for adult females (Institute of Medicine 1998). The Tolerable Upper Intake Level (UL) is 80 to 100 mg/day for adult males and females. Median intake of vitamin B6 for men and women in the US population age 20 years and older ranges from 1.8 to 2.0 mg/day and 1.3 to 1.4 mg/day, respectively (Ervin et al. 2004). Median intakes for men indicate that many men are meeting daily vitamin B6 intake recommendations on any given day, however many women may not. The vitamin B6 content of citrus fruits and juices is presented in Table 6.

Table 6. Vitamin B6 content of citrus fruits and juices

Product	Portion	% Daily Value*	Amount (approximate) (mg)
Orange juice	8 oz	6	0.12
Grapefruit juice	8 oz	4	0.08
Orange	154 g	4	0.08
Grapefruit	154 g	4	0.08
Tangerine	109 g	4	0.08

* The Daily Value for vitamin B6 is 2.0 mg.
Source: www.floridajuice.com.

Vitamin B6 and Immunity

Severe deficiency of vitamin B6 has been associated with changes in the structure and function of various lymphoid organs. Specifically, studies in animals have demonstrated that vitamin B6 deficiency results in atrophy or other physical alterations of the thymus gland, lymphoid organs, and spleen in rats, monkeys, chickens, and dogs (Chandra et al. 1990;

Grimble 1997). The reasons for this may be related to the depletion of lymphocytes. In animals, vitamin B6 deficiency also has been associated with alterations in humoral and cell-mediated immune responses, including a reduction in the number of circulating antibodies, reduced levels of immunoglobulins, decreased lymphocyte proliferation and cytotoxicity, decreased cell-mediated cytotoxicity, and reduced delayed-type hypersensitivity (Chandra et al. 1990; Rall et al. 1993). In many cases, abnormalities were reversed with vitamin B6 treatment. Animal studies also have demonstrated that vitamin B6 deficiency resulted in the decreased biosynthesis of DNA, RNA and messenger RNA. Because these molecules are composed of nucleic acids and vitamin B6 is essential to nucleic acid synthesis, it is postulated that this is one of the primary mechanisms by which vitamin B6 deficiency impairs immune function (Chandra et al. 1990).

Observational and intervention studies in humans support a role for vitamin B6 in immune function. Intervention studies show that vitamin B6 supplementation can affect immune response, particularly lymphocyte response. The effects of pyridoxine supplementation on lymphocyte response was evaluated in 15 healthy elderly persons aged 65 to 81 years (Talbott et al. 1987). Eleven subjects received 50 mg/day pyridoxine (over 25 times the RDA) and four subjects received a placebo. Immune responses were measured after one and two months of supplementation. Plasma vitamin B6 status and lymphocyte proliferation in response to T- and B-cell mitogens was higher in the supplemented group compared to control subjects. Subjects with initially lower plasma vitamin B6 had a more enhanced response to some mitogens compared to subjects with plasma vitamin B6 concentrations in the normal range at baseline. In a study of 33 males and females (30 to 75 years of age), 9 subjects were administered 200 mg/day vitamin B6 for 2 months. Another group was administered 200 mg/day of CoQ10 and 300 mg/day vitamin B6. In the group receiving vitamin B6 supplementation only, the percentage of T4 (helper) lymphocytes and the T4:T8 ratio was significantly higher after 2 months compared to baseline. However, vitamin B6 supplementation had no effect on immunoglobulin G (IgG) concentrations or T8 (suppressor) lymphocyte percentage (Folkers et al. 1993).

In a vitamin B6 depletion-repletion study, eight healthy elderly adults (age 61 years and older) followed a vitamin B6 depletion diet for ≤ 20 days, followed by three stages of vitamin

B6 repletion for 21 days each (Meydani et al. 1991). Repletion period vitamin B6 intakes ranged from 0.10 to 1.9 mg/day for women and 0. 17 to 2.88 mg/day for men (amounts similar to the current RDA). All subjects received 50 mg/day during the final 4 days of the study. During the depletion phase, lymphocyte counts and percentage decreased significantly and n eutrophil percentage increased significantly compared to baseline. Repletion with vitamin B6 did not reverse all of the aberrations and at the end of the study percent lymphocyte counts were still significantly lower and percent neutrophils significantly higher when compared to baseline. Total white blood cell counts did not change significantly during the course of the study. Both T- and B-cell mitogenic responses decreased significantly during depletion and returned to baseline values following the repletion period. Vitamin B6 status affected IL-2 production, which was significantly reduced during depletion and restored following B6 repletion.

Not all studies show improved immune response with B6 supplementation. A small study in men demonstrated that four men on a vitamin B6-deficient diet or induced vitamin B6 deficiency for 12 weeks had only slightly impaired antibody responses compared to two men consuming a v itamin B6 sufficient diet (Hodges et al. 1962). In an observational study, healthy elderly individuals age 65 years and older who were taking megadoses of vitamin or mineral supplements and who were in the upper quartile or decile of vitamin B6 intake (substantially above the RDA) did not have significantly different immune parameters including lymphocyte mitogen response and neutrophil counts compared to subjects with lower B6 intake (Goodwin et al. 1983). Actually, higher vitamin B6 intake was associated with significantly lower lymphocyte counts.

Vitamin B6 supplementation has been found to enhance immune response in special populations, including critically ill patients (Huang et al. 2005; Cheng et al. 2006), patients on hemodialysis (Kleiner et al. 1980; Casciato et al. 1984), and individuals with HIV infection (Baum et al. 1991). However, interpretation of these studies can be difficult since the disease process by itself can affect overall immune function. Along with folate, vitamin B6 can influence homocysteine status (Institute of Medicine 1998). Refer to the previous section on folate concerning the role of homocysteine status in immune response.

Vitamin B6 and Cancer

A World Cancer Research Fund/American Institute for Cancer Research report (World Cancer Research Fund 1997) does not mention vitamin B6 in relation to reducing the risk for cancer. However, high levels of vitamin B6 have been reported to suppress the growth of animal and human cancer cells in vitro and moderate doses of vitamin B6 were shown to have a preventive effect on colon tumorigenesis in mice (Komatsu et al. 2003). Several epidemiological studies have associated vitamin B6 intake (Slattery et al. 1997; Jansen et al. 1999; Le Marchand et al. 2002; Larsson et al. 2005) or status (Wei et al. 2005) with a reduced risk for colorectal cancer. Potential mechanisms by which vitamin B6 reduces cancer risk include reducing the disruption of DNA synthesis, repair, and methylation associated with inadequate vitamin B6 intake. Vitamin B6 also may reduce cancer cell proliferation, reduce oxidative stress, suppress nitric oxide, or have antiangiogenic properties (Matsubara et al. 2003). It is not known whether the potential effects of vitamin B6 on immune parameters contribute to cancer risk reduction.

Summary of Vitamin B6 and Immune Function

Both animal and human studies strongly support a role for vitamin B6 in both cell-mediated and humoral immune function. Intervention studies in humans typically evaluated immune response of vitamin B6 at doses much higher than the RDA amount for vitamin B6 (2.0 mg/day). Only one study (Meydani et al. 1991) used repletion amounts consistent with the RDA and reported that these amounts were not able to restore immune parameters to baseline following vitamin B6 depletion. It is possible that a study longer in duration may have accomplished this. Many intervention studies have been conducted in older individuals, so it is unclear what effects may be observed in younger subjects. Because certain aspects of immune function decrease with age (Chandra 2002; Marcos et al. 2003), it is possible that older individuals may be more responsive to nutritional intervention.

Carotenoids and Vitamin A

Background

Carotenoids are natural fat-soluble pigments found in plants that help with photosynthesis reactions and protect plants against photosensitization. Carotenoids provide the bright red, yellow, and orange coloration of many fruits and vegetables. The prevalent carotenoids in the human diet include beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin. Beta-carotene is one of the most prevalent carotenoids in the US diet and is most readily obtained from darkly colored fruits and vegetables. In fact, blood concentrations of beta-carotene are one of the best indicators of fruit and vegetable consumption (Institute 2000). Beta-carotene has other possible actions in the body, including acting as an antioxidant and as an enhancer of immunity (Institute 2000). In humans, the carotenoids beta-carotene, alpha-carotene, and beta-cryptoxanthin have provitamin A activity, that is, they are converted to retinol, the active form of vitamin A, in the body. The carotenoids lutein, lycopene, and zeaxanthin do not have provitamin A activity. The primary carotenoid in oranges is beta-cryptoxanthin and pink and red grapefruit contain the carotenoids beta-carotene and lycopene.

Vitamin A compounds are important for a variety of functions including normal vision, gene expression, reproduction, embryonic development, growth, and immune function (Institute of Medicine 2001). Specifically, retinoids (retinol and its metabolites) are necessary for the maintenance of immune function, which depends on cell differentiation and proliferation. Therefore, vitamin A plays an important and recognized role in both humoral and cell-mediated immunity and is one of the most widely studied nutrients with regard to immune function. Dietary sources of preformed vitamin A include foods of animal origin, including liver, eggs, and dairy products. The RDA for vitamin A is 700 µg retinol activity equivalents (RAE)/day and 900 µg RAE/day for adult women and men, respectively (Institute of Medicine 2001). Median intakes of vitamin A for men and women in the US population age 20 years and older ranges from 618 to 759 RAE and 574 to 670 RAE, respectively (Ervin et al. 2004). Median intakes of vitamin A are below the RDA for both men and women. Median intakes of carotenes (beta-carotene and other provitamin-A carotenoids for individuals aged 20 years and older range from 160 to 202 retinol equivalents (RE) for men and 160 to 233 RE for women (Ervin et al. 2004). Data summarized from NHANES III (1988-1994) for 50th

percentile intakes of beta-carotene, beta-cryptoxanthin, and lycopene are as presented in Table 7 (Institute of Medicine 2001).

Table 7. Carotenoid intake in the US population

Carotenoid	Daily Intake Range–Men (µg)	Daily Intake Range-Women (µg)
Beta-carotene	1793 – 2261	1359 - 2351
Beta-cryptoxanthin	19 – 39	13 – 52
Lycopene	1376 – 5079	842 – 2420

Note: daily intake range is for individuals age 19 years and older. Ranges are 50th percentile intake.

There is currently no established daily dietary requirement for carotenoids per se. However, data are strongly supportive for increased consumption of carotenoid-rich fruits and vegetables (Institute of Medicine 2001). Provitamin A carotenoids have been identified as a nutrient of concern in the 2005 Dietary Guidelines for Americans (USDHHS 2005). Intake of the carotenoids beta-carotene and beta-cryptoxanthin must be converted into RAE units because in the body the conversion of provitamin A carotenoids is inefficient. Twelve (12) µg of dietary beta-carotene is equivalent to 1 µg RAE and 24 µg beta-cryptoxanthin is equivalent to 1 µg RAE. Because citrus fruits and juices are a source of carotenoids and not preformed vitamin A, this review will be limited to those studies evaluating the role of carotenoids in immune function. The carotenoid and vitamin A content of citrus fruits and juices is presented in Table 8.

Table 8. Carotenoid and vitamin A content of citrus fruits and juices

Product	Portion	Beta-carotene (µg)	Beta-cryptoxanthin (µg)	Lycopene (µg)	Vitamin A (IU)*
Orange juice	8 oz	65	338	0	351
Grapefruit juice (colored)	8 oz	ND	ND	ND	1087
Orange	154 g	109	179	0	357
Grapefruit (colored)	154 g	1056	9	2185	856

Tangerine	109 g	169	444	0	742

* The Daily Value for vitamin A is 5,000 IU.
ND = no data available in the USDA database for this product and nutrient.
Beta-carotene and beta-cryptoxanthin are provitamin A carotenoids.
Source: USDA Database for Standard Reference, Release 18 (juices; average of like products) and "Fresh Citrus Fruit-Nutrition and Health Benefits" (Zhang 2005) (fresh fruit, except vitamin A in IU – USDA Database, average of like products)

Beta-carotene

Beta-carotene has been reported to have immunomodulatory activities in both animal and human studies. In rats, beta-carotene supplementation enhanced B and T cell responses to mitogens (Bendich et al. 1986), augmented tumor immunity in mice (Tomita et al. 1987) and inhibited or prevented gross development of squamous cell carcinoma in hamster buccal cells (Schwartz et al. 1990). In dogs, supplementation with beta-carotene resulted in higher CD4+ cell counts, higher CD4:CD8 cell ratio, increased IgG, and heightened delayed-type hypersensitivity response (Chew et al. 2000). However, beta-carotene supplementation did not enhance lymphocyte proliferation or IL-2 production in this study. Supplementation with beta-carotene increased lymphocyte percentage, number of white blood cells, and lymphocyte stimulation to mitogens in pigs (Zomborszky-Kovacs et al. 2000). Supplementation with beta-carotene resulted in no enhancement of lymphocyte proliferation in chicks (Sklan et al. 1989), did not reduce mortality in immunized and unimmunized chickens from challenge with *E. coli*, and did not enhance agglutinating antibody production (Tengerdy et al. 1990).

In human cross-sectional studies, serum beta-carotene was found to be significantly lower in children with various acute infections (Cser et al. 2004). However, plasma beta-carotene concentrations were not related to antibody titer response to influenza vaccine in healthy elderly individuals (mean age 81 years) (Gardner et al. 2000). There was no significant correlation between plasma beta-carotene and the proliferative response of lymphocytes in healthy elderly (Gardner et al. 1997). In a study of 652 non-institutionalized elderly age 60 years and older, beta-carotene status was significantly inversely related to the incidence of acute respiratory infections but not illness severity (van der Horst-Graat et al. 2004).

Short term and dose-response human intervention studies provide some support for an affect of beta-carotene supplementation on certain immune parameters, including production of cytokines, surface cell markers, immune cell numbers, and NK cell cytotoxicity. In a double-blind placebo-controlled crossover trial with 25 healthy male nonsmokers (19 to 58 years of age), beta-carotene supplementation of 15 mg/day for 26 days, an amount that is achievable through diet, resulted in an increased percentage of major histocompatibility complex II (MHCII) surface presenting monocytes and an increased ex vivo production of TNF-α compared to placebo (Hughes et al. 1997). In a study of 25 men (age 35 to 79 years) with premalignant oral or esophageal lesions, 30 mg/day beta-carotene for 2 months increased NK cell cytotoxicity and NK cell percentage (Prabhala et al. 1991). In a study coupling diet with supplementation, nine healthy women consumed a low beta-carotene diet for 68 days, followed by the same diet with 15 mg/day beta-carotene supplementation for 28 days (Daudu et al. 1994). Neither the depletion nor repletion phase of the study affected proliferation of peripheral blood mononuclear cells, in vitro production of IL-2 receptor, or the number of circulating lymphocytes or their subsets. These were healthy adults consuming adequate vitamin A. In a study of 52 free-living healthy elderly, beta-carotene supplementation of 8.2 mg/day for 12 weeks had no effect on cell-mediated immune parameters (Corridan et al. 2001). Short-term beta-carotene supplementation (30 mg/day for 28 days) in young post-partum women age 19 to 39 years did not effect T lymphocyte proliferation in response to mitogens (Gossage et al. 2000). Several of the before-mentioned studies used beta-carotene amounts achievable through diet, yet these amounts were associated with little or no significant effects on immune function markers.

In dose-response studies, beta-carotene supplementation at higher amounts (i.e., 30, 45 or 60 mg/day) for 3 months increased the percentage of cells displaying the CD4+ protein marker, percent of NK cells, and cell activation markers, where there was no change in these immune parameters at intakes of 0 or 15 mg/day (Watson et al. 1991). However, this was a small study with only four subjects in each treatment group. Fifty healthy males and females who ingested either 0, 15, 45, 180, or 300 mg/day beta-carotene for one month had no changes in lymphocyte proliferation or cytokine (IL-2) production (Ringer et al. 1991). The difference in the second study was the intervention time, that is, one month (Ringer et

al. 1991) versus three months (Watson et al. 1991), which may not have been long enough to show significant effects of supplementation, even at higher intake amounts.

Long-term intervention studies provide support for a role of beta-carotene, particularly with regard to NK cell function, in healthy older men. Several studies have been published which evaluated immune function in older physicians who consumed a 50 mg beta-carotene supplement every other day for approximately 12 years as part of a larger beta-carotene intervention study. In this study group, natural killer cell activity was found to be higher in men aged 65 to 88 years who took beta-carotene supplements compared to men taking a placebo (Santos et al. 1996; Santos et al. 1998). This could not be explained by an increased number of NK cells or upregulation of the cytokines IFN-α, IFN-γ, IL-12, or IL-2. In another study using this subject group, long-term beta-carotene supplementation did not significantly affect delayed-type hypersensitivity response, lymphocyte proliferation, IL-2 production, or number of NK cells compared to the placebo group (Santos et al. 1997). A short-term experiment, reported in this same publication, administered 90 mg/day of beta-carotene for 3 weeks and reported no significant impact on these same immune parameters (Santos et al. 1997). In a study of long-term supplementation in younger persons, ten young healthy individuals (age 20 to 25 years) were supplemented with 60 mg/day of beta-carotene for 44 weeks. The supplemented group had an increased CD4+/CD8+ cell ratio (ratio of helper T to cytotoxic T cells) compared to placebo. However there was no change in number of NK cells, T cells, memory T cells, or cytotoxic T cells (Murata et al. 1994). In a group of healthy individuals age 57 to 84 years, subjects received either a placebo, selenium, beta-carotene (45 mg/day) or both selenium and beta-carotene for 6 months (Wood et al. 1999). Subjects receiving only beta-carotene showed no changes in cell-mediated immunity. The results of long-term supplementation studies suggest that the positive effects of beta-carotene supplementation on immune function may be limited to enhancement of NK cell activity in older individuals.

Beta-cryptoxanthin

There are very few studies evaluating the association between beta-cryptoxanthin and immune function. In a human cross-sectional study, serum beta-cryptoxanthin was found to

be significantly lower in children with various acute infections (Cser et al. 2004). Because this study is cross-sectional in nature, the results could be explained as a consequence of the infection condition. In a cross-sectional study of 652 non-institutionalized elderly age 60 years and older, beta-cryptoxanthin status was not significantly associated with the incidence of acute respiratory infections (van der Horst-Graat et al. 2004).

Lycopene

As with beta-cryptoxanthin, there are few studies regarding the role of lycopene and immune function. In a study of 52 free-living healthy elderly, lycopene supplementation of 13.3 mg/day for 12 weeks had no effect on cell-mediated immune parameters (Corridan et al. 2001). In an intervention study that included 23 healthy male nonsmokers age 25 to 58 years, subjects were provided 15 mg lycopene/day for 26 days. Lycopene supplementation resulted in a significant increase in the percentage of monocytes expressing MHC class II molecules, but not the relative number of expression molecules, suggesting that lycopene may help enhance some aspects of antigen-presenting cell function (Hughes et al. 2000).

Carotenoids and Cancer

A report by the World Cancer Research Fund and American Institute for Cancer Research (World Cancer Research Fund 1997) concludes that the intake of carotenoids may reduce the risk of cancer of several sites. Specifically, the evidence suggests it is “probable” that carotenoids (specifically beta-carotene) reduce the risk of lung cancer, and “possible” that they may help reduce the risk of esophageal, gastric, colorectal, breast and cervical cancer. There is insufficient evidence for a link between carotenoid intake and risk of cancers of the larynx, ovary, endometrium, and bladder. There is also evidence suggesting that diets high in carotenoids protect against cancer as a whole (World Cancer Research Fund 1997).

Consumption of beta-carotene containing foods has been significantly associated with a reduced risk of lung cancer in a variety of epidemiologic studies (Hughes 2001). However, intervention trials with beta-carotene supplements were not effective in reducing the

incidence of lung cancer and, in fact, resulted in an increased risk for lung cancer in smokers. Based on long-term intervention studies, beta-carotene may enhance NK cell activity, which may improve tumor surveillance and reduce cancer risk, although this effect was observed only in older men. However, the failure of supplementation to reduce lung cancer risk highlights the importance of the amount of supplementation (intervention studies routinely use amounts higher than obtained from the normal diet) and the fact that the cancer protective effects of beta-carotene may be independent of its possible role in the enhancement of immune function. It also supports the idea that consuming a carotenoid-rich diet consisting of a variety of fruits and vegetables may be the best way for reducing the risk of cancer.

Lycopene has been shown to have high singlet oxygen quenching capability and has been associated with anti-tumor promoting activities in various tissues in animal studies (Nishino et al. 2002). Epidemiological studies have reported associations between lycopene intake, primarily from tomato products, and reduced risk for cancers at all sites and in the digestive tract and prostate (Nishino et al. 2002).

Beta-cryptoxanthin also exhibits anti-tumor promoting activity in animal studies and has been associated with a reduced incidence of colon cancer formation in rats (Nishino et al. 2002). Epidemiological studies have found significant associations between higher plasma beta-cryptoxanthin and a reduced risk for gastric adenocarcinomas (Jenab M 2006) and lung cancer in men (Ito et al. 2005). Dietary beta-cryptoxanthin has been inversely associated with risk of lung cancer (Yuan et al. 2003; Mannisto S 2004) and cervical neoplasia (Garcia-Closas et al. 2005).

Summary of Carotenoids and Immune Function

Some carotenoids present in oranges (beta-carotene and beta-cryptoxanthin) and grapefruit (beta-carotene) have pro-vitamin A activity. Vitamin A has been clearly associated with modulating immune function. The results of various human intervention studies with beta-carotene suggest that beta-carotene may enhance NK cell activity in older individuals. The immune enhancing functions of beta-carotene appear to be at least in part independent of

its function as a provitamin A carotenoid (Bendich 2001). There are very few studies regarding beta-cryptoxanthin and immune function and those existing are cross-sectional in nature and provide little support for a direct role of beta-cryptoxanthin in immune function. A single intervention study suggested that supplementation with lycopene may affect antigen presentation of cells. Clearly there needs to be more studies regarding the role of carotenoids in enhancing immune function.

Thiamin

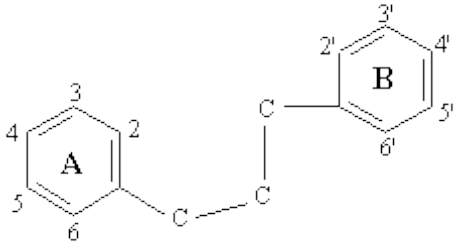
Thiamin is a water-soluble B vitamin also known as vitamin B1. Thiamin functions as a coenzyme in carbohydrate and branched-chain amino acid metabolism. The RDA for thiamin is 1.2 mg/day for men and 1.1 mg/day for women (Institute of Medicine 1998). Median intake of thiamin for men and women in the US population age 20 years and older ranges from 1.6 to 1.8 mg/day and 1.2 to 1.3 mg/day, respectively (Ervin et al. 2004). Median intakes for men and women in all age groups fall above the RDA.

Although an 8-ounce glass of orange juice provides approximately 10 percent of the Daily Value for thiamin, there is very little data linking thiamin to modulation of immune function. The polymorphonuclear leukocytes from sheep on thiamin supplemented diets were more readily able to kill phagocytosed *Candida albicans* compared to sheep on diets not supplemented with thiamin (Olkowski et al. 1990). Limited clinical observations in humans link thiamin deficiency with the development of some tumor types (cyst) and case studies have reported the use of thiamin locally as a therapeutic agent (Lee et al. 2005). Several case control studies have reported significant associations between thiamin intake and reduced risk of several cancers including colon (Tuynes 1986; Slattery et al. 1997), rectal (Tuynes 1986), colorectal (Jedrychowski et al. 2002), and prostate (Du et al. 1977). However, a potential mechanism of action is unknown and it's possible that thiamin may serve as a marker for other nutrients or foods that contribute to cancer risk reduction.

Citrus Phytochemicals

Background

Flavonoids are polyphenolic compounds found readily in plants. These compounds are comprised of 15 carbon atoms; two benzene rings joined by a linear three-carbon chain, as shown in the figure below.



Polyphenols were traditionally employed as natural food coloring agents but new studies have recently found potent antioxidative properties in polyphenol extracts.

Epidemiological studies have indicated the role of flavonoids in human health due to their antioxidant properties and their involvement in antiproliferation processes, antioxidation, regulation of host immune functions and other mechanisms. There is strong and consistent evidence that consumption of foods rich in flavonoids may prevent against several degenerative pathologies including cardiovascular diseases, atherosclerosis, cataract and several forms of cancer.

The genus Citrus is overall characterized by a substantial accumulation of flavonoids; their presence is, however, highly variable among the different species and varieties and each genotype is characterized by a particular flavonoid pattern. Citrus species showing the highest quantitative values (mg/100 mL of juice) have been sweet orange and grapefruit (De Leo et al. 2005).

In citrus, four types of flavonoids (flavanones, flavones, flavonols, and anthocyanins) are found and more than 60 individual flavonoids have been identified (Ooghe et al. 1994). Flavanones are the most predominant flavonoids in citrus fruits and juices and they also

contribute to their flavor (Ranganna et al. 1983). Hesperidin is the major flavonoid in orange/orange juice, and naringin is the main flavonoid in grapefruit/grapefruit juice. Other commonly found flavonoids in citrus fruits and juices include, neohesperidin, tangeretin, narirutin, poncirin, neoponcirin, quercetin, didymin, eriocitrin, sinensetin, and nobiletin.

Phytochemicals and Immunity

Lyu & Park (Lyu et al. 2005) studied the effects of flavonoids on TNF-α, a proinflammatory cytokine, which is important in the inflammatory stages of several chronic inflammatory diseases. Their results showed that flavonoids have the capacity to modulate the immune response and have a potential anti-inflammatory activity. They also reported that the capability of flavonoids for modulating the immune system cannot be predicted on the basis of their chemical composition and structure.

Choe et al. (Choe et al. 2001) evaluated the effect of naringin on blood lipid levels and aortic fatty streaks, and its action mechanism in hypercholesterolemic rabbits. Naringin treatment inhibited the hypercholesterolemia-induced intercellular adhesion molecule-1 (ICAM-1) expression on endothelial cells. The study, which included feeding a 0.25% cholesterol diet to rabbits, was divided into 3 groups: an untreated group, a naringin-treated group and a lovastatin-treated group. Hypercholesterolemia causes fatty liver and elevation of liver enzymes, which was prevented by naringin but not by lovastatin. Naringin significantly reduced fatty streak formation and neointimal macrophage infiltration and also inhibited the expression of the ICAM-1 in endothelial cells, suggesting that suppression of ICAM-1 contributed to the antiatherogenic effect.

Lee et al. (Lee et al. 2001) studied the anti-atherogenic effect of citrus flavonoids, naringin and naringenin, associated with hepatic ACAT (acyl-CoA:cholesterol acyltransferase), aortic VCAM-1 (vascular cell adhesion molecule-1), and MCP-1 (monocyte chemotactic protein-1) in high cholesterol-fed rabbits. The results of these studies found that naringin and naringenin significantly inhibited the aorta fatty streak formation in rabbits on high cholesterol diets. This anti-atherogenic effect seems to be closely involved with a decreased hepatic ACAT activity, and the down-regulation of VCAM-1 and MCP-1 genes. Systemic ACAT inhibition reduces

circulating tumor necrosis factor-alpha levels in hypercholesterolemic subjects and improves resistance-vessel endothelial function, with small effects on circulating cholesterol (Kharbanda et al. 2005). Modern genetic technology has identified specific molecules involved in the early phases of atherosclerosis. VCAM-1 and allied adhesion molecules cause binding of inflammatory leukocytes to the endothelium. Chemoattractants such as MCP-1 cause the directed migration of leukocytes into the intima.

It has been reported that flavonoids exert their anti-inflammatory actions by inhibiting IL-8 (Sato et al. 1997) and PGE_2 (prostaglandins are chemical messengers that cause the pain and swelling of arthritis inflammation) production (Chung et al. 1995) in fibroblasts and synovial cells. According to Lin et al. (Lin et al. 2003) nobiletin interferes with the production of PGE_2 by selectively downregulating COX-2 gene expression and protein in human synovial fibroblasts. The authors also reported that nobiletin down regulated the production of proMMPs-1 and –3 (proMMP: precursors of matrix metalloproteinase) in human synovial fibroblasts but up regulated the expression of TIMP-1 (tissue inhibitor of metalloproteinases). The authors concluded that nobiletin exerts both anti-inflammatory and immunemodulatory actions.

Battinelli et al. (Battinelli et al. 2003) studied the effect of limonin and nomilin on HIV-1 replication on infected human mononuclear cells. Results showed that both limonin and nomilin were found to inhibit HIV-1 replication in fresh PHA (phytohemagglutin)-activated PBMC (peripheral blood mononuclear cells, these cells are critical components of the defense mechanism against infection) from health donors and HIV-1 infected patients. In general, the results obtained pointed out a similar anti-HIV activity of limonin and nomilin indicating that this activity is not drastically influenced by the structural difference between the two compounds. As for the mechanism of anti-HIV-1 effect of limonin and nomilin, an inhibition of HIV-1 protease was observed for both limonoids. The effects of nomilin, a naturally occurring triterpenoid found in citrus seeds and grapefruit juice, glycyrrhizic acid, usrolic acid and oleanolic acid on the immune system were studied on mice by Raphael & Kuttan (Raphael et al. 2003). The effect of triterpenoids on the production of bone marrow cells and alpha-esterase positive cells showed that the terpenoid treated group had a remarkable increase in bone marrow cell number compared to control animals. The maximum increase in the number

of bone marrow cells was observed in animals treated with oleanolic acid followed by nomilin. The number of alpha-esterase positive cells was again significantly increased in animals by the same two compounds. Results showed that triterpenoids remarkably inhibited delayed type hypersensitive reaction. These results indicate the immunemodulatory activity of naturally occurring triterpenoids such as nomilin.

Phytochemicals and Cancer

Chemoprevention is considered to be one of the most promising propositions for prevention of human cancers (Sakata et al. 2003). Dietary hesperidin has been reported to exert anticarcinogenic actions in the tongue (Tanaka et al. 1997), colon (Tanaka et al. 1997; Tanaka et al. 2000), esophageal (Tanaka et al. 1997), and urinary bladder (Yang et al. 1997) in rats. There have also been studies showing that some types of flavonoids including hesperidin have anti-inflammatory activities (Meloni et al. 1995). A study by Koyuncu et al. (Koyuncu et al. 1999) showed that hesperidin has a preventive effect against inflammation in mouse skin caused by a tumor promoter.

There are abundant data indicating a positive association between chronic inflammation and carcinogenic risk (Rabinovitch et al. 1989; Ernst et al. 2001; Prinz et al. 2001; Shacter et al. 2002). Sakada et al. (Sakata et al. 2003) investigated the modulating effects of hesperidin on the expression and activity of COX-2 and iNOS enzymes induced by LPS (lipopolysaccharide) in RAW macrophage cells, suggesting hesperidin is a natural-occuring COX-2 and iNOS inhibitor. Recent studies have shown that some inducible enzymes such as COX-2 and/or inducible nitric oxide synthase (iNOS), in association with inflammatory responses play a key role in carcinogenesis (Sakata et al. 2003).

Naringin has been found to scavenge free radicals. Radiation is a well-known inducer of free radicals, and compounds that can scavange free radicals may reduce radiation-induced DNA damage. Jagetia et al. (Jagetia et al. 2003) evaluated the radioprotective action of naringin in the bone marrow of mice exposed to different doses of radiation. The authors concluded that naringin could protect mouse bone marrow cells against radiation-induced chromosomal damage.

Tangeretin is a polymethoxylatedflavone accumulating in the peel oil of citrus fruits. It has been tested in a screen program for the detection of anti-invasive compounds among flavonoids and other polyphenolics. The assay was based on the invasion of human breast carcinoma cells (MCF-7/6) into a fragment of normal tissue in vitro. Tangeretin inhibited the invasion of the MCF-7/6 cells. According to Bracke et al. (Brack et al. 2002) in addition to its anti-invasive effects, tangeretin possesses an anti-proliferative activity on the tumor cells. The observations with tangeretin are similar to those with tamoxifen, a synthetic antiestrogen that is currently prescribed worldwide as a successful adjuvant therapy and even as a preventive agent in human breast cancer. However, this beneficial effect of the host's immune defense against the tumor neutralizes the growth inhibitory effect of tamoxifen. There is no data to support the idea that drinking citrus juices or eating citrus fruit can provoke any harm to breast cancer patients under tamoxifen.

Hirano et al. (Hirano et al. 1995) studied the effect of tangeretin on the inhibition of leukemic HL-60 cell growth. The growth of HL-60 cells in vitro assessed by [^{3}H]thymidine incorporation or tetrazolium crystal formation was strongly suppressed in the presence of tangeretin. Apotosis of HL-60 cells, assessed by cell morphology and DNA fragmentation, was demonstrated in the presence of greater than 2.7 µmol of tangeretin. Apoptosis was evident after 24 hours of incubation with tangeretin. Tangeretin showed no cytotoxicity against either HL-60 cells or mitogen-activated PBMCs (peripheral blood mononuclear cells). PBMCs are cells in the bloodstream with one nucleus, generally refers to lymphocytes and macrophages.

In a population-based case-control study, a statistically significant inverse association was reported between lung cancer risk and white grapefruit, the main food source of the flavonoids naringin (Le Marchand et al. 2000). The odds ratio for the highest compared with the lowest tertile of intake for white grapefruit was 0.5 (95% CI = 0.2-0.9) (P for trend =.02).

Summary of Phytochemicals and Immune Function

Citrus fruits and juices contain a variety of flavonoids that have been associated with modulating immune function and tumorigenesis in animal and in vitro studies. However, there are no data to indicate that, contrary to the vitamins reviewed in this report, flavonoids

are required for normal baseline immune function. To date, no intervention studies have been conducted to show that the intake of citrus flavonoids affect immune parameters in humans, which limits the conclusions that can be drawn from this research. However, the positive results of animal and in vitro studies should pave the way for human studies in the near future.

The Role of Multivitamin/Multimineral Supplements in Immune Function

Several short- and long-term studies have evaluated the effects of a multivitamin and/or multimineral supplement on immune response. The majority of studies have been conducted in older individuals, some of which have single or multiple micronutrient deficiencies or otherwise are at higher risk for infection or disease.

In a randomized controlled trial in 119 nursing home residents 60 years of age and older, subjects consumed for 8 weeks a micronutrient supplement tablet that provided the reference nutrient intakes for all vitamins and trace minerals. After 4 weeks, subjects were vaccinated for the influenza virus. There was no difference in antibody response to influenza vaccine in the treatment group compared to the untreated group, despite significant increases in serum vitamins A, C, D, E, folate and selenium (Allsup et al. 2004). Eighty healthy free-living individuals age 50 to 87 years were randomized to consume a multivitamin/multimineral preparation or placebo for 8 weeks. The supplemented provided 100 percent of the Daily Value for most vitamins and minerals. There was no effect of supplementation on production of the cytokines IL-2, IL-6, IL-10 and prostaglandin E2 (McKay et al. 2000). In a study of 51 subjects over 60 years of age, 21 of which had nutrition deficiencies, 8 weeks of consuming a dietary supplement that included vitamins and minerals improved the results of the delayed-type hypersensitivity test compared to baseline (Chandra et al. 1982). Twelve elderly subjects at high risk for protein-calorie malnutrition were provided with a nutrition supplement for a period of 16 weeks. However, following supplementation, there were no changes in total lymphocyte count or number of T cells, T suppressor cells, T helper cells or B cells compared to baseline (Lipschitz et al. 1985). An intervention study was conducted in elders, aged 65 and older, residing in assisted- and independent-living facilities in Florida (Langkamp-Henken et al. 2004). Subjects received 8

ounces/day of a nutritional supplement that included folic acid (361 dietary folate equivalents), vitamin B6 (2.0 mg), antioxidants (including 281 mg vitamin C and 8 mg beta-carotene), zinc, selenium, fermentable oligosaccharides, and structured triacylglycerol or a control beverage for 183 days. Subjects consuming the nutritional supplement had fewer days of upper respiratory tract infection, better antibody response to vaccine, and greater lymphocyte proliferative response to influenza vaccine compared to control subjects.

The results of longer-term supplementation studies are generally more positive. In a randomized double-blind trial, 756 institutionalized (long term hospitalized) elderly subjects in France were randomized to receive a vitamin supplement (vitamin C, beta-carotene, alpha tocopherol), trace mineral supplement (zinc and selenium), vitamin + trace mineral supplement, or placebo for 1 year. Immune parameters were measured after 6 months in a subsample of 134 subjects. Mitogen stimulated IL1 production was significantly higher in the groups receiving vitamins only or vitamins plus trace elements (Galan et al. 1997). However, there was no effect of supplementation on lymphocyte proliferation. Forty-four healthy subjects, age 50 to 65 years, were randomized to receive a daily nutrient supplement or placebo for 12 months (Chandra 2002). The supplement contained RDA-like values for many vitamins and minerals. Subjects in the treatment group had higher percentage T lymphocytes, higher CD4+ cells (T helper) and higher amounts of IL-2 compared to placebo group at 6 months. Antibody levels to influenza virus were higher and infection-related illness (number of sick days) was lower in the supplemented compared to placebo groups. Subjects with nutrient deficiencies at baseline had a greater improvement in immunity. A multiple regression analysis showed that no one nutrient was responsible for the improvement.

In a study of 60 healthy subjects 70 years of age or older, half of which consumed a special nutritional formula in addition to their regular diet for 12 months, the supplemented subjects reported higher NK cell activity, higher IL-2 production, and fewer infections compared to non-supplemented individuals (Bunout et al. 2004). In a placebo-controlled double-blind trial, subjects 59 to 85 years of age were randomly assigned to consume a supplement (containing physiological amounts of vitamins and minerals) or placebo for 12 months (Bogden et al. 1994). Subjects in the treatment group displayed enhanced delayed-

hypersensitivity skin test responses compared to the placebo group. In a study of healthy free-living elderly, some with nutritional deficiencies, 96 subjects were randomized to receive a nutritional supplement or placebo for 12 months. Those in the supplement group had higher numbers of certain T cell subsets, NK cell %, lymphocyte proliferation, IL-2 production, NK cell activity, and antibody response to influenza vaccine. Absolute numbers of neutrophils or lymphocytes did not change in either group (Chandra 1992).

A large, randomized, double-blind, placebo-controlled intervention study was carried out with 477 healthy men and women (mean age 36 years-a group considerably younger than previously reviewed studies) in order to investigate whether consumption of a dietary supplement containing probiotic bacteria plus vitamins and minerals over a period of at least three months in winter/spring affects the duration, frequency, and severity of symptoms of naturally acquired common cold infections as well as cellular immune parameters (Winkler et al. 2005). Subjects were randomly assigned to a group who received daily a probiotic multivitamin and mineral supplement or a placebo for three or just under 6 months. Cellular immune response was determined in 60 participants per study group before and after 14 days of supplementation. The incidence of respiratory tract infections was almost 14% lower in the supplemented group compared to the placebo group, although this difference was not statistically significant. Infection symptoms were reduced in the supplemented as evidenced by a 19% reduction in the total symptom score ($p = 0.12$), 25% reduction in influenza symptoms ($p = 0.09$), and 54% reduction in the number of days with fever ($p = 0.03$). The duration of the infections was not affected. Leukocytes, lymphocytes, in particular T-lymphocytes including CD4+ and CD8+ cells, as well as monocytes were significantly increased in the supplemented group during the first 14 days of supplementation compared to placebo. This study suggests that supplementation also may be effective in younger individuals.

The results of nutritional supplementation studies indicate that supplementation, especially for time periods exceeding 6 months, can enhance immune parameters sometimes resulting in desirable clinical outcomes such as decreased infection rates. Many of these studies use supplements that contain physiological doses or amounts of a variety of vitamins and minerals. The supplements are consumed in addition to the subjects' normal diets which can

result in moderate increases in nutrient intake and a reversal of nutrient deficiencies. The limitations of these studies are that because many nutrients may be provided in the supplements, it is impossible to single out any one or group of nutrients that may be responsible for the positive effects. Also, many of these studies were conducted in older individuals who are more likely to have nutrient deficiencies and weaker immune function in general and may especially benefit from such supplementation. It is not known whether similar results would be seen in healthy younger individuals. Nonetheless, these data do provide some support for the enhancement of immune function with the intake of physiological amounts of a variety of nutrients (in addition to the basal diet) over longer time periods (i.e., greater than 6 months).

The Role of Individual Foods or Dietary Patterns in Immune Function

Many studies have reported associations between the consumption of various foods, food groups, and dietary patterns with modulating the risk of chronic diseases such as cardiovascular diseases and cancer. Foods and dietary patterns can exert preventive effects on cardiovascular disease through a variety of mechanisms, including modulation of oxidative stress, endothelial function, plasma lipids, inflammation, blood pressure, insulin sensitivity, and coronary blood flow.(Roberts et al. 2005) Food and diet can modify cancer risk through the ingestion of mutagens and carcinogens, alterations in oxidant/antioxidant status, and effects on metabolic hormones such as insulin and growth factors (Roberts et al. 2005).

Diet also may influence immune function, which can affect the initiation and progression of cancer. A limited number of studies have evaluated the intake of particular foods on immune parameters. A randomized controlled trial examined the effects of a diet that included carotenoid-rich fruits and vegetables on various immune functions (Watzl et al. 2005). Sixty-three nonsmoking men consumed a diet low in fruits and vegetables (i.e., ≤ 2 servings/day) for 4 weeks and were then randomized to receive 2, 5, or 8 servings/day of carotenoid-rich fruits and vegetables for another 4 weeks. It should be noted that no citrus fruits were included as an intervention food item. Following the treatment period, no changes in immune parameters were noted, including the production of several cytokines, NK cell

cytotoxicity, NK cell number, and lymphocyte proliferation. There were no changes in plasma vitamin C after 4 weeks of treatment, although total serum carotenoids increased significantly in the 5 and 8 servings/day groups only. Another study examined the effects in younger men of a low-carotenoid diet supplemented for 2 week intervals (with 2-week depletion periods in between) with 330 mL/day of either carrot or tomato juice (Watzl et al. 2003). Plasma carotenoid concentrations responded immediately to the increased intake of carotenoids. Immune response as measured by lymphocyte proliferation, NK cell cytotoxicity, and IL-2 production significantly increased following the juice supplementation periods, but lagged behind the serum response.

Briviba et al. (Briviba et al. 2004) examined the effects of supplementing a low-carotenoid diet with capsulated tomato extract for a period of 2 weeks in 27 male smokers and nonsmokers. Twenty-eight control subjects received placebo capsules. Consumption of the tomato extract capsules resulted in no effects on lymphocyte proliferation, NK cell activity, and IL-2 or TNF-α production. However, there was a significant lowering of the cytokine IL-4 in smokers at the end of the study compared to baseline. Tomato extract is rich in lycopene, which is also found in pink and red grapefruit. However this study provided no support for a role for tomato extract (and possibly lycopene) on immune function.

One study examined the effects of including polyphenol-rich fruit juices on immune function (Bub et al. 2003). Twenty-seven nonsmoking men participated in a 2 week washout period, following by 2 weeks of consuming 330 mL/day of one of two juices, then another 2 week washout period, followed by the consumption of the opposite juice (crossover design) for 2 weeks, followed by a final 2-week washout period. The juices contained a mixture of apple, mango and orange juice. Additionally, one of the juices included anthocyanin-rich aronia, blueberries, and boysenberries and the other juice included flavanol-rich green tea, apricot, and lime. Consumption of either juice resulted in an increase in lymphocyte proliferation and some increase in NK cell cytolytic activity. However, there was a time delay between the increased serum carotenoid response and the immune response. There was no clear difference between the juices with regard to effects on immune function.

Kossoy et al. (Kossoy et al. 2001) evaluated the effects of an orange-pulp supplemented diet on tumorigenesis and immune response in rats. Rats were fed a control diet or a diet supplemented with 15% orange pulp. They were then injected with a carcinogen to induce colon tumors. Rats fed the supplemented diet had a reduced number of adenocarcinomas compared to controls. Supplemented rats also had increased activity of T cells in tumors and a higher level of apoptosis-related proteins, suggesting a tumor suppressor effect of the orange pulp supplemented diet.

Surprisingly few studies have been conducted evaluating the effects of dietary patterns on immune function parameters. Haddad et al. (Haddad et al. 1999) conducted an observational study in nonvegetarians and vegans. Dietary intake was measured in 25 vegans and 20 nonvegetarians, along with various hematologic and immune status markers. Vegans consumed significantly higher amounts of dietary fiber, vitamin C, thiamin, folate, magnesium, copper, and manganese, and lower amounts of protein, saturated fat, total fat, cholesterol and vitamin B12 compared to nonvegetarians. Many of the micronutrients consumed in higher amounts by vegans are found in orange juice and other citrus products. However, vegans had significantly lower counts of leukocytes, lymphocytes, and platelets, and lower levels of complement factor 3 compared to nonvegetarians. There were no differences between the groups with respect to NK cell cytotoxicity, immunoglobulin concentration, or mitogen stimulation. The authors postulated that the lower BMIs of the vegan subjects may account for the differences in immune cell counts. However, BMI was not associated with immune function independent of diet.

A clinical trial in 18 subjects (age 50 years and older) with moderately elevated LDL cholesterol evaluated the effects of fat content in the diet on immune function (Han et al. 2003). Subjects consumed their habitual diet followed (or preceded) by either a high-fat (38% of calories as fat) or low-fat (28% of calories as fat) diet during two 32-day study phases with a minimal 2-week interval between phases. Delayed-type hypersensitivity skin response increased significantly following consumption of the low-fat compared to the high-fat diet, as did lymphocyte proliferative response to T-cell mitogens. There was no diet effect on lymphocyte response to B-cell mitogens, production of various cytokines, or serum levels of C-reactive protein.

In summary, studies provide some support for an effect on immunity by including particular foods into the diet, especially when intakes of the test food nutrients are limited in the remainder of the diet (e.g., carotenoids). More studies are clearly needed to examine the effects of the inclusion of single or multiple foods on immune markers. In particular, intervention studies with citrus fruit and juices are currently non-existent and s hould be supported. With regard to cancer, it is well established that the intake of diets high in fruits and vegetables is associated with a reduced risk for several types of cancer (Steinmetz et al. 1996; 1997). The cornerstone of current dietary recommendations emphasize the inclusion of a variety of fruits and vegetables into a healthy diet (USDHHS 2005), thereby supporting the inclusion of citrus fruits and juices into the diet throughout the life span to help reduce cancer risk.

Summary and Conclusions

Many animal, in vitro, and human intervention studies have reported positive effects of several nutrients found in citrus fruits and juices - vitamin C, folate, vitamin B6, carotenoids, and other phytochemicals – on parameters of immune function. However, there are no human studies that test the effects of citrus fruit or juice consumption on markers of immunity. Studies typically use micronutrient supplements as opposed to foods. But it must be remembered that humans don't consume individual micronutrients, but rather whole foods. Nutrients may compete with one another for absorption or one nutrient may interact with others for digestion and/or absorption (Rivlin 1990). In addition, an excess of certain nutrients may even be detrimental to immune function (e.g., vitamin E, zinc) (Rivlin 1990).

Based on this review, the following conclusions can be made:

- Several micronutrients provided in substantial amounts by citrus fruit and juices, specifically vitamin C, folate, vitamin B6, and some carotenoids, appear to be required for proper functioning of the immune system. This is clearly supported by studies where severely deficient animals have overt aberrations in immune function. Many times the aberrations are reversed with nutrient restoration.
- Supplementation with single nutrients, some of which are found in citrus, often at supraphysiological doses, appears to improve the function of some components of the immune system, although the data are equivocal. Supplementation with individual nutrients at lower amounts (i.e., at or near the RDA) may be less or not effective in modulating immune response.
- Although they may play a role in modulating some aspects of immune function, there are no data to support that citrus flavonoids are required for adequate immune function.
- Several studies report that the consumption of a nutritional supplement providing a variety of vitamins and minerals at physiological doses into the diet over an extended period of time (i.e., greater than 6 months) may enhance certain aspects of immune function, particularly in older individuals. These data help support the concept that consumption of a varied diet that includes nutrient-rich foods, such as citrus fruits and juices, can contribute to maintaining a healthy immune system.

- Studies show that the inclusion of foods that provide particular nutrients in the diet may help enhance immune response. However, no studies have been conducted regarding citrus fruits or juices.
- From a research standpoint, it appears to be very difficult to consistently demonstrate immune enhancement in healthy individuals through the supplementation of single or multiple nutrients. Despite a vast body of research on various aspects of nutrition and immune function, it is unclear at this time which immune markers or how many markers might signal or present significant enhancements to immune function that may translate into lower disease rates.

The studies reviewed in this report bring to light several issues that must be taken into account when interpreting the research results:

- ***What marker(s) of immune function are most important for predicting enhanced immune response on a day-to-day basis?*** The complexity of the immune system and numbers and types of various immune cells and functions make this a difficult question that has yet to be answered.
- ***How does a moderate rather than overt deficiency in certain nutrients affect immune parameters?*** Studies, particularly in animals, show detrimental effects of overt nutrient deficiencies on immune function. This indicates that these nutrients are required for proper immune system function. However, since individuals in the US are more likely to have moderate rather than extreme deficiencies of nutrients, it is unclear whether immune function is significantly compromised, and to what extent, with moderate deficiencies and whether the deficiency negatively impacts health.
- ***Does supplementation with nutrients at or moderately above the RDA enhance immune function in otherwise healthy individuals with near-normal nutritional status?*** Human intervention studies most often use nutrient amounts that far exceed amounts recommended for healthy individuals on a daily basis. It is unclear whether slight or moderate increases in nutrient intake, as represented by consuming a glass of orange juice, could influence immune response to a significant degree.
- ***Would the consumption of foods containing these nutrients, such as orange juice or citrus fruit, enhance immune function in healthy individuals?*** There are no

studies testing the effects of consumption of citrus products on immune function and these studies are needed. Studies using other whole foods provide some limited support for their effectiveness.

- ***Does enhanced immune function translate into clinical outcomes such as short-term resistance to infection or long-term reduced risk for chronic disease?*** Studies using vitamin/mineral supplements are encouraging. However, long-term studies are needed in healthy individuals to determine such an association.

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