SYMPOSIUM: ANTIOXIDANTS, IMMUNE RESPONSE, AND ANIMAL FUNCTION

Physiological Role of Antioxidants in the Immune System

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ABSTRACT

Diets contain naturally occurring antioxidant compounds that can stabilize highly reactive, potentially harmful molecules called free radicals. Free radicals are generated during normal cellular metabolism and result from the metabolism of certain drugs or xenobiotics. Exposure to UV light, cigarette smoke, and other environmental pollutants also increases the body's free radical burden. The harmful activities of free radicals are associated with damage to membranes, enzymes, and DNA. The ability of antioxidants to destroy free radicals protects the structural integrity of cells and tissues. This review focuses on data indicating that the functions of the human immune system depend on the intake of micronutrients, which can act as antioxidants. Recent clinical trials have found that antioxidant supplementation can significantly improve certain immune responses. Specifically, supplementation with vitamins C, E, and A or β-carotene increased the activation of cells involved in tumor immunity in the elderly. Supplementation with the antioxidant vitamins also protected immune responses in individuals exposed to certain environmental sources of free radicals. Supplementation with vitamin A, a relatively weak antioxidant, decreases morbidity and mortality associated with measles infections in children.
(**Key words**: vitamin A, vitamin C, vitamin E, β-carotene)

Abbreviation key: **DTH** = delayed-type hypersensitivity.

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IMMUNE SYSTEM FUNCTIONS

The immune system is responsible for protection against infection by pathogens such as bacteria, viruses, and protozoan parasites. Pathogens, recognized as invaders of the body and as "non-self", are then destroyed by immune cells and their secretions. The immune system responds similarly to cells of the body that have undergone changes that can lead to cancer. The body's immune system no longer recognizes certain precancerous cells as "self". In essence, the altered cells are considered to be invaders and are destroyed. Conditions that depress immune functions consequently increase the risks of infection and development of certain cancers. Conversely, factors that can enhance immunity may lower these risks (27).

FREE RADICALS AND IMMUNE CELL FUNCTION

Many of the protective functions of immune cells depend on the fluidity of the membranes of the cell. As the concentration of polyunsaturated fatty acids in the membranes is increased, the potential for membrane lipid peroxidation mediated by free radicals also is increased. Lipid peroxidation decreases membrane fluidity, which adversely affects immune responses. Mice fed oxidized lipids show marked atrophy of the thymus and T-cell dysfunction. Loss of membrane fluidity has been related directly to the decreased ability of lymphocytes to respond to challenges to the immune system (3).

The degree of unsaturation of the lipids incorporated into membranes alters the exposure of membrane receptors and their activities. The synthesis of metabolites of arachidonic acid, such as immunosuppressive prostaglandins, is increased as the degree of unsaturation of membrane lipids increases, the concentration of antioxidants decreases, or both (22).

DIETARY ANTIOXIDANT MICRONUTRIENTS

Vitamins E and C and β-carotene are potent, direct-acting antioxidants; vitamin A is a less active antioxidant. Minerals such as Zn, Cu, and Mn are essential for the activity of superoxide dismutases; Se is required for glutathione peroxidase activity, and Fe is required for catalase activity. In general, minerals do not act directly as antioxidants but are critical components of the antioxidant enzymes.

Three essential micronutrients can interfere directly with the propagation stage of free radical generation and scavenge free radicals. Vitamin E (α-tocopherol), the major lipid-soluble antioxidant present in all cellular membranes, protects against lipid peroxidation and prevents the loss of membrane fluidity. Vitamin C (ascorbic acid) is water-soluble and can quench free radicals and singlet oxygen, as can vitamin E. Ascorbate also can regenerate the reduced antioxidant form of vitamin E (20).

Carotenoids are red and yellow pigments naturally occurring in all photosynthetic plants and organisms. Of the more than 600 characterized compounds, less than 10% can serve as precursors of vitamin A. β-Carotene, the most commonly available carotenoid in human diets, also is the major carotenoid precursor of vitamin A (5). Recent work (5) has shown that β-carotene is an efficient quencher of singlet oxygen and can function as an antioxidant. Vitamin A cannot quench singlet oxygen and has less antioxidant activity than the other antioxidant nutrients discussed; however, its importance to the immune system is well recognized.

VITAMIN A AND IMMUNE FUNCTION RELATED TO MEASLES INFECTIONS

Vitamin A has been called the anti-infective vitamin for many decades (23). Overt vitamin A deficiency has been associated with increased risk of infections. Vitamin A is critical for the development and functioning of T and B lymphocytes. Thus, low vitamin A status understandably results in a reduction of cell-mediated immune responses and decreased specific antibody responses following immunization. Even marginal deficiency, with no clinical signs of deficiency, decreased immune responses to vaccines and production of pathogen-specific antibodies (30). Vitamin A supplementation also acts as an adjuvant, enhancing immune responses when it is administered at immunization.

Recently (9, 10, 11), the importance of vitamin A in the recovery from measles in children has been examined. Evidence suggests that the body stores of vitamin A may be unavailable during measles infection. Supplementation with high doses of vitamin A, administered during the first few days of hospitalization, significantly reduced mortality and morbidity in young children. Improvement in outcomes has been linked to increased specific measles antibody titers in children given vitamin A supplementation.

In 1932, Ellison (13) treated 600 children hospitalized with measles complications; one group of 300 received orally high doses of vitamin A in the form of cod liver oil for a minimum of 7 d, and the control and treated groups were fed the same diets; 26 deaths occurred in the control group and 11 in the treated group. In 1987, Barclay et al. (2), in a hospital-based study, treated 88 children with measles with two oral doses of 200,000 IU of vitamin A; the control group of 92 did not receive the supplement; 12 deaths occurred in the controls and 6 in the treated group (Table 1).

In 1990, Hussey and Klein (18) treated 92 children hospitalized for complications associated with measles with two doses of 200,000 IU of vitamin A and gave a placebo to 97 matched control children; all children were monitored for morbidity and mortality in this hospital-based study. Ten deaths in the placebo group and 2 deaths in the treated group occurred. Average hospital stay was reduced 30% from 15 d in the placebo group to 10 d in the children treated with vitamin A. The incidence of pneumonia and diarrhea lasting ≥10 d was significantly decreased by 60% in the supplemented group. Coutsoudis et al. (9, 10) reported that, in hospitalized children infected with measles that were given 160,000 IU (<12 mo of age) or 300,000 IU (>12 mo of age) of vitamin A on 3 consecutive d, incidence of pneumonia and diarrhea was significantly reduced from that of the placebo group for up to 6 mo following supplementation. The reduced morbidity was associated with a significant increase in total number of circulating

TABLE 1. Effects of high dose vitamin A supplementation in hospitalized children that are infected with measles with no overt signs of vitamin A deficiency.

Supplement	Significant effects	Reference
200,000 IU on 2 consecutive d	Reduced mortality by 50%	(2)
200,000 IU on 2 consecutive d	Reduced mortality by 80%, morbidity from pneumonia and diarrhea by 60%, and hospital stay by 30%	(18)
160,000 IU (<12 mo) on 3 consecutive d or 300,000 IU (>12 mo) on 3 consecutive d	Reduced morbidity from pneumonia and diarrhea for up to 6 mo and increased measles antibody concentrations	(9, 10)

immune cells and a significant increase in the concentration of measles-specific antibodies. In both of the placebo-controlled, double-blind studies discussed (9, 10), no overt signs of vitamin A deficiency were present in the children infected with measles, nor was vitamin A deficiency common in the populations examined (11, 18). No evidence of vitamin A toxicity was found in either study.

The importance of vitamin A supplementation and its reduction in mortality and morbidity in children infected with measles has relevance to children in the US. Since 1989, measles epidemics have occurred yearly in the US; the epidemics were mainly confined to neighborhoods with unvaccinated children. Over 50,000 cases of measles have been reported; 19% of cases required hospitalization. Over 100 deaths from measles complications were reported in 1989 and 1990 (1).

For the past 60 yr, clinical studies consistently have found a strong association of vitamin A supplementation and significant reduction in adverse measles outcomes, especially in young children. However, in 1992, no US physician policy recommends the use of vitamin A supplementation in children hospitalized with measles complications.

β-CAROTENE AND IMMUNE RESPONSES

Nutritionists traditionally have viewed β-carotene solely as a source of vitamin A activity in the diet. However, several studies have shown that carotenoids can enhance immune functions independently of any provitamin A activity (4). The mechanisms of immunoenhancement may include the capacities of a number of carotenoids to quench antioxidants and singlet oxygen.

In laboratory studies, comparisons between β-carotene and the nonvitamin A carotenoid, canthaxanthin, have shown that both carotenoids enhanced proliferative responses of T and B lymphocytes to mitogens (6); increased cytotoxic T-cell (32) and macrophage tumor-killing activities; and stimulated the secretion of tumor necrosis factor-α and simultaneously lowered the tumor burden (29).

In a clinical study (21), vegetarians had similar serum concentrations of all of the vitamins compared with a matched nonvegetarian population. However, serum β-carotene concentrations were twice as high in the vegetarian group. Natural killer cells from the vegetarian group lysed twice the number of tumor cells as did natural killer cells from the nonvegetarian group, suggesting that β-carotene may enhance natural killer cell functions independently of its provitamin A activity.

FACTORS AFFECTING IMMUNITY

Certain environmental factors can decrease immune function, including exposure to UV light, cigarette smoking, and infection with viruses such as human immunodeficiency virus. The aging process is associated with a loss of cell-mediated immune responses and a concomitant increase in infections and cancer incidence.

UV Exposure

Exposure to UV light directly increases the free radical burden imposed on the body, decreases immune responses, especially cell-mediated responses, and significantly increases the risk of skin cancer (12). Experimental studies (26) clearly have linked the immunosuppressive effects of UV exposure with

TABLE 2. Effects of vitamin supplementation on immune responses.

Oral supplement	Duration	Immune effects[1]	Reference[2]
Vitamin E, 800 IU	30 d	Enhanced DTH, IL-2, and proliferation	(22)
β-Carotene, 30 mg	70 d	Prevented UV-induced depression in DTH in young adults	(14)
β-Carotene, 45 to 60 mg	60 d	Increased markers for helper T cells, NK cells, and IL-2 receptors	(34)
Vitamin A, 800 IU Vitamin E, 50 mg Vitamin C, 100 mg	28 d	Increased markers for helper T cells and total T cells; enhanced proliferation	(25)
Multivitamin-mineral supplement	16 mo	Enhanced DTH, enhanced proliferation	(7)

[1]DTH = Delayed-type hypersensitivity; IL-2 = interleukin-2; NK = natural killer.

[2]All studies were double-blind and placebo-controlled and, except that of Penn et al. (25), used healthy elderly individuals.

increased development of skin tumors and other tumors.

β-Carotene and certain other carotenoids can block the formation of UV-induced singlet oxygen. Singlet oxygen can initiate the generation of immunosuppressive, reactive free radical species containing oxygen. Recent laboratory studies (14) have shown that dietary intake of β-carotene or canthaxanthin, a carotenoid lacking vitamin A activity, can significantly reduce the immunosuppressive effects of UV (Table 2). Supplemental intakes of canthaxanthin and vitamin A blocked the loss of tumor immunity in mice exposed to UV and reduced the growth of experimentally implanted tumors (15).

In a placebo-controlled, double blind study involving adult male humans (14), β-carotene supplementation prior to exposure to UV light prevented UV-induced depression of delayed-type hypersensitivity responses (**DTH**), a clinically important index of cell-mediated immunity.

Cigarette Smoking

The risk of lung cancer is approximately 15 times greater for smokers than for nonsmokers (8). Along with many other known harmful chemicals in cigarette smoke, there are thousands of free radicals per puff. Reports (28) based on the data from the second National Health and Nutrition Examination Survey of the American population show that serum ascorbic acid concentrations are consistently lower in smokers than nonsmokers. Smokers need to consume well over twice the recommended dietary allowance of vitamin C daily as nonsmokers to have similar concentrations of serum vitamin C (28).

Serum concentrations of folic acid and β-carotene and vitamin E concentrations in the lung are significantly lower in smokers than in nonsmokers (17, 24, 31). The reduction of antioxidant status in smokers may favor cellular damage attributed to the increased free radical burden imposed by smoking (20). Chronic exposure to cigarette smoke significantly lowers lymphocyte functions, such as proliferation and antibody production. Decreased natural killer cell activity and significantly increased risk of infection and precancerous lesions are well documented in chronic smokers. Cigarette smoke also contributes to the development of local inflammation in the lung, resulting in increased production of reactive oxygen radicals from activated leukocytes. Several laboratory studies (19) found that experimentally induced tumors grew faster in animals exposed to cigarette smoke; concomitant immunosuppression also has been documented in several animal models. The combined effects of increased free radical burden and increased damage to DNA and the decreased status of vitamins C and E, folic acid, and β-carotene in smokers may interfere sufficiently with normal tumor immunity to increase risk of carcinogenesis in smokers.

Aging

Cancer risk increases significantly in individuals over age 65. For example, the risk of cancer of the large intestine increases 1000 times between age 30 and 80; the majority of the increased risk occurs after age 65 (33).

One possible factor affecting the increased occurrence of cancer in the elderly could be the well-established decrease in immune functions as individuals age (16). The cell-mediated immune responses involving T-lymphocyte functions (cytotoxicity, production of interleukin-2, and proliferation) are the most sensitive to the age-related decline in immune responses. As a consequence, DTH to skin test antigens are diminished significantly in the elderly, resulting in anergy, a complete lack of skin test responses, in the most immunosuppressed individuals.

Recent studies (35) have shown that DTH can be used as a predictor of morbidity and mortality in the elderly; i.e., elderly with reduced DTH responses (anergy) had twice the risk of death from all causes as did elderly that responded to the antigens. Of great importance are recent data from four placebo-controlled, double-blind studies (7, 22, 25, 34) that indicate that certain micronutrient supplements can significantly enhance DTH responses, T-cell subpopulations, or both, proliferative responses, and interleukin-2 activities in the elderly. Vitamin E supplementation (800 IU/d) for 1 mo significantly increased DTH responses of healthy elders (22). Supplementation of healthy elderly individuals for 2 mo with 45 or 60 mg/d of β-carotene increased the concentration of peripheral blood cells bearing markers for natural killer cells and three markers of immune cell activation (34). A high potency multiple vitamin and mineral supplement taken for 16 mo significantly enhanced DTH in healthy elderly persons (7). A supplement containing 8000 IU of vitamin A, 50 mg of vitamin E, and 100 mg of vitamin C taken for 28 d increased total T lymphocytes and T helper cell markers in a long-term study of hospitalized elderly persons (Table 2) (25).

CONCLUSIONS

These data strongly suggest that supplementation with vitamins A, C, and E or β-carotene provides a safe and effective means to enhance clinically relevant immune functions. Vitamin A supplementation significantly reduced morbidity and mortality associated with measles in children. The protective effects of vitamin A were associated with enhancement in immune responses and a reduction in the rate of secondary infections for up to 6 mo.

Supplementation with vitamins A, C, and E and β-carotene was associated with enhancement of immune responses in several population groups. Many of the immune responses that were enhanced are necessary for destruction of cancerous cells. In addition, the antioxidant micronutrients protected immune responses from the immunosuppressive effects of environmental factors, such as exposure to UV and cigarette smoke, that also increase the risk of cancer.

Thus, antioxidant vitamins enhance immune responses that are involved in protection from infection and malignancies. The data strongly suggest that the intake needed to enhance immune responses may be many times greater than the currently recommended allowances.

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