Role of Carotenoids In the Immune Response

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ABSTRACT

The well-established provitamin A activities of certain carotenoids have hampered past research into their possible specific functions. More recently, research interests on carotenoids have been revived, largely because of their anticancer activities. β-Carotene and other carotenoids have been reported to possess immunomodulatory activities in humans and animals. These carotenoids enhance lymphocyte blastogenesis, increase the population of specific lymphocyte subsets, increase lymphocyte cytotoxic activity, and stimulate the production of various cytokines. In addition, carotenoids also stimulate the phagocytic and bacteria-killing ability of blood neutrophils and peritoneal macrophages. The action of these carotenoids is widely accepted to be independent of their provitamin A activity. The immunostimulatory action of carotenoids may be translated into improved health, including mammary and reproductive health in dairy cattle. Studies on carotenoids other than β-carotene are needed.

(Key words: carotenoids, immunity, health)

INTRODUCTION

Occurrence and Metabolism of Carotenoids

Carotenoids consist of a group of over 600 colored pigments found in nature (42). Most carotenoids have 40 carbon atoms. Also, most have oxygen functions (oxycarotenoids); less than 10% are hydrocarbons. Even though carotenoids are recognized mainly for their provitamin A functions, less than 10% possess provitamin A activities in mammals. Of these carotenoids, β-carotene (Figure 1) has the highest provitamin A activity. This situation is complicated by the differing abilities of animals to convert carotenoids to vitamin A. For example, within the low range of β-carotene intake, 1 mg of β-carotene is equivalent to 400 IU of vitamin A in dairy cattle compared with 1667 IU of vitamin A in rodents (5). The efficiency of β-carotene conversion to vitamin A is lower with higher β-carotene intakes (49).

In addition to differences in conversion efficiency of β-carotene to vitamin A, large species differences also occur in an animal's ability to absorb and to transport carotenoids. Cattle (and humans, chickens, and horses) absorb large amounts of different carotenoids from the diet, transport these carotenoids (primarily β-carotene) in the blood, and transfer them into milk (7). The carotenoids present in milk and milk products include β-carotene (the major carotenoid) and lutein (4, 31, 33). Zeaxanthin, cryptoxanthin, lycopene, and α-carotene also are present, but in much lower concentrations because of lower concentrations in feeds and different rates of absorption across the intestinal wall. Different breeds of dairy cattle also differ in their ability to transfer carotenoids into milk. For instance, Guernsey cows transfer more carotenoids into their milk than do Holsteins (44).

In practical dairy diets, carotenoids are primarily derived from forages. However, considerable variations occur in the carotenoids
Figure 1. The structures of β-carotene, lycopene, lutein, and canthaxanthin. MW = Molecular weight.
content of feedstuffs because carotenoids are oxidized readily, especially under conditions of high heat and humidity. Certain processing methods and prolonged storage (8) can reduce carotenoid content drastically. Therefore, stored forage is not a reliable source of dietary carotenoids in practical dairy diets.

The possible specific physiological roles of carotenoids have been ignored previously because of their assumed sole function as provitamin A. However, interest in the role of carotenoids on immunity has increased in recent years as a result of reports (54) on the association between high concentrations of β-carotene and high intakes of β-carotene with lower incidences of certain types of cancers in human. As this review makes apparent, most present studies deal with β-carotene. However, carotenoids other than β-carotene have received greater attention in recent years. The present discussion deals primarily with the effects of carotenoids on immunity in dairy cows; however, pertinent studies using other animal models are cited.

**Immunity**

In early studies, β-carotene supplementation increased resistance to infection for children (22) and for rats (15). These observations were strengthened in later studies (40), which reported a marked stimulatory action of β-carotene on the growth of the thymus gland. Furthermore, mice fed β-carotene (4.3 mg of β-carotene/kg of diet) had large increases in the number of thymic small lymphocytes and hastened skin graft rejection response. In contrast, retinoic acid supplementation reduced the number of thymic lymphocytes and delayed the skin graft rejection response (40). Tomita et al. (48) reported that β-carotene fed to mice for 9 d augmented tumor immunity against syngeneic Meth A fibrosarcoma cells. Tomita et al. (48) further demonstrated that the action of β-carotene on tumor rejection might have been specific to tumor-specific antigens, as evidenced from the failure of β-carotene to augment mice inoculated with Meth A to reject Meth 1 fibrosarcoma cells (another BALB/c mouse syngeneic tumor cell) when the latter were used as a rechallenge tumor.

**Specific Cellular Host Defense.** Both in vitro and in vivo studies have reported enhanced host defense functions in animals and humans supplemented with β-carotene and other carotenoids. For instance, Holstein cows supplemented with 300 to 600 mg/d of β-carotene from wk 4 prepartum through wk 4 postpartum showed increased mitogen-induced (concanavalin A, phytohemagglutinin, and pokeweed mitogen) lymphocyte proliferation (24) during the peripartum period. Cows supplemented with 120,000 IU/d of vitamin A did not show a similar response profile. Likewise, blood lymphocytes isolated from Holstein cows during the peripartum period and incubated with $1 \times 10^{-9} M$ β-carotene had higher lymphocyte proliferation induced by concanavalin A than did unsupplemented cultures; retinol had no effect on lymphocyte proliferation, whereas retinoic acid was suppressive (19). The stimulatory effects of β-carotene ($1 \times 10^{-8}$ to $1 \times 10^{-6} M$) on bovine lymphocytes in vitro have similarly been demonstrated in nonlactating, primiparous Holsteins (17). That group of studies suggested a specific effect of β-carotene. In contrast, Tjoelker et al. (46) reported that $1 \times 10^{-6}$ to $1 \times 10^{-5} M$ of β-carotene inhibited lymphocyte proliferation in vitro in dairy cows immediately prior to and after drying off. This discrepancy likely is due to differences in the concentration of β-carotene used in the incubation mixture, the concentration of blood β-carotene and vitamin A of the donor cows, or the lactation status of the experimental cows. The effects of blood β-carotene and vitamin A concentrations and lactational status of the cow on the in vitro effects of β-carotene on lymphocyte proliferation have been demonstrated (47).

The stimulatory activity of β-carotene on lymphocyte blastogenesis has similarly been demonstrated in other species. An intramuscular injection of 20 or 40 mg of β-carotene into mature (25) and newborn (26) pigs enhanced blood lymphocyte proliferation compared with that of pigs injected with the vehicle. A similar, heightened response in lymphocyte proliferation induced by concanavalin A was observed in rats fed a diet that contained .2% β-carotene by weight and in lymphoproliferation induced by lipopolysaccharides (B-cell mitogen) in rats fed canthaxanthin, a 4,4'-diketo analog of β-carotene (6). Because canthaxanthin has little or no provitamin A activity, the authors (6) concluded that the immunoenhancing activities of the carotenoids
Alexander et al. (2) reported a 30% increase in the number of helper and inducer T lymphocytes (OKT4+) in healthy human adults after 7 d of oral β-carotene supplementation (180 mg/d). In contrast, β-carotene supplementation did not alter the cytotoxic and suppressor T lymphocyte (OKT8+) population. The increased frequency of T helper and inducer T cells was diminished greatly 7 d after β-carotene supplementation ended. An increase in the number of T helper cells (CD4+) was similarly reported in healthy human volunteers fed 35 to 60 mg/d of β-carotene for 2 mo (30). In the latter study, the number of lymphoid cells with surface markers for natural killer (NK) cells and for interleukin-2 and transferrin receptors also was increased substantially with β-carotene. Watson et al. (30) noted that the concentration of plasma β-carotene, but not retinol, was elevated in individuals with supplemented β-carotene, suggesting a specific immunomodulatory action of the carotenoid itself. The ability of β-carotene to stimulate increases in the percentage of peripheral mononuclear cells expressing surface markers for T helper and NK cells and for IL-2 and transferrin receptors were similarly reported in an in vitro study (35) using human leukocytes and in in vivo studies (34) with human subjects with premalignant lesions or individuals infected with human immunodeficiency virus (21). The NK cells cytotoxicity was enhanced in human subjects given oral β-carotene (34). Tomita et al. (48) reported that the primary effector cells responsible for the antitumor activity in mice supplemented with β-carotene was Thy-1+, Lyt-1−, and Lyt-2+ lymphocytes, which are presumed to by cytotoxic T lymphocytes.

Secretion and Action of Cytokines. In vitro, physiological concentrations of β-carotene inhibited the cytostatic action of interferon (IFN)-α on monocyte expression of Fcy and human lymphocyte antigen DR receptors (36). In contrast, retinol and retinoic acid reversed the actions of β-carotene on these cells. Abril et al. (1) reported that 10−6 to 10−10 M β-carotene in vitro stimulated human peripheral blood mononuclear cells to secrete a novel cytokine that possesses cytotoxic activity against human cancer cell lines. The cytokotoxic activity induced by β-carotene was different from that of IFN, interleukins, lymphotoxin, or tumor necrosis factor. Using the hamster bucal pouch model, Shklar and Schwartz (41) demonstrated that β-carotene, but not retinoic acid, could induce macrophages to produce tumor necrosis factor-α.

Nonspecific Cellular Host Defense. In addition to modulating lymphocyte function, β-carotene also modulates other host defense cells. In the presence of 10−8 to 10−7 M β-carotene in vitro, bovine blood and mammary polymorphonuclear leukocytes (PMN) isolated from cows during the peripartum period showed enhanced ability to kill Staphylococcus aureus (18). Similarly, blood PMN isolated from Holstein cows fed 300 to 600 mg/d of β-carotene from wk 4 prepartum through wk 4 postpartum showed higher killing ability against bacteria during the peripartum period (28). The increased bacterial killing could be accounted for partly by increased myeloperoxidase activity in the PMN and seems to be unrelated to changes in the production of superoxides (28). The latter observations are in agreement with a study with PMN from humans (3). β-Carotene increased PMN function in cows during the dry-off period. In vitro, Tjoelker et al. (45) reported that 1 × 10−6 M β-carotene stimulated PMN phagocytic and bacterial killing ability in dairy cows before and after drying off. This finding is in contrast to the effects of retinol and retinoic acid, which generally decreased phagocytosis and had no effect on killing activity, suggesting a possible specific action of the intact carotenoid molecule as opposed to its provitamin A activity. In vivo, blood PMN from cows supplemented with 400 mg of β-carotene during the period around dry-off maintained their phagocytic ability, as opposed to the decreased phagocytic ability observed with PMN from cows fed only preformed vitamin A (47). Human PMN incubated with β-carotene similarly showed enhanced killing of bacteria in vitro (51). Schwartz et al. (38) reported increased histochemical and functional changes with mouse peritoneal exudate macrophages incubated in vitro with several carotenoids. For example, cytochrome oxidase and peroxidase activities were enhanced in macrophages incubated with canthaxanthin, β-carotene, and α-carotene compared with incubation with
13-cis retinoic acid. The stimulatory activity of canthaxanthin was greater than that of β-carotene and α-carotene. Phagocytosis similarly was stimulated by these carotenoids, even though to a lower degree. All of these changes indicate increased respiratory bursts by the macrophages when they are exposed to carotenoids (38).

Mechanism of Carotenoid Action

The mechanisms by which carotenoids regulate immunity largely are unclear. The most widely recognized mechanism of carotenoids action is its antioxidant function (27). Singlet oxygen species and preoxyl radicals are generated constantly in biological systems. These reactive species are capable of disrupting cell membrane function and inducing DNA single-strand breaks (51). β-Carotene possesses potent activities that scavenge singlet oxygen and quench peroxyl radicals, especially under low oxygen tension (9, 32). Several naturally occurring carotenoids and xanthophylls exceeded β-carotene in their physical activity of quenching singlet oxygen (20). For instance, lycopene has over twice the activity of β-carotene in quenching singlet oxygen and over 10 times that of vitamin E. The activity of other carotenoids, such as γ- and α-carotene, astaxanthin, and canthaxanthin, also exceeded that of β-carotene. β-Carotene protects host defense cells against destruction from reactive oxygen species. For example, Weitberg et al. (51) reported that β-carotene reduced sister chromosomal damage produced by phagocytically and enzymatically generated oxygen metabolites in human blood PMN. Blood phagocytes isolated from cigarette smokers supplemented with 40 mg/d of β-carotene for 6 wk showed progressive inhibition of chemiluminescence enhanced by activated luminol, which suggested that β-carotene functions as an intracellular and an extracellular scavenger of oxidants generated by the myeloperoxidase-hydrogen peroxide-halide system (3, 37).

Young male human subjects supplemented with either 15 or 150 mg/d of β-carotene for 4 wk had reduced serum lipid peroxide concentrations (29). However, supplemental β-carotene did not affect superoxide production by neutrophils, suggesting that β-carotene inhibits the by-products of cellular oxidative metabolism at a different stage of the lipid peroxidation process. Zamora et al. (52) showed that β-carotene (30 mg/kg of diet) was as effective as vitamin E and Se in preventing liver damage, as indicated by decreased plasma aspartate aminotransferase. Furthermore, β-carotene also was as effective as vitamin E and Se in preventing lipid peroxidation and oxidative damage in erythrocytes, as measured by decreases in substances reactive to thiobarbituric acid in hemolysis, and in proteolysis (52).

Carotenoids also may regulate immune cell function by regulating a number of other separate or interrelated cell events. These carotenoids (especially the polar forms) can regulate the fluidity of biological membranes by intercalating into the cell lipid bilayer, thereby increasing the mobility of the polar head groups of the membrane, and increasing accessibility to that region (43). In addition, carotenoids also may alter the metabolic state of these cells, possibly through the induction of heat shock protein (39), increase gap-junctional intercellular communication (53), and inhibit arachidonic acid oxidation, which are reactions initiated by free radicals (23).

The possibility that β-carotene and other carotenoids may regulate nuclear events has not been considered previously. Evidence from calves (14) and pigs (11, 12) has shown significant uptake of β-carotene by blood lymphocytes, but not by blood neutrophils or erythrocytes in both species. Subcellular fractionation of lymphocytes from animals treated with β-carotene revealed significant uptake of β-carotene by the nuclei, microsomes, and mitochondria; concentrations detected in the cytosol were very low. The rate of uptake and depletion of β-carotene among the various subcellular fractions differed. These data showed the presence of β-carotene in cellular organelles and suggest that β-carotene may act at these sites.

Implications for Animal Health

As apparent from discussion already presented, β-carotene and other carotenoids modulate the immune system. Although their immunomodulatory activity clearly has direct implications on cancer etiology, how carotenoids affect animal health is less clear. Lactat-
ing dairy cows supplemented with 300 mg/d of β-carotene during the first 10 wk of lactation had lower milk SCC than did cows fed an equivalent amount as preformed vitamin A (10). Similarly, cows fed the same amount of β-carotene around dry-off had lower rates of new IMI during early dry-off (16). Another study (30) failed to show a beneficial effect of β-carotene supplementation on the rate of IMI. However, in the latter study, concentration of β-carotene in all cows were approximately four- to fivefold higher than the proposed optimal blood concentration of 200 μg of β-carotene/100 ml for dairy cows (10, 16). Further studies therefore are warranted.

In addition to mammary health, carotenoids also may improve reproductive health, which may or may not be related to improved immunity associated with β-carotene supplementation. Dairy cows fed 600 mg/d of β-carotene from about wk 4 prepartum had lower rates of postpartum retained placenta than cows fed an equivalent amount as preformed vitamin A (28). Improvements in other aspects of reproduction also have been reported (13).

CONCLUSIONS

β-Carotene and other carotenoids possess immunoregulatory activities. Furthermore, the biological actions of these carotenoids likely are independent of their provitamin A activity. The enhanced host defense from dietary use of carotenoids may translate into improved health. Therefore, more studies are warranted to establish this relationship. Research on the effects of carotenoids on immunity and health has concentrated mainly on β-carotene, whereas the effects of other carotenoids largely have been ignored. Even though β-carotene is the predominant carotenoid in bovine blood and milk (mainly because of its high concentration in feeds), several other carotenoids, such as lutein, zeaxanthin, cryptoxanthin, α-carotene, and γ-carotene, also occur in significant concentrations. Future studies should include these potentially important carotenoids.

REFERENCES


