Effects of Various Carotenoids on Cloned, Effector-Stage T-Helper Cell Activity

Harumi Jyonouchi, Sining Sun, Munetaka Mizokami, and Myron D. Gross

Abstract

Astaxanthin, a carotenoid without provitamin A activity, enhances murine T-helper (Th) cell clone-mediated antibody (Ab) production with suboptimal antigen (Ag) challenges. It also suppresses interferon-γ (IFN-γ) production by cloned murine Th1 cells. β-Carotene is less effective than astaxanthin. This study evaluates the effects of various carotenoids with various relative polarity, provitamin A activity, and antioxidant activity. Carotenoids tested include astaxanthin, cantaxanthin, zeaxanthin, lutein, and lycopene, and their effects were tested at a concentration at which astaxanthin's effect was most potent. A.E7 and CDC35 cells are used as representative type 1 and type 2 Th cell (Th1 and Th2) clones, respectively. In the Th1 clone, astaxanthin, but not other carotenoids, suppressed IFN-γ production and increased the number of Ab-secreting cells with the use of primed spleen cells. With cultures of Th1 cells and unprimed spleen cells, astaxanthin and zeaxanthin augmented the number of immunoglobulin M Ab-secreting cells. In the cultures of Th2 clone and primed spleen cells, astaxanthin, but not other carotenoids, enhanced the number of Ab-secreting cells. With unprimed spleen cells, lycopene suppressed Th2 clone-mediated Ab production. Interleukin-5 production by the Th2 clone was not significantly altered with the carotenoids tested, irrespective of the use of unprimed or primed spleen cells. Carotenoid actions on Th cells may vary in each carotenoid and do not seem to be closely associated with carotenoid antioxidant activity or relative polarity.

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Introduction

Carotenoid intake from diets rich in fruits and vegetables is positively correlated with the chemoprevention of cancer and other degenerative diseases (1–4). Carotenoids may exert a protective effect through several mechanisms; they are known to exert actions other than the well-recognized vitamin A and antioxidant activities. These include enhancement of gap-junctional communications and modulation of immune functions (5–11). However, little is known regarding the carotenoid mode of action in relation to their apparent chemopreventive actions. More than 600 carotenoids are present naturally; many are contained in regular diets (12). The action of each carotenoid may differ depending on its molecular structure, its biochemical nature, and its metabolism in the body. Comparison of the actions of various carotenoids commonly present in regular diets may help further evaluate the role of dietary carotenoids in disease prevention.

The immune system is one of the major defenses against tumorigenesis, and the mode of

H. Jyonouchi, S. Sun, and M. Mizokami are affiliated with the Department of Pediatrics, School of Medicine, and M. D. Gross is affiliated with the Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN 55455.

carotenoid chemopreventive action may be through its action on this system. Our previous studies focused on antigen (Ag)-mediated T-dependent immune responses. We have shown that astaxanthin, a carotenoid without provitamin A activity, enhances T-dependent humoral immunity *in vivo* and *in vitro* in rodent models (13–15). The similar enhancing action of astaxanthin was shown on T-dependent immunoglobulin (Ig) production with use of human peripheral blood mononuclear cells (9). Recently, we showed that astaxanthin enhances T-helper (Th) clone-mediated antibody (Ab) production with suboptimal Ag challenges (10). Our results indirectly suggest that astaxanthin modulates the processes of Ag presentation between Ag-presenting cells (APC) and Th cells (14,15). One of astaxanthin's chemical characteristics is its relatively high polarity; astaxanthin may affect cellular/plasma membranes because of its high polarity and may modify the cell-cell interactions.

In this study we compared the actions of carotenoids with various degrees of antioxidant activity, provitamin A activity, and relative polarity to determine whether astaxanthin's action on the immune system can also be demonstrated by other dietary carotenoids and whether there is an association between carotenoid immunomodulating activity and other carotenoid functions and chemical characteristics.

Materials and Methods

Mice

B10 and DBA/2 female mice (5–6 wks old) were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained in the animal facility at the University of Minnesota (Minneapolis, MN). The mice were generally housed in groups of five per cage and fed a nonpurified diet[1] (Purina Lab Chow 5010, Purina Mills, Richmond, IN). The mice were killed in a CO_2 chamber, as approved by the Laboratory Animal Medicine Ethics Committee, University of Minnesota. Two- to four-month-old mice were used.

Experimental Design

Selection of carotenoids: We selected carotenoids on the basis of relative polarity, antioxidant activity, provitamin A activity, and gap-junctional enhancement activity (Table 1).

Experiment 1: This experiment examines the effects of various carotenoids on Th clone-mediated Ab production [A.E7 cells, type 1 Th (Th1) cells; CDC35 cells, type 2 Th (Th2) cells]. Spleen cells were obtained from syngeneic primed or unprimed mice (B10 mice for A.E7 cells and DBA/2 mice for CDC35 cells), depleted of $CD4^+$ cells, and used as the source of APC and B cells (10). The cell mixture of the Th cell clones and $CD4^-$ spleen cells were stimulated by Ag for five days in a culture supplemented with carotenoids. Ab production was evaluated by measuring the number of Ab-secreting cells. When unprimed spleen cells were used, only the number of IgM Ab-secreting cells was measured; primary immune responses are mainly mediated by IgM Ab. IgM and IgG Ab-secreting cells were measured when primed spleen cells were used. On the basis of our previous results (10), carotenoid effects were tested at 10^{-8} mol/l in most experiments. There was no significant change in cell viability at the end of culture at a carotenoid concentration of 10^{-8} mol/l compared with controls cultured without carotenoids. The ratio of $CD4^-$ spleen cells to Th clones was adjusted

[1]Dietary composition of a nonpurified diet (Purina Lab Chow 5008, Purina Mills) is as follows (per 100 g diet): 23.5 g protein, 6.5 g fat, 3.8 g fiber, and 6.8 g ash, with gross energy of 17.44 KJ/g. The carotene content of this diet provides approximately 4.5 times the daily requirement of vitamin A recommended for rodents (39).

Table 1. Summary of Actions of Carotenoid From Our Studies and Others

Carotenoid	Relative Polarity	Vitamin A Activity	Gap-Junctional Enhancement[a]	Antioxidant Activity[b]
β-Carotene	++	++++	++++	++
Astaxanthin	++++	No[c]	ND	+++
Cantaxanthin	+++	+	++++	+++
Lutein	++++	No	+++	+++
Lycopene	+	No	++	++
Zeaxanthin	++++	No	ND	++

a: Adapted from Ref. 40; ND, not determined.
b: Relative inhibition of lipid peroxidation (34,40).
c: No vitamin A activity in mammals.

to 1:16 for A.E7 cells and to 1:80 for CDC35 cells; at these APC and Th cell mixture rates, we observed the most optimal Th cell activity in preliminary experiments. Ag concentration was adjusted to show the most optimal enhancing actions of astaxanthin (cytochrome *c*, 0.5 μmol/l; rabbit γ-globulin, 5 mg/l)(10).

Experiment 2: The objective of this experiment is to examine the effects of various carotenoids on Ag-mediated cytokine production by Th1 and Th2 clones. The ratio of APC to Th cells and concentrations of Ag were the same as in Experiment 1. The mixture of Th cells and irradiated syngeneic spleen cells was stimulated with Ag in carotenoid-supplemented cultures. At Day 3 of culture, culture supernatants were harvested and tested for cytokine levels. Interferon-γ (IFN-γ) levels were tested for Th1 cells, and interleukin-5 (IL-5) levels were tested for Th2 cells.

Analytic Measures

Cell suspensions: Spleen cell suspensions were prepared by crushing a spleen between two sterile glass slides and suspending cells in RPMI 1640 with 50 ml/l fetal calf serum (FCS). Debris was removed by passing cell suspensions through coarse filters. $CD4^+$ Th cells were depleted by treatment with monoclonal rat anti-mouse L3T4 Ab (specific for murine CD4) (16) and guinea pig complement (1:5 dilution; Pel-Freeze, Rogers, AR). More than 98% of the spleen Th cells were removed by this procedure when examined by flow cytometry.

Reagents: β-Carotene, lutein, lycopene, and zeaxanthin were obtained from Sigma Chemical (St. Louis, MO). Astaxanthin and cantaxanthin were kindly provided by Hoffmann-La Roche (Basel, Switzerland). All carotenoids were dissolved in 5 ml of an ethanol-hexane mixture (49 parts absolute ethanol and 1 part hexane) and filtered through a 0.45-μm filter (Millex-AP, Milipore, Bedford, MA). The carotenoid concentration was measured spectrophotometrically on the day of each experiment; concentrations of the stock solution were usually around 10^{-4}–10^{-5} mol/l. The prepared stock solutions were further diluted by culture media containing 100 ml/l of FCS. The amount of hexane-ethanol mixture used for preparing carotenoid stock solution did not interfere with the assays employed in the study. The solubility of astaxanthin and β-carotene in the same medium used for this study was tested using higher concentrations of carotenoids (10^{-7}–10^{-6} mol/l) because of the limited sensitivity of high-performance liquid chromatography methodology. With these concentrations, >80% of astaxanthin and 50% of β-carotene were solubilized and stable in the medium for several days. When spleen cells were incubated at a carotenoid concentration of 10^{-6} mol/l, 3–4% of astaxanthin or β-carotene was detected in cell pellets.

Maintenance of Th1 cell clones: A.E7 cells (Th1 clone) were kindly provided by Dr. Marc K. Jenkins (Dept. of Microbiology, University of Minnesota) and maintained in our laboratory. A.E7 cells were stimulated periodically (every 2–3 wks) with Ag (pigeon cytochrome *c*, 3 μmol/l; Sigma Chemical) and irradiated B10.A spleen cells in Dulbecco's modified Eagle's medium supplemented with FCS (100 ml/l), penicillin G (10^5 U/l), streptomycin (100 mg/l), and 2-mercaptoethanol (10^{-5} mol/l); A.E7 cells were derived from Th cells from a female B10.A mouse (17). CDC35 cells (Th2 clone) were kindly provided by Dr. David C. Parker (Dept. of Molecular Genetics and Microbiology, University of Massachusetts, Worcester, MA) and maintained in our laboratory with stimulation by Ag (rabbit γ-globulin, 25–50 mg/l, Sigma Chemical) and irradiated DBA/2 spleen cells every two weeks in the same medium used for A.E7 cells; CDC35 cells were derived from Th cells of a DBA/2 mouse (18).

In vivo Ag priming: Ag was given twice subcutaneously two to three weeks apart [pigeon cytochrome *c* (25 μmol/l) or rabbit γ-globulin (50 mg/l) in 1:1 phosphate-buffered saline (PBS)-Freund's complete adjuvant, 0.5 ml/dose]. Then another booster dose of Ag (the same dose) was given intraperitoneally five to seven days before the experiment (19); spleen cells from *in vivo* immunized mice were used as the source of APC.

Enzyme-linked immunospot assay for IgM and IgG antibody-secreting cells: Numbers of Ag-secreting cells were measured by enzyme-linked immunospot (ELISPOT) assay (20,21). Cloned Th1 (A.E7 cells) and Th2 (CDC35) cells were incubated with Th cell-depleted syngeneic spleen cells (1:16 at a final concentration of 2.7×10^6 cells for A.E7 cells and 1:80 at a final concentration of 1.05×10^6 cells for Th2 cells) for five days in the same medium used for maintaining A.E7 cells in a 24-well tissue culture plate (Costar, Cambridge, MA). Then the cells were harvested, counted, and resuspended in Iscove's modified Dulbecco's medium supplemented with FCS (50 ml/l), penicillin G (10^5 U/l), streptomycin (100 mg/l), and glutamine (2 mmol/l). The harvested cell mixture (100 μl/well) was incubated overnight at 37°C in a 5% CO_2 incubator in a 96-well microtiter plate (Costar) coated with Ag (pigeon cytochrome *c*, 5 mmol/l) and preincubated with a blocking buffer (PBS, pH 7.4, with 10 g/l bovine serum albumin) for 30 minutes at 37°C. The cells were removed by vigorous washing on the next day, and goat anti-mouse IgG or IgM antibody-alkaline phosphatase conjugate [1:1,000 in dilution buffer used for enzyme-linked immunosorbent assay, (ELISA), 100 μl/well] was added to the well. The plate was incubated overnight at 4°C and washed again. A spot of Ab secreted by cells was detected by adding gel substrate solution [5-bromo-4-chloro-3-indolyl phosphate (75 mg/l) and nitro blue tetrazolium chloride (150 mg/l) in 50 mmol/l $NaHCO_3$, pH 9.8, with $MgCl_2$ (5 mmol/l) and agar (5 g/l), 100 μl/well] and incubating the plate at 37°C for four to five hours. Triplicates for each sample were tested and expressed as mean number of triplicate samples per 10^6 viable cells based on the cell count after five days of culture. Cell viability declined when cells were cultured for more than six days in this experimental system.

Cytokine production: A.E7 cells were mixed with irradiated B10 spleen cells at 1:16 with a final concentration of 2.7×10^9 cells/l. CDC35 cells were mixed with irradiated DBA/2 spleen cells at 1:80 with a final concentration of 1.05×10^9 cells/l. The cells were cultured in the same medium used for maintaining these clones. Cytokine levels in the culture supernatants were measured after three days of culture.

ELISA for IL-5 and IFN-γ: IL-5 and IFN-γ levels in the culture supernatants were measured by an ELISA. We used unconjugated anti-mouse IL-5 or IFN-γ (2–3 mg/l in the coating buffer; Pharmingen, San Diego, CA) as the first Ab and biotinylated anti-murine IL-5 or IFN-γ (2 mg/l in PBS supplemented with 100 ml/l calf serum; Pharmingen) as the second Ab. ELISA plates (Nunc, Naperville, IL) were coated with the first Ab overnight at 4°C; then

samples were incubated overnight in the plate at 4°C. The plate was washed with rinse buffer (PBS, pH 7.4, with 0.5 ml/l Tween 20) and incubated with the second Ab at room temperature for 1–1.5 hours. The plate was washed again and incubated with avidin peroxide (Sigma Chemical) in PBS with 100 ml/l calf serum at room temperature for 30 minutes. The color was developed by addition of substrate buffer [azino-bis-3-ethylbenzthiazolin-6-sulfonic acid (200 mg/l; Sigma Chemical) in 0.1 mol/l citric acid, pH 4.34, with 0.3 ml/l H_2O_2].

Statistical analysis: The statistical analysis was done by Student's two-tailed *t*-test using raw or log-transformed data (22); $p < 0.05$ was considered to be significant.

Results

Effects of Various Carotenoids on Th Clone-Mediated Ab Production

The clone-mediated Ab production was measured using syngeneic primed and unprimed spleen cells.

Ab production using unprimed syngeneic spleen cells [primary (IgM Ab) responses]: In Th1 (A.E7) and Th2 (CDC35) cells, astaxanthin significantly enhanced the number of IgM Ab-secreting cells at a 10^{-8} mol/l (Figures 1 and 2). Among other carotenoids tested, zeaxanthin significantly increased the number of IgM Ab-secreting cells in Th1-mediated Ab production (Figure 1). In the Th2 clone-mediated Ab production, the number of IgM Ab-secreting cells was significantly lower in the lyopene-supplemented cultures (Figure 2).

Ab production with the use of primed syngeneic spleen cells (secondary Ab responses): Astaxanthin enhanced the numbers of IgM Ab-secreting cells in Th1 and Th2 clone-mediated Ab production assay (Figures 3 and 4). Other carotenoids did not alter the number of IgM Ab-secreting cells significantly. However, the number of IgM Ab-secreting cells tended to be lower with the use of lycopene and cantaxanthin in the Th1 clone-mediated Ab production (lycopene, $p = 0.087$ vs. control; cantaxanthin, $p = 0.079$ vs. control). The number of IgG

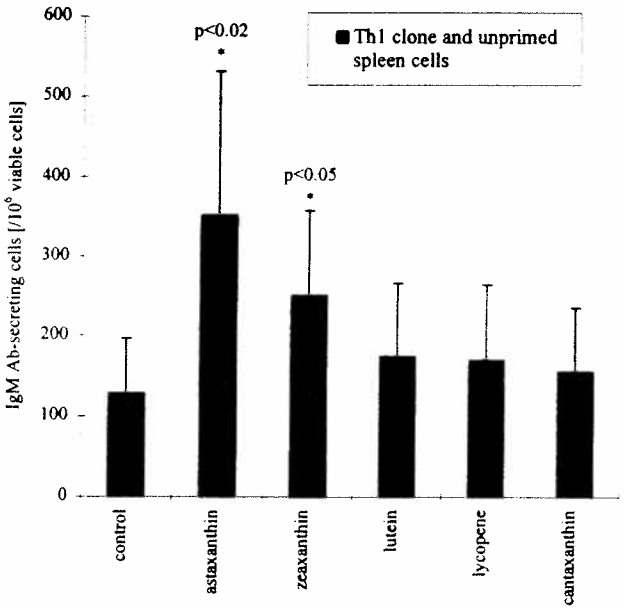

Figure 1. Effects of various carotenoids on number of immunoglobulin M (IgM) antibody (Ab)-secreting cells formed in cultures of Th1 cells and unprimed $CD4^-$ B10.A spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

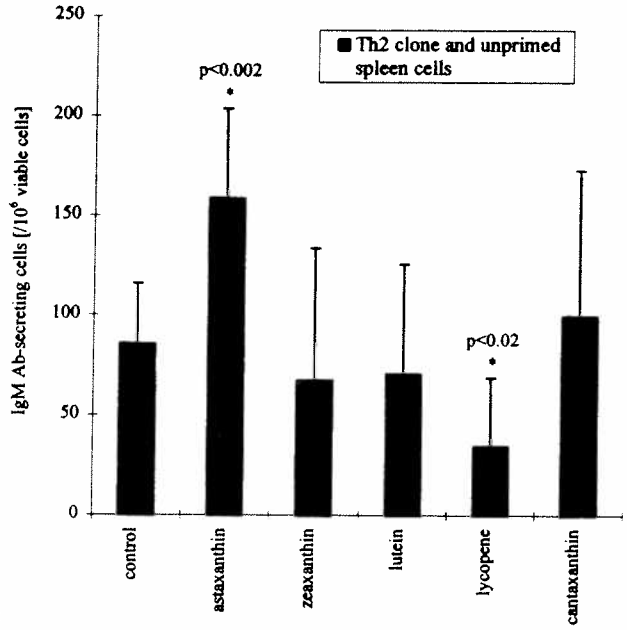

Figure 2. Effects of various carotenoids on number of IgM Ab-secreting cells formed in cultures of Th2 cells and unprimed CD4⁻ DBA/2 spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

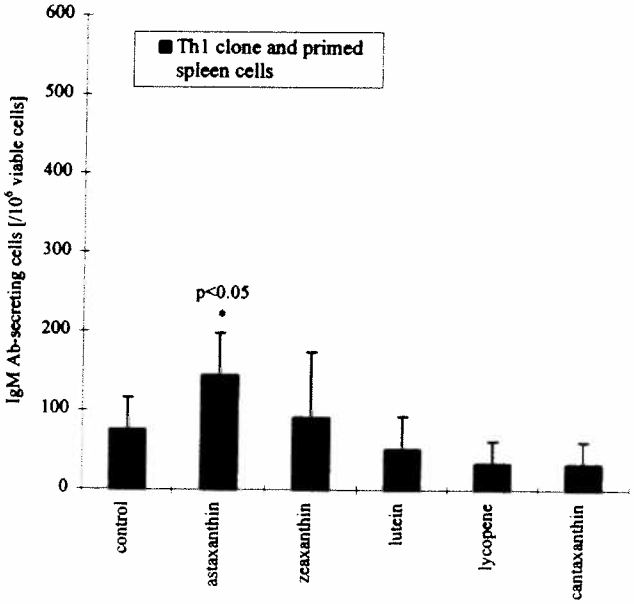

Figure 3. Effects of various carotenoids on number of IgM Ab-secreting cells formed in cultures of Th1 cells and primed CD4⁻ B10.A spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

Ab-secreting cells was higher with astaxanthin for both Th clones, which is consistent with our previous results (9,10) (Figures 5 and 6). The number of IgM and IgG Ab-secreting cells tended to be slightly higher with zeaxanthin in the Th2 clone-mediated Ab production (IgM Ab-secreting cells, $p = 0.25$; IgG Ab-secreting cells, $p = 0.32$ vs. control). The number of IgG Ab-secreting cells tended to be lower in the lycopene-supplemented culture with the use of the Th2 cells ($p = 0.15$ vs. control).

Effects of Various Carotenoids on Ag-Potentiated Cytokine Production by Th Clones

Th1 and Th2 clones in the carotenoid-supplemented cultures were stimulated with Ag specific for each clone, and cytokine levels were measured for each set of cultures.

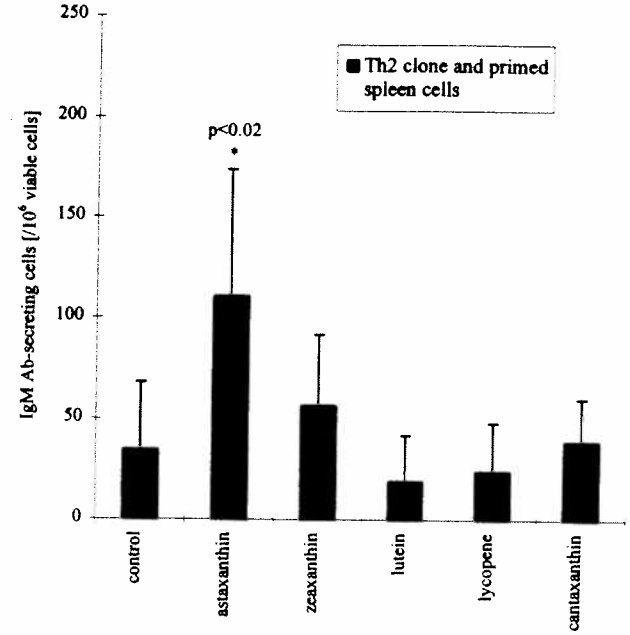

Figure 4. Effects of various carotenoids on number of IgM Ab-secreting cells formed in cultures of Th2 cells and primed CD4⁻ DBA/2 spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

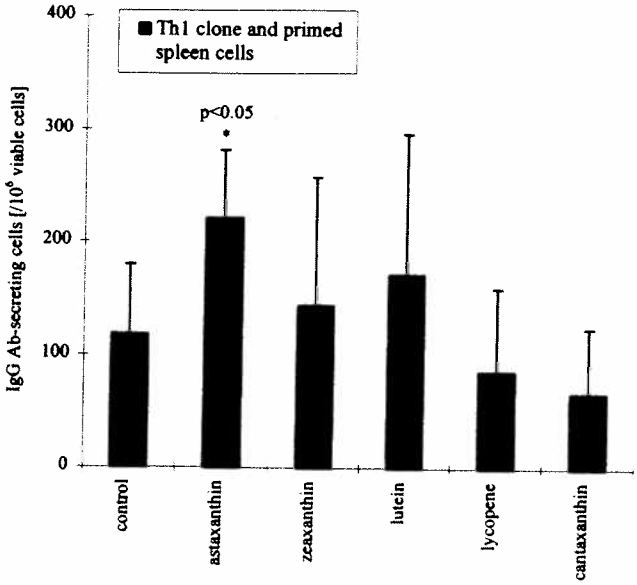

Figure 5. Effects of various carotenoids on number of immunoglobulin G (IgG) Ab-secreting cells formed in cultures of Th1 cells and primed CD4⁻ B10.A spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

Cytokine production with the use of unprimed syngeneic spleen cells: IFN-γ produced by the Th1 clone was not altered significantly with carotenoid supplementation (data not shown), nor did IL-5 levels change in the carotenoid-supplemented cultures (Figure 7). However, lycopene tended to decrease IL-5 levels ($p = 0.11$ vs. controls).

Cytokine production with the use of primed syngeneic spleen cells: IFN-γ levels produced by the Th1 clone in the presence of primed spleen cells were significantly lower with supplemental astaxanthin (Figure 8). Other carotenoids did not significantly alter the levels of IFN-γ produced. IL-5 levels produced by the Th2 clone were not altered by supplementation of the culture with any of the carotenoids (data not shown).

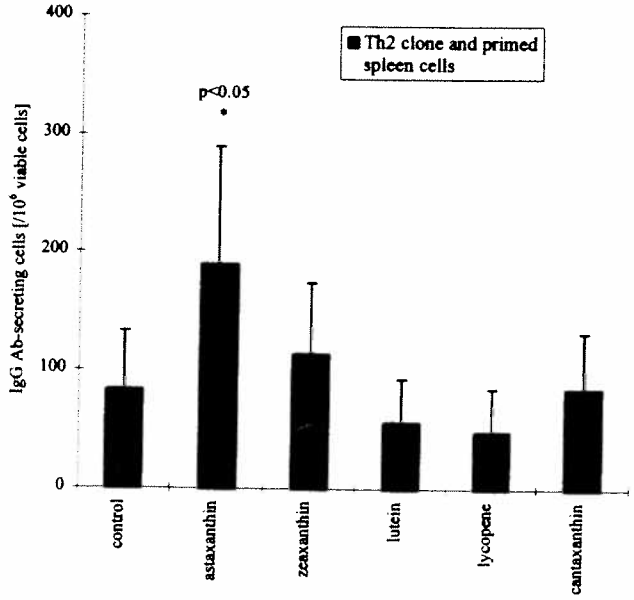

Figure 6. Effects of various carotenoids on number of IgG Ab-secreting cells formed in cultures of Th2 cells and primed CD4⁻ DBA/2 spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

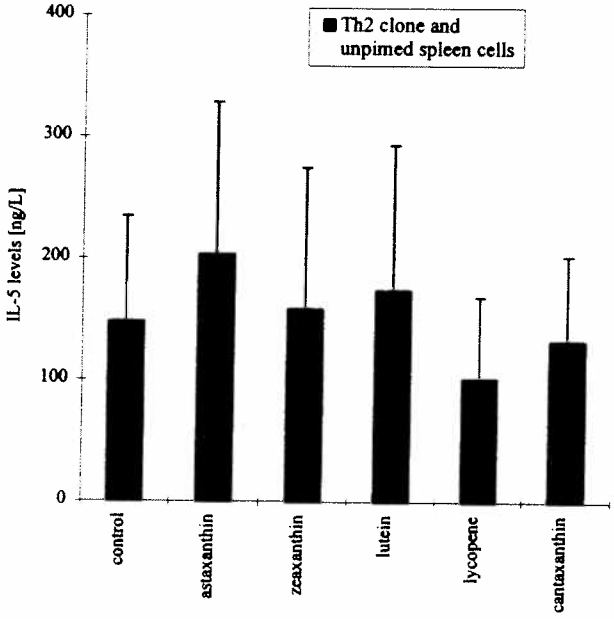

Figure 7. Interleukin-5 (IL-5) levels in supernatant of cultures of Th2 cells and unprimed CD4⁻ DBA/2 spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

Discussion

Carotenoids are colored pigments widely distributed in plants, animals, fish, and microorganisms. They appear to exert a wide variety of actions in plants and animals. In humans, interest focuses on their activities in the prevention of degenerative diseases; the intakes of dietary carotenoids are positively correlated with chemopreventive effects of diets rich in fruits and vegetables (2,4,6). However, recent intervention studies with supplemental β-carotene did not show a beneficial effect in cancer prevention (23,24).

More than 600 naturally present carotenoids have been isolated and characterized; they differ widely in their physical and chemical properties (12). In some instances, various carotenoids may have specific biologic effects. For example, the carotenoid protective action

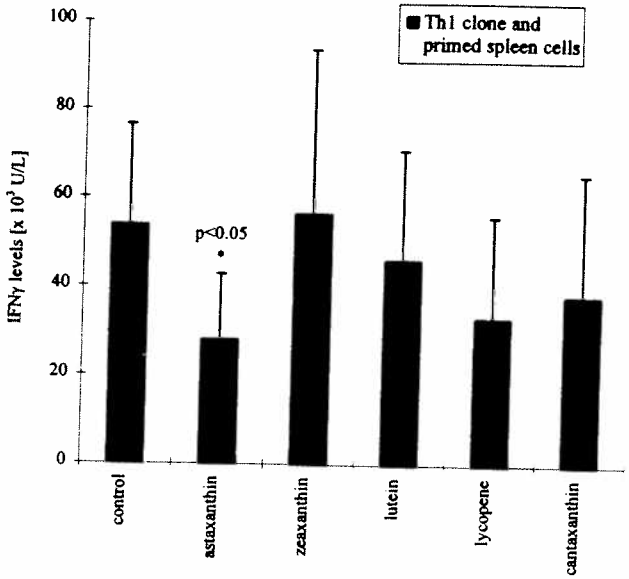

Figure 8. Interferon-γ (IFN-γ) levels in supernatant of cultures of Th1 cells and primed CD4⁻ B10.A spleen cells. Carotenoids (10^{-8} mol/l) were added to culture at Day 0.

against macular degeneration is due to the light-absorption properties of zeaxanthin and lutein (25). In other cases, the mechanisms of carotenoid action, such as in chemopreventive action of cancer and other degenerative disease, are still poorly understood, partly because of the lack of good biomarkers. The presence of a large number of carotenoids makes the analysis of carotenoid action difficult, and a good experimental system is needed.

In our previous studies, we focused on carotenoid action in the immune system and showed that carotenoids without provitamin A activity can modulate T-dependent humoral immunity (13–15). The immune system is an intricate regulatory network providing a powerful means of protection against pathogens, tumorigenesis, and autoimmunity. On the basis of our results and those of others (6,9,10,13–15), we reasoned that a likely mode of carotenoid action in the prevention of degenerative diseases is its effect on the immune system. A key regulator of the immune network is Th cells, which are mainly activated by Ag presented by APC (26–28). The processes of Th cell activation through APC, including dendritic cells, activated B cells, activated macrophages, and Langerhans cells (29), are considered to be the key event in the immune network and have been a focus of intense research. Moreover, Ag-mediated T cell responses are shown to be affected by multiple immunomodulating nutrients; they may affect Th cell functions directly or indirectly through APC (10,30–32).

Because the frequency of Ag-specific T cells in unprimed animals is <10,000 in purified T cells (33), the measurement of changes of Th cell activities is extremely difficult with the use of conventional methodology. Therefore, Ag-mediated Th cell responses have been analyzed using Ag-specific cloned Th cells or purified Th cells from Ag-primed mice (17,26–28). Thus the Th cell clones have been essential for analysis of mechanisms of Th cell activation and the following signal transduction. In the previous studies, we developed a well-defined experimental system in which we can assess Ag-potentiated Th cell activities in a quantitative manner employing Th cell clones (10).

Upon Ag stimulation, naive Th cells differentiate into subsets of effector stage Th cells, which are classified into at least three subsets, Th0, Th1, and Th2 cells, on the basis of cytokine production pattern (26–28). Uncommitted Th0 cells produce a wide variety of cytokines, whereas Th1 and Th2 cells have a more skewed cytokine production pattern but produce them in large quantities (26–28). Th1 cells produce IL-2 and IFN-γ, whereas Th2 cells produce IL-4, IL-5, and IL-13 (28). We constructed the experimental system replacing

Th cells with cloned Ag-specific Th cells to test carotenoid actions on Th cell activities (10). Using this experimental system, we have shown that astaxanthin, a carotenoid without provitamin A activity and relatively high polarity, can enhance Th clone-mediated Ab production with suboptimal Ag challenges (10) and also modulate IFN-γ production by the Th1 clone (unpublished observations). This astaxanthin action was demonstrated at 10^{-8} mol/l, a physiologically feasible tissue concentration in lymphoid organs (12). The actions of β-carotene at the same concentration are either less than the actions of astaxanthin or are minimal compared with astaxanthin; it is unlikely that this enhancing action of carotenoids is associated with provitamin A activity. In this study we further addressed the question of whether other carotenoids with various degrees of relative polarity and antioxidant activity affect the activities of cloned Th1 and Th2 cells. The clone-mediated Ab production and cytokine production, the two major functions of Th cells, were used as markers for Th cell activity in the culture.

Our results have shown that astaxanthin has significant enhancing actions on Th clone-mediated Ab production compared with other carotenoids tested. Zeaxanthin showed a less but demonstrable enhancing effect on Ab production. Cantaxanthin and lutein, whose antioxidant activity against lipid peroxidation was as potent as that of astaxanthin (12,34,35), showed no effect on activities of the Th clones in our experimental system. Lycopene significantly suppressed Th2 clone-mediated Ab production with unprimed spleen cells. With primed spleen cells, lycopene tended to suppress Th2 clone-mediated Ab production, but this was not statistically significant. These results did not suggest a close association between carotenoid action on Th cell activities (demonstrated in our experimental system) and antioxidant activity and/or gap-junctional enhancement by carotenoids. The carotenoid concentrations in mammalian tissues are generally much lower than those used in the cell-free model system for carotenoid antioxidant activity and may be close to the concentrations employed in our experimental system in the culture (12). Relative polarity of carotenoids is also unlikely to be associated with carotenoid actions on cloned Th cells.

In vivo, carotenoids are commonly located in membranes, and orientation of carotenoid within the bipolar membranes depends on the structure of the individual carotenoids (12). For example, zeaxanthin, with two polar end groups, may increase membrane rigidity and mechanical strength (12). This may also be true for astaxanthin. In fact, our results showed a more potent modulating action in astaxanthin than in zeaxanthin. Changes in membrane physical features at the site of Ag presentation may have a significant influence, because Ag presentation and subsequent Th cell activation heavily rely on cell-cell interactions through cell surface molecules (36,37). Once such changes take place, they may be related to changes in cell surface molecule expression associated with Th cell activation (expression of costimulatory molecules) or, alternatively, in transmembrane signal transduction processes. It may be interesting to examine carotenoid effects on cell surface molecule expression in Ag-mediated Th cell activation processes. Another possibility we have to consider is that degradation of astaxanthin may have major effects, given the facts that astaxanthin's effect appears exceptionally potent compared with the effects of other carotenoids. However, we compared carotenoid actions in our experimental system at one selected concentration at which astaxanthin actions are most potent. This is due to the relatively complex labor-intensive assay system. It is necessary to examine effects of carotenoids such as zeaxanthin and lycopene at various concentrations, because these carotenoids demonstrated detectable actions on the Th clones.

In summary, our results indicate no close relationship between carotenoid-modulating action on Th cell functions and other known carotenoid properties (provitamin A activity, antioxidant activity, and gap-junctional enhancement). It seems necessary to carefully examine each carotenoid action in a well-defined experimental system in association with its molecular structures and biochemical properties.

Acknowledgments and Notes

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