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ARTICLE

Dietary Carotenoids Inhibit Oral Sensitization and the Development of Food Allergy

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Type-I allergic disorders and particularly food hypersensitivities are becoming increasingly common worldwide. This study investigated whether dietary enrichment with carotenoids inhibited oral sensitization to an antigen and the development of food allergies. The effects of a diet high in carotenoids were investigated in B10A mice that were orally sensitized to ovalbumin (OVA). The serum titers of OVA-specific immunoglobulin E (IgE), IgG1, and IgG2a were inhibited in mice fed *ad libitum* on a diet high in α- or β-carotene compared to the control mice when orally sensitized to OVA. High α- and β-carotene diets inhibited the immediate reduction in body temperature and rise in serum histamine associated with active systemic anaphylaxis in OVA-sensitized B10A mice. After re-stimulation with OVA *in vitro*, the production of T-helper 2-type cytokines by splenocytes from mice fed a diet high in carotenoids was lower than in control mice. Furthermore, the proportion of $CD4^+$ $CD103^+$ T cells in Peyer's patches of mice fed a carotenoid-rich diet was significantly lower than in control mice. These results suggest that an increased oral intake of carotenoids inhibits OVA-specific IgE and IgG1 production and antigen-induced anaphylactic responses by inhibiting specific T-cell activation in the mucosal immune system. A diet high in carotenoids might therefore prevent the development of food allergies.

KEYWORDS: β-Carotene; food allergy; IgE; oral sensitization; prevention

INTRODUCTION

It is estimated that up to 8% of children and 2% of adults in industrialized countries are affected by food allergies (*1*−*3*). The clinical manifestations of food allergies vary from mild symptoms, such as oral allergy syndrome or mild urticaria, to severe anaphylactic reactions that can have fatal consequences. Young children frequently have food allergies because of naïve immune systems or developing gastrointestinal epithelium and gut-associated lymphoid tissue (GALT), which may make them susceptible to oral sensitization.

The gastrointestinal tract mucosal immune system includes GALT that discriminates among harmless foreign proteins, commensal organisms, and dangerous pathogens (*4*, *5*). In humans, although intact foreign food antigens routinely penetrate the gastrointestinal tract, they rarely induce clinical symptoms because of oral tolerance. Food allergies can develop in genetically predisposed individuals, presumably when oral tolerance either fails to develop normally or breaks down. Once these allergies have developed, specific associated diseases can occur in different age groups, including eczema, gastrointestinal diseases, and asthma and other upper respiratory diseases. Food allergies mark the beginning of a progression known as the "allergy march" (*6*, *7*). Early inhibition of the development of food allergies might be the key to managing the allergy march; hence, there is a need for a treatment plan that could prevent the development of food allergies.

Carotenoid pigments provide the yellow, orange, and red coloration seen in fruit and vegetables, with β- and α-carotene being the most abundant in human foods. Several epidemiological studies have shown that carotenoid intake is associated with the prevention of certain degenerative diseases (*8*−*15*). These reports suggest that carotenoids have the potential to modulate beneficially or regulate host immune responses. In addition, certain antioxidants, including vitamin C and β-carotene, might have anti-inflammatory protective effects, and the reduced consumption of fresh fruit and vegetables in the Western diet has been suggested as a potential reason for the rise of allergies in the U.K. (*16*). Thus far, there has been no adequate biological explanation as to how this could affect IgE sensitization to food.

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We previously showed that B10A mice were efficiently sensitized to an antigen administered by oral gavage (*17*−*20*). These mice are therefore good models for studying the effects of food and drugs on the development of oral sensitization to food allergens. Here, we report the effects of carotenoids on oral sensitization to food allergens and on intestinal lymphocytes using flow cytometry.

MATERIALS AND METHODS

Animals and Reagents. Female, 6-week-old B10A mice were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan). They were kept in our animal facility and fed a commercial Mouse Flat (MF) diet (Japan SLC) for 1 week prior to the study. They were maintained in a temperature- and light-controlled environment and were allowed free access to water throughout the study period. MF diets containing α- and β-carotene were prepared by mixing the standard MF diet with 20 mg/kg of the carotenoids, as reported in many previous experiments (*8*−*15*).

The control mice, which were fed the standard MF diet ($n = 5$), the α-carotene-enhanced mice (α-carotene group, $n = 5$), and the β-carotene-enhanced mice (β-carotene group, $n = 5$) had free access to the relevant diet for 11 weeks. After 2 weeks, all three groups underwent oral sensitization to ovalbumin (OVA) over the remaining 9 weeks. The null group ($n = 5$) had free access to water and the standard MF diet but did not undergo oral sensitization. The care and use of the experimental animals in this study followed "The Ethical Guidelines of Animal Care, Handling and Termination" published by the National Institute of Health Sciences.

Oral Immunization and Induction of Active Systemic Anaphylaxis (ASA). Mice were orally sensitized by the administration of 1.0 mg of OVA by daily gavage for 9 weeks (*15*, *18*, *21*). ASA was elicited by an intraperitoneal injection of 1 mg of OVA 1 day later. The changes in body temperature associated with ASA were monitored using a mouse rectal thermometer (Shibaura Electronics Co., Ltd., Saitama, Japan) without general anesthesia. At 10 min after the antigen challenge, mice were sacrificed and blood and liver tissue were collected. Serum prepared from the blood was used to measure histamine levels by means of the postcolumn high-performance liquid chromatography (HPLC) method (*15*, *20*, *21*).

Serum Anti-OVA IgE, IgG1, and IgG2a and Fecal Anti-OVA IgA Titers. Serum titers of anti-OVA IgE, IgG1, and IgG2a antibodies were determined experimentally, in triplicate, using 96-well microtiter plates, as previously reported (*15*, *20*). Fecal samples were used to determine OVA-specific IgA titers (*17*).

Cytokine Production by Splenocytes *in Vitro*. Splenocytes were collected from OVA-immunized mice ($n = 5$), and 5×10^6 cells/mL were re-stimulated with OVA *in vitro* at a final concentration of 100 μg/mL in 24-well culture plates at 37 °C for 3 days (*15*, *20*). The cytokine levels in the RPMI 1640 culture medium after 3 days of co-culture with OVA were measured with an OptEIA mouse cytokine enzyme-linked immunosorbent assay (ELISA) kit (BD Pharmingen, San Jose, CA). The absorbance was measured at 450 nm with a microplate reader (E-max, Molecular Devices, Sunnyvale, CA).

Isolation of Peyer's Patches (PP) and Mesenteric Lymph Nodes (MLN). PP and MLN were dissected from the small intestines of mice that were orally sensitized to OVA. Single-cell suspensions were prepared by passing the PP and MLN through a nylon mesh cell strainer (Becton Dickinson, Franklin Lakes, NJ), centrifuging, and then washing 3 times in RPMI 1640 containing 10% fetal bovine serum (*21*).

Determination of α- and β-Carotene Levels in Animal Diets and Livers. The α- and β-carotene levels in the animal diets and mouse livers were assayed by HPLC, as previously described (*15*).

Determination of Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), GATA-Binding Protein 3 (GATA3), and T-Helper (Th)1-Specific T-Box Transcription Factor (T-bet) Messenger Ribonucleic Acid (mRNA) Levels. Total RNA was extracted from purified splenic $CD4^+$ T cells using TRIzol (Life Technologies, Basel, Switzerland) according to the protocol of the manufacturer. Reverse transcription was performed using a high-capacity complementary deoxyribonucleic acid (cDNA) reverse-transcription kit (Applied Biosystems, Foster City, CA) according to the protocol of the manufacturer. Reverse-transcribed cDNA was analyzed immediately or stored at −20 °C until use. Real-time TaqMan Applied Biosystems polymerase chain reactions (PCRs) for mouse GAPDH, GATA3, and T-bet were run in separate wells. The specific primers and probes were as follows: GAPDH forward, 5′-CCAGCCTCGTCCCGTAGA-3′; GAPDH reverse, 5′-ACTGCAAATGGCAGCCCT-3′; GAPDH probe, FAM-AGGTCGGTGTGAACGGATTTGGCC-BHQ-1; GATA3 forward, 5′-AGGGCTACGGTGCAGAGGT-3′; GATA3 reverse, 5′-GCAGAGATCCGTGCAGCA-3′; GATA3 probe, FAM-ACCCACCACGGGAGCCAGGTATG-BHQ-1; T-bet forward, 5′-GCACCAGACAGAGATGATCATCA-3′; T-bet reverse, 5′-TAATGGCTTGTGGGCTCCA-3′; and T-bet probe, FAM-AGCAAGGACGGCGAATGTTCCCA-BHQ-1. The PCRs contained 300 nM of each primer, 250 nM TaqMan probe, 12.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems), and 5 μL diluted cDNA sample, in a final volume of 25 μL. Samples were amplified in 96-well plates using the ABI Prism 7900 sequence-detection system (Applied Biosystems) and the following conditions: 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 60 s at 60 °C.

$CD4^+$ and $CD11c^+$ Cell Isolation. Splenocytes, PP, and MLN were removed, teased into single-cell suspensions, and filtered through a 40 μm cell strainer (BD Pharmingen). $CD4^+$ and $CD11c^+$ cells were isolated using an EasyStep Positive Selection mouse allophycocyanin (APC) selection kit (Stem Cell Technologies, Inc., Vancouver, British Columbia, Canada) using APC-conjugated anti-mouse CD4 (L3T4) and APC-conjugated anti-mouse CD11c (integrin αx chain or HL3) antibodies (BD Pharmingen) according to the instructions of the manufacturer.

Flow Cytometry. PerCP-conjugated anti-mouse CD8α antibody (Ly-2, 53−6.7), phycoerythrin (PE)-conjugated anti-mouse CD25 antibody (IL-2 receptor α chain, p55, PC61), fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD122 antibody (IL-2 receptor β chain), propidium iodide (PI) solution (all purchased from BD Pharmingen), and PE-conjugated anti-mouse CD103 antibody (2E7, eBioscience, San Diego, CA) were used in three-color analyses of splenocyte subsets, as previously reported (*16*, *20*). $CD4^+$ and $CD11c^+$ cells isolated from PP and MLN were stained with PE-conjugated anti-CD103 (2E7) antibody.

To analyze intercellular forkhead box P3 (Foxp3) protein, isolated $CD4^+$ cells were stained using a FITC anti-mouse/rat Foxp3 staining kit (eBioscience) according to the protocol of the manufacturer. Cells were analyzed using a FACS Caliber flow cytometer (BD Pharmingen) with CellQuest software (BD Pharmingen) and by means of PI fluorescence to exclude dead cells. Data analysis was performed using FlowJo software (TreeStare, Inc., Ashland, OR).

Statistical Analysis. All values are expressed as the mean ± standard error (SE). A p value < 0.05 was considered statistically significant. Differences between values were tested using Student's t test or the Scheffe method after analysis of variance (ANOVA).

RESULTS

Body Weight and Diet. No significant differences were observed in body weight and overall food intake between the control and carotenoid-enhanced mice. No specific symptoms were observed in the carotenoid-enhanced groups during the study. To examine the absorption of α- and β-carotene from the diet, we determined their levels in the liver. Both α- and β-carotene were undetectable in the livers of control mice (detection limit = 15 ng/g), even though the standard MF diet contained a trace amount of β-carotene (approximately, 300 μg/kg), whereas they were present at significantly higher levels in the livers from the α-carotene (42.4 ± 7.1 ng/g) and β-carotene (97.0 ± 63.4 ng/g) groups.

OVA-Specific IgE, IgG1, and IgG2a Antibody Titers in Serum and IgA Antibody Titer in Feces. To investigate whether a diet high in carotenoids affected the development of oral sensitization in B10A mice, OVA-specific IgE, IgG1, and IgG2a antibody titers in serum from mice orally sensitized with OVA were determined using indirect ELISAs (**Figure 1**). The OVA-specific IgE, IgG1, and IgG2a titers were significantly lower in the carotenoid-enhanced groups than in the control mice, suggesting that the increased carotenoid intake inhibited OVA oral sensitization in

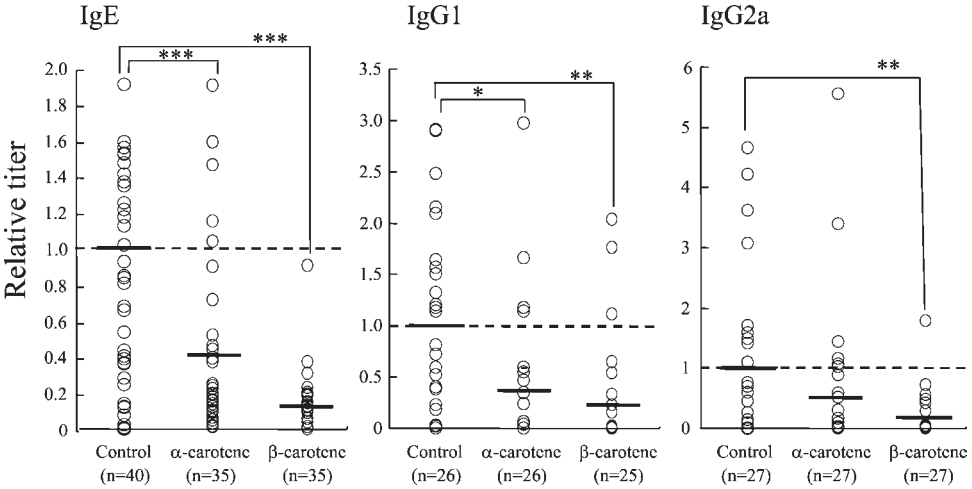

Figure 1. Effects of dietary carotenoids on serum OVA-specific (A) IgE, (B) IgG1, and (C) IgG2a antibody titers in B10A mice orally sensitized to OVA. Circles denote individual animals. Horizontal bars indicate median values [(∗) $p < 0.05$, (∗∗) $p < 0.01$, and (∗∗∗) $p < 0.001$]. Data were combined from six to eight separate experiments.

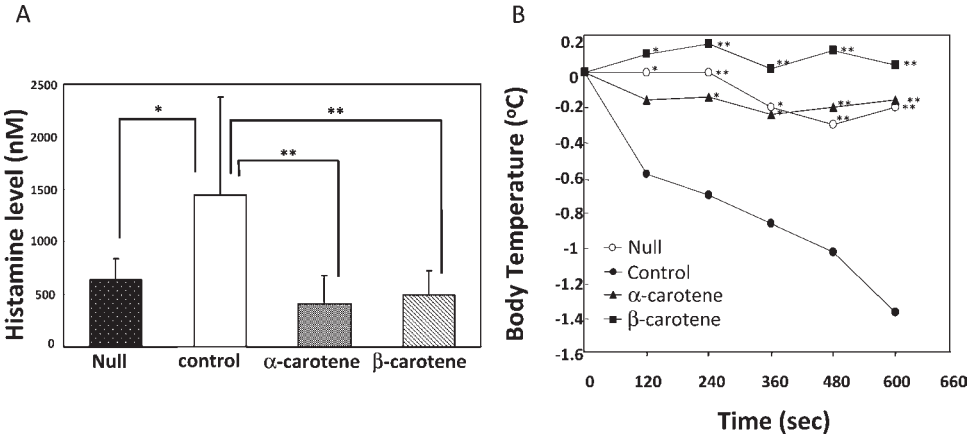

Figure 2. (A) Serum histamine levels and (B) body temperature of B10A mice after inducing ASA. Asterisks indicate significant differences from control values [(∗) $p < 0.05$ and (∗∗) $p < 0.01$]. Bars represent means ± SEs ($n = 5$).

B10A mice. Furthermore, the OVA-specific IgA antibody titers were significantly lower in the feces from the α-carotene (0.13 ± 0.04) and β-carotene (0.43 ± 0.11) groups than in the control mice (1.00 ± 0.24).

Body Temperature and Serum Histamine Levels after Antigen Stimulation. The effects of carotenoids on the OVA-induced hypersensitivity reaction in sensitized B10A mice were assessed by measuring their body temperature once every minute for 10 min after intraperitoneal challenge with 1 mg of OVA. Intense hypothermia developed in only the control group but not in the null group and the carotenoid-enhanced groups (**Figure 2**). A marked increase in serum histamine levels was also seen in the control group mice challenged with OVA, whereas the levels in the null group and the carotenoid-enhanced mice were significantly lower (**Figure 2**).

Cytokine Production by Splenocytes *in Vitro*. To clarify the mechanisms involved in the inhibition of OVA-specific IgE production in mice, we investigated cytokine production by splenocytes from mice stimulated with OVA *in vitro*. Levels of interferon (IFN)-γ, interleukin (IL)-2, and IL-12 produced by splenocytes from the carotenoid-enhanced groups appeared higher than those from the control mice (**Figure 3**). In contrast, the levels of Th2-type cytokines (IL-5, IL-6, IL-10, and IL-13) produced by splenocytes from the carotenoid-enhanced groups were significantly lower than those from the control mice, although not significantly different in IL-10 for the β-carotene group and IL-13 for both groups (**Figure 3**).

Phenotypic Analysis of Splenocytes. We investigated the effect of an enhanced carotenoid intake on the differentiation of splenocytes in OVA-sensitized mice by analyzing their phenotypes using flow cytometry. The percentages of splenocyte $CD4^+$ and $CD8^+$ T cells were both significantly higher in the carotenoid-enhanced groups than in the control mice (**Figure 4**). However, the percentages of $CD4^+$ $CD25^+$ (2.6 ± 0.2, α-carotene group; 2.5 ± 0.1, β-carotene group; 2.7 ± 0.3, control group) and $CD8^+$ $CD122^+$ (1.6 ± 0.2, α-carotene group; 1.6 ± 0.2, β-carotene group; 1.5 ± 0.2, control group) regulatory T cells showed no significant differences.

Determination of mRNAs of Th1 and Th2 Master Regulators. The mRNA levels of the Th1 master regulator T-bet and the Th2 master regulator GATA3 were determined in purified splenic $CD4^+$ T cells using the real-time PCR. The expression levels of GATA3 (43.3 ± 18.8, α-carotene group; 33.0 ± 10.2, β-carotene group) and T-bet (5.6 ± 0.8, α-carotene group; 4.9 ± 1.8, β-carotene group) were lower in both carotenoid-enhanced groups than in the control mice (50.3 ± 19.1, GATA3; 7.3 ± 2.8, T-bet) but not significantly. These results suggest that an enhanced carotenoid intake might reduce the differentiation of Th1 and Th2 cells in mice orally sensitized to OVA.

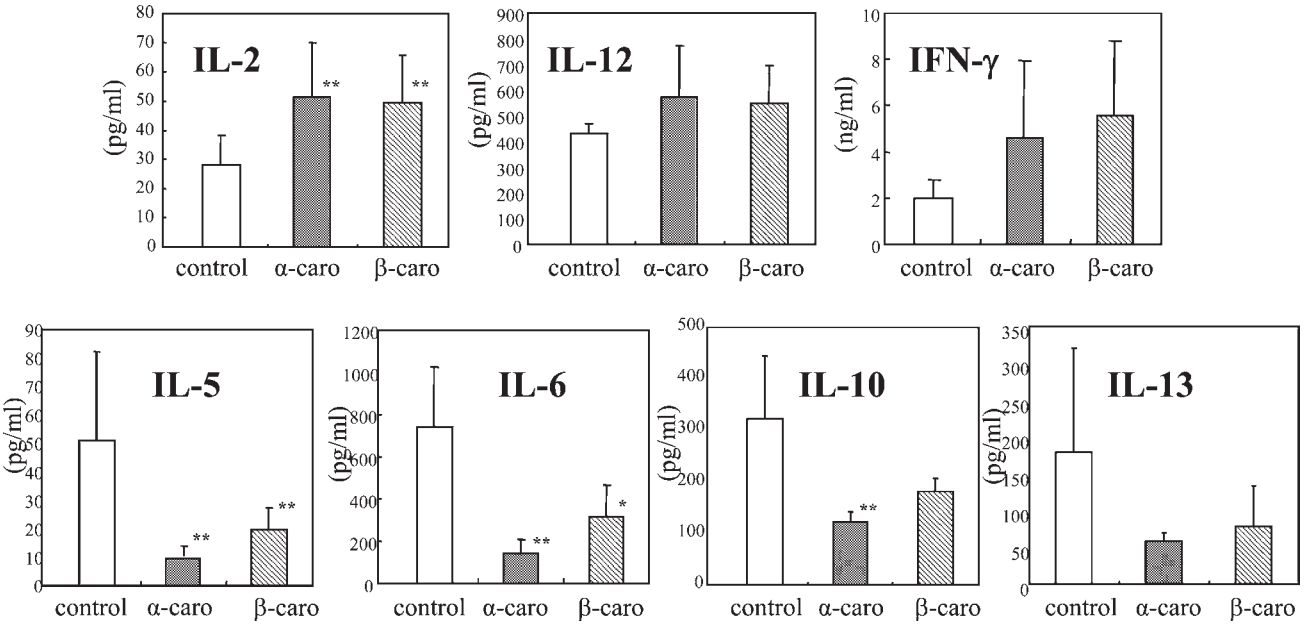

Figure 3. Effects of dietary α-carotene (α-caro) and β-carotene (β-caro) on cytokine production by splenocytes *in vitro*. Splenocytes were collected, and 5.0×10^6 cells/mL were co-cultured with 100 μg/mL OVA. Cytokines were measured in the supernatants using ELISAs. Asterisks indicate significant differences from control values [(**) $p < 0.01$ and (*) $p < 0.05$]. Bars represent means ± SEs ($n = 5$).

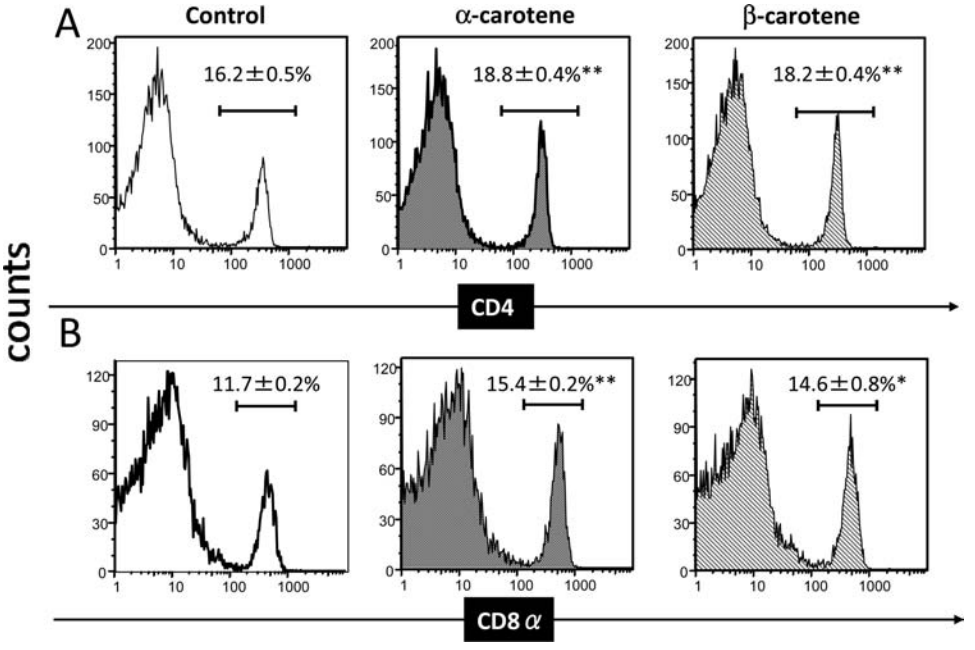

Figure 4. $CD4^+$ and $CD8^+$ T-cell populations in splenocytes from mice fed different diets. (A) Surface staining of splenocytes for CD4. (B) Surface staining of splenocytes for CD8α. All data shown are means ± SEs from at least six independent experiments.

Phenotypic Analysis of PP and MLN Cells. To examine the inhibition of specific IgE production in mice orally sensitized to OVA while consuming a high carotenoid diet, PP and MLN cells were isolated from the small intestine and their lymphocytes were analyzed by flow cytometry. PP cells from orally sensitized mice contained a higher proportion of $CD4^+$ $CD103^+$ $Foxp3^-$ T cells than those from the null, unsensitized mice (**Figure 5A**). In contrast, the PP cells from carotenoid-enhanced groups had a significantly lower proportion of $CD4^+$ $CD103^+$ $Foxp3^-$ cells than those from the control mice (**Figure 5A**). However, the proportions of $CD4^+$ $CD103^+$ $Foxp3^+$ T cells from carotenoid-enhanced groups were not significantly different from those of the control mice. This suggests that a high carotenoid intake might inhibit the differentiation of $CD4^+$ $CD103^+$ $Foxp3^-$ T cells induced in PP cells by oral sensitization and have no effect on the differentiation of $CD4^+$ $CD103^+$ $Foxp3^+$ T cells.

The MLN cells from the control group contained similar proportions of $CD4^+$ $CD103^+$ $Foxp3^-$ and $CD4^+$ $CD103^+$ $Foxp3^+$ T cells to the carotenoid-enhanced groups (**Figure 5B**). In addition, the proportions of $CD11c^+$ $CD103^+$ dendritic cells (DCs) in the carotenoid-enhanced mice and control mice were not significantly different, although the population of $CD11c^+$ DCs in the control group was higher than in the null group (**Figure 6**).

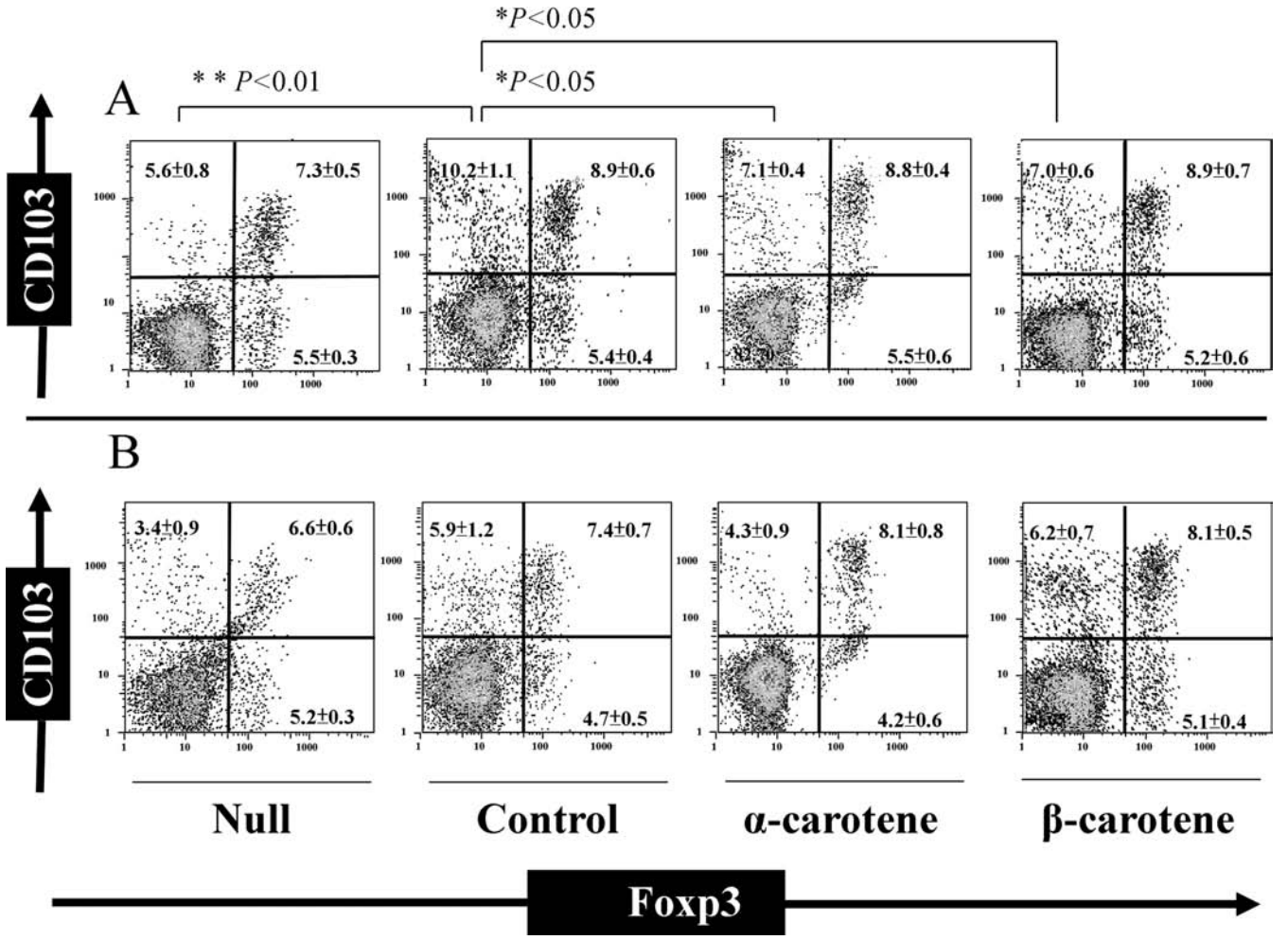

Figure 5. Phenotypic characterization of $CD4^+$ T cells isolated from (A) PP and (B) MLN cells. Isolated $CD4^+$ T cells were stained for intracellular Foxp3 and surface CD103. Numbers represent percentages of events in each quadrant. Data are means ± SEs from at least six independent experiments.

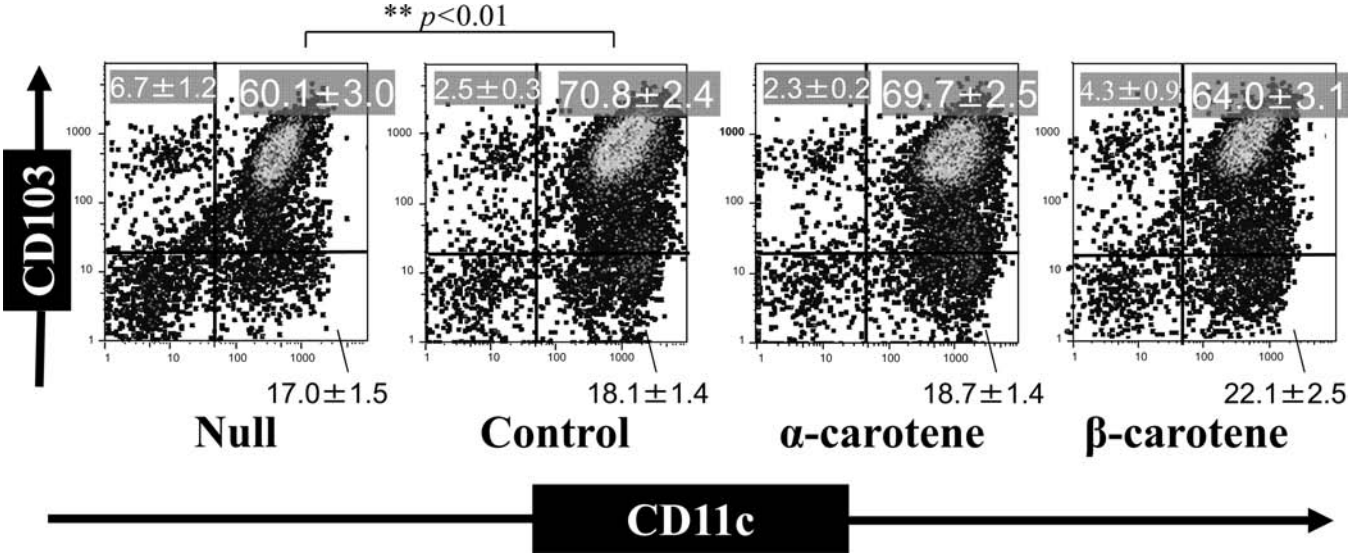

Figure 6. Percentages of $CD11c^+$ $CD103^+$ DCs in MLN cells. Numbers represent percentages of events in each double-positive quadrant. Data are means ± SEs from at least three independent experiments.

DISCUSSION

This study showed that feeding diets high in carotenoids to B10A mice inhibited OVA-specific IgE and IgG1 production in response to oral sensitization *in vivo*. The carotenoid-rich diets also significantly inhibited anaphylaxis induced by antigen stimulation in OVA-sensitized B10A mice. We also showed that an enhanced carotenoid intake might reduce the differentiation of Th1 and Th2 cells in mice orally sensitized to OVA. These results

suggest that an enhanced carotenoid intake could prevent the development of food allergy in this model and might be involved in the inhibition of oral sensitization.

Dietary α- and β-carotene are converted to vitamin A (retinol) *in vivo* (*22*) and provide a potential source of retinol for infants in breast milk. They might also confer long-term protection against chronic disease and contribute to the immunoprotective effects of human breast milk (*23*, *24*). Retinol is required for antibody responses to bacterial polysaccharide antigens, the prevention of activation-induced T-cell apoptosis, and normal phagocytic functions (*25*). Retinoids enhance the numbers and effector functions of various immune cell types and are reportedly required for the B-cell Ig switch to IgA (*26*). The GALT DCs can produce retinoic acid (RA) from retinol and upregulate the gut-homing receptors CCR9 and α4β7 on B and T cells (*27*). In contrast, retinol and retinoids suppress inflammatory responses and tissue damage. Supplementation with retinol has been reported to suppress inflammation in experimental autoimmune encephalomyelitis (*28*). Moreover, retinol supplementation in animals has been shown to reduce serum concentrations of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IL-1, but to increase levels of suppressive cytokines, such as IL-10 (*29*).

Recently, Mucida et al. identified RA as a key regulator of transforming growth factor-β (TGF-β)-dependent immune responses, which was capable of inhibiting the IL-6-driven induction of naive T cells into pro-inflammatory IL-17 cytokine-producing Th cells (Th17 cells) and promoting the differentiation of anti-inflammatory regulatory T cells (Treg cells) (*30*). Benson et al. suggested that $CD103^+$ gut-associated DCs expressed relatively high levels of retinal dehydrogenase and that RA enhanced the induction and upregulation of α4β7 integrin and CCR9, permitting newly formed Treg cells to accumulate preferentially in GALT (*31*). Furthermore, RA can reduce the negative impact of co-stimulation on the TGF-β-dependent conversion of T cells into Foxp3 Treg cells. Finally, Coombes et al. and Sun et al. showed that RA conversion takes place more effectively in GALT than in other lymphoid tissues (*32*, *33*).

The previous reports showed that RA regulates the balance between pro-inflammatory and anti-inflammatory immune responses. However, the studies were performed either *in vitro* or *in vivo*, with cells induced *in vitro* transferred into animal models. Hence, there is a need to examine whether the oral uptake of retinol or RA can enhance Treg cell induction in PP and MLN *in vivo*. However, such experiments would be difficult to perform because of the side effects associated with excess dietary retinol and RA. We therefore investigated whether an enhanced carotenoid intake induced the differentiation of Foxp3 Treg cells, because no adverse affects have been associated with dietary carotenoids. We found that enhanced carotenoid intake did not increase the number of Foxp3 Treg cells or $CD103^+$ DCs in PP and MLN, although it did inhibit OVA-specific IgE and IgG1 production in response to oral sensitization and the anaphylaxis induced by antigen stimulation. Although oral sensitization appeared to increase the population of $CD103^+$ $CD4^+$ T cells, suggesting that the proportion of $CD4^+$ $CD103^+$ T cells might be a marker for oral sensitization to an antigen in PP, an enhanced carotenoid intake inhibited the increase of the $CD103^+$ $CD4^+$ T-cell population caused by oral sensitization. The integrin CD103 is highly expressed at mucosal sites, although the role of $CD103^+$ T cells in mucosal immune regulation remains poorly understood (*34*). This study suggests that the effects of high dietary carotenoids on intestinal mucosal immune regulation might be related to the function of $CD4^+$ $CD103^+$ T cells at intestinal mucosal sites.

We also showed that feeding carotenoids to mice that were orally sensitized to OVA reduced the OVA-specific IgA titers in the feces. Weiner suggested that the primary mechanisms by which oral tolerance is mediated include deletion, anergy, and active suppression (*35*). In light of this, our results suggest that a high carotenoid diet might inhibit oral sensitization by a mechanism involving anergy or the clonal deletion of sensitized T cells rather than by the active suppression of Foxp3 Treg cells. We postulate that the inhibition of oral sensitization involved the antioxidant and singlet-oxygen quenching properties of both carotenoids rather than their pro-vitamin A activity, because dietary carotenoids inhibited the increase in the $CD4^+$ $CD103^+$ $Foxp3^-$ T-cell population in PP and the CD11ct DCs inMLN induced by oral sensitization (*36*, *37*).

In conclusion, an enhanced α- and β-carotene intake could be used to prevent the development of food allergies and the allergy march. These results open the way for understanding the mechanism of oral tolerance. Further studies will be needed to clarify the mechanism underlying the inhibition of the development of food allergies and the regulation of the intestinal immunological system. We postulate that high levels of dietary carotenoids provided by breast milk or other supplementation during early infancy might prevent the development of food allergies and the subsequent allergy march. In addition, dietary supplementation with carotenoids could help to stimulate a balanced and effective mucosal immune system.

ABBREVIATIONS USED

ANOVA, analysis of variance; APC, allophycocyanin; ASA, active systemic anaphylaxis; cDNA, complementary deoxyribonucleic acid; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; Foxp3, forkhead box P3; GALT, gut-associated lymphoid tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GATA3; GATA-binding protein 3; HPLC, high-performance liquid chromatography; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MF, Mouse Flat; MLN, mesenteric lymph node; mRNA, messenger ribonucleic acid; OVA, ovalbumin; PCR, polymerase chain reaction; PE, phycoerythrin; PI, propidium iodide; PP, Peyer's patch; RA, retinoic acid; SE, standard error; T-bet, T-helper 1-specific T-box transcription factor; TGF-β, transforming growth factor-β; Th, T-helper; TNF-α, tumor necrosis factor-α; Treg cells, regulatory T cells.

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NOTE ADDED AFTER ASAP PUBLICATION

The ASAP publication of May 10, 2010, contained an older version of Figure 5. The latest version is included in the ASAP publication of May 19, 2010.

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