Fucoxanthin is a non-provitamin A carotenoid contained in brown seaweeds. We found that it suppressed interleukin-17 secretion from CD4+ T cells under IL-17-producing T (Th17) cell development conditions. By evaluating T cell differentiation in vitro, fucoxanthin and its metabolite fucoxanthinol inhibited T cell differentiation into Th17 cells. This suggests that fucoxanthin can improve inflammatory diseases due to Th17 cells.

Key words: fucoxanthin; interleukin-17; interleukin-17-producing T (Th17) cell; regulatory T cell

Fucoxanthin is a non-provitamin A carotenoid in brown seaweeds, Undaria pinnatifida. There are many reports on the beneficial effects of fucoxanthin on human health including anti-tumor and anti-obesity effects.1–3) Recently, a protective effect of topical fucoxanthin treatment against skin photodamage was reported.4) As for immunological functions, it has been found that intravenous injection of fucoxanthin protects the eyes from lipopolysaccharide-induced inflammation in rats.5) We hypothesized that non-provitamin A carotenoids, including fucoxanthin, can affect T cell differentiation due to a chemical structure similar to vitamin A, which is metabolized into retinoic acid (RA) and induces forkhead box P3 (Foxp3+) regulatory T (Treg) cells, resulting in inhibition of interleukin 17-producing T (Th17) cell development in vitro and in vivo.6–8)

Foxp3+ Treg cells are induced in the presence of transforming growth factor (TGF)-β and RA in vitro, while Th17 cell development is induced in the presence of IL-6 and TGF-β.6,7) Elevated levels of IL-17 are associated with the pathogenesis of many inflammatory diseases, including autoimmune diseases, inflammatory bowel diseases, allergic asthma, and atopic dermatitis.9–12) Therefore, Th17 cell differentiation or IL-17 secretion is a target of therapeutic drugs and foods to be used against these inflammatory diseases. Clinical effects of synthetic retinoid Am80, an agonist of RA receptors, on autoimmune diseases has been found in mice,13,14) and a commensal bacterium in the intestine of infants, Bifidobacterium infantis, has been reported to suppress IL-17 production ex vivo using a dextran sodium sulfate-treated colon as a model of inflammatory bowel diseases.15) Recently, a functionally distinct subset of integrin α chain CD103-expressing dendritic cells (DCs) was identified in murine mesenteric lymph nodes.15) CD103+ DCs promote the development of Foxp3+ Treg cell responses depending on TGF-β and the dietary metabolite RA.16) There are many reports on the immunological effects of vitamin A and its metabolite, RA, as described above, while no report on the inhibitory effect of non-provitamin A carotenoids against Th17 cell development has appeared. Hence we evaluated the impact of these carotenoids on Th17 cell differentiation in vitro.

To assess the effect of non-provitamin A carotenoids as to the suppression of IL-17 production, CD4+ T cells were collected from spleens of C57BL/6 mice (Japan Crea, Tokyo) using CD4 microbeads (Miltenyi Biotec, Bergish Gladbach, Germany) and MACS (Miltenyi Biotec). Anti-CD3 and anti-CD28 monoclonal antibodies for T cell activation and proliferation were purchased from eBioscience (San Diego, CA). After culturing CD4+ T cells with non-provitamin A carotenoids (fucoxanthin, astaxanthin, lycopene, and lutein, from Wako, Osaka, Japan) in the presence of IL-6 (20 ng/mL, R&D Systems, Minneapolis, MN) and TGF-β (2 ng/mL, R&D Systems) for 3 d, IL-17 production was measured by a Mouse IL-17 ELISA Set (eBioscience). All-trans RA (ATRA, Sigma, St. Louis, MO) was added as positive control of suppression of Th17 cell differentiation. ATRA and carotenoid solutions were prepared at 5 and 10 μM in dimethyl sulfoxide (DMSO). Then these solutions were diluted and added to a culture medium at 2 or 4 μM (final concentration). As control, the same concentration of DMSO (0.04% v/v) was added to the culture medium. This concentration of DMSO did not affect the experimental model as to cytotoxicity or cytokine production. IL-17 secretion was suppressed only by the addition of fucoxanthin in a dose-dependent manner, while the other carotenoids did not suppress IL-17 production by CD4+ T cells (Fig. 1). To analyze the gene expression of retinoic acid receptor-related orphan nuclear receptor (ROR)γt, a transcription factor expressed in Th17 cells,17) and Foxp3, quantitative RT-PCR was performed using PrimeScript RT reagent (Takara, Ohtsu, Japan), SYBR Premix Ex Taq (Takara), and specific primers as follows: 5'-TGTCCTGGGGCTACCCTACTG-3' and 5'-GTGCAAGGATAGCCACATT-3' for RORγt, 5'-
Fucoxanthin Inhibits Th17 Cell Differentiation

CD4⁺ T cells (5 x 10⁵) from the spleens of C57BL/6 mice were cultured with all-trans retinoic acid (ATRA) or carotenoids in the presence of anti-CD3 monoclonal antibody (αCD3, plate-bound, 5 μg/mL), anti-CD28 monoclonal antibody (αCD28, soluble, 5 μg/mL), IL-6 (20 ng/mL), and TGF-β (2 ng/mL) for 3 d. The IL-17 concentration in culture supernatants was determined by ELISA. The data are shown as mean ± SD of triplicate cell cultures. They are representative of three independent experiments. *p < 0.05, **p < 0.01 (Student’s t-test).

Next we evaluated the T cell differentiation of naïve T cells (CD4⁺ CD62L⁺ T cells) into Th17 cells. Naïve T cells were prepared from C57BL/6 mice by negatively isolating CD4⁺ T cells using CD4⁺ T cell Isolation Kit II (Miltenyi Biotec), followed by the collection of CD62L⁺ cells using fluorescein isothiocyanate (FITC) conjugated CD62L antibody (eBioscience) and FITC-labeled microbeads (Miltenyi Biotec). Naïve T cells were cultured with fucoxanthin in the presence of IL-6 and TGF-β for 3 d, and then the cells were fixed and permeabilized with a Foxp3 Staining Set (eBioscience) and stained with FITC conjugated IL-17 antibody (eBioscience) and phycoerythrin (PE) conjugated Foxp3 antibody (eBioscience). In an analysis of Th17 (IL-17⁺) and Treg (Foxp3⁺) cell populations by FACS (BD Bioscience), fucoxanthin suppressed Th17 cell development and increased the number of Foxp3⁺ Treg cells in a dose-dependent manner (Fig. 3). Other carotenoids (astaxanthin, lycopene, and lutein) did not affect T cell differentiation like fucoxanthin (data not shown). This result is in accordance with the results for the suppressive effect on IL-17 production (Fig. 1). In an analysis of T cell differentiation, fucoxanthinol (Wako), a metabolite of fucoxanthin by gut microflora, was also tested. It was found that fucoxanthinol inhibited Th17 cell development as well as fucoxanthin did (Fig. 3). This indicates that oral administration of fucoxanthin should exert the same effect as to T cell differentiation after absorption in the intestine.

Under Th17 cell development conditions, RORγt expression was suppressed by fucoxanthin (Fig. 2A). Moreover, in the presence of TGF-β without IL-6, gene expression encoding Foxp3 was upregulated by fucoxanthin supplementation (Fig. 2B). These results indicate that fucoxanthin suppresses Th17 cell development and induces Foxp3⁺ Treg cell differentiation as RA.

Next we evaluated the T cell differentiation of naïve T cells (CD4⁺ CD62L⁺ T cells) into Th17 cells. Naïve T cells were prepared from C57BL/6 mice by negatively isolating CD4⁺ T cells using CD4⁺ T cell Isolation Kit II (Miltenyi Biotec), followed by the collection of CD62L⁺ cells using fluorescein isothiocyanate (FITC) conjugated CD62L antibody (eBioscience) and FITC-labeled microbeads (Miltenyi Biotec). Naïve T cells were cultured with fucoxanthin in the presence of IL-6 and TGF-β for 3 d, and then the cells were stimulated with phorbol-12-myristate-13-acetate (PMA)/ionomycin (100 ng/mL and 500 ng/mL, respectively, Sigma) and GolgiStop (BD Bioscience, San Jose, CA) for intracellular cytokine analysis. Then the cells were fixed and permeabilized with a Foxp3 Staining Set (eBioscience) and stained with FITC conjugated IL-17 antibody (eBioscience) and phycoerythrin (PE) conjugated Foxp3 antibody (eBioscience). In an analysis of Th17 (IL-17⁺) and Treg (Foxp3⁺) cell populations by FACS (BD Bioscience), fucoxanthin suppressed Th17 cell development and increased the number of Foxp3⁺ Treg cells in a dose-dependent manner (Fig. 3). Other carotenoids (astaxanthin, lycopene, and lutein) did not affect T cell differentiation like fucoxanthin (data not shown). This result is in accordance with the results for the suppressive effect on IL-17 production (Fig. 1). In an analysis of T cell differentiation, fucoxanthinol (Wako), a metabolite of fucoxanthin by gut microflora, was also tested. It was found that fucoxanthinol inhibited Th17 cell development as well as fucoxanthin did (Fig. 3). This indicates that oral administration of fucoxanthin should exert the same effect as to T cell differentiation after absorption in the intestine.

We found that fucoxanthin and its metabolite fucoxanthinol in the gut inhibited Th17 cell differentiation and induced Treg cell development, resulting in the suppression of inflammatory IL-17 production. Many studies have found that RA receptor agonists such as
**Fig. 2.** Fucoxanthin Suppressed ROR\(_{\gamma}t\) mRNA Expression and Increased Foxp3 mRNA Expression in CD4\(^+\) T Cells.

A. CD4\(^+\) T cells (5 \(\times\) 10\(^5\)) from the spleens of C57BL/6 mice were cultured with all-trans retinoic acid (ATRA) or fucoxanthin in the presence of anti-CD3 monoclonal antibody (\(\alpha\)CD3, plate-bound, 5 \(\mu\)g/mL), anti-CD28 monoclonal antibody (\(\alpha\)CD28, soluble, 5 \(\mu\)g/mL), IL-6 (20 ng/mL), and TGF-\(\beta\) (2 ng/mL) for 3 d. ROR\(_{\gamma}t\) mRNA expression was determined by quantitative RT-PCR. The data are representative of three independent experiments. B. CD4\(^+\) T cells (5 \(\times\) 10\(^5\)) were cultured with ATRA or fucoxanthin in the presence of \(\alpha\)CD3, \(\alpha\)CD28, and TGF-\(\beta\) for 3 d. Foxp3 mRNA expression was measured by quantitative RT-PCR. The data are representative of three independent experiments. A and B. The data are shown as expression relative to cells stimulated with \(\alpha\)CD3/\(\alpha\)CD28 only.

**Fig. 3.** Fucoxanthin and Fucoxanthinol Inhibited Th17 Cell Development and Induced Foxp3\(^+\) Regulatory T Cell Differentiation.

Naive T cells (5 \(\times\) 10\(^5\)) from the spleens of C57BL/6 mice were cultured with all-trans retinoic acid (ATRA), fucoxanthin, and fucoxanthinol in the presence of anti-CD3 monoclonal antibody (\(\alpha\)CD3, plate-bound, 5 \(\mu\)g/mL), anti-CD28 monoclonal antibody (\(\alpha\)CD28, soluble, 5 \(\mu\)g/mL), IL-6 (20 ng/mL), and TGF-\(\beta\) (2 ng/mL) for 3 d. The cells were collected, incubated with PMA/ionomycin and GolgiStop, and fixed and permeabilized. They were analyzed by FACS staining with FITC conjugated IL-17 antibody and PE conjugated Foxp3 antibody. The data are representative of three independent experiments. Values represent ratios of cell populations.
ATRA and synthetic retinoids suppress Th17 cell development, and hence we assume that fucoxanthin and fucoxanthinol exert activity similar to ATRA via RA receptors. Although fucoxanthin and fucoxanthinol were effective at higher concentrations than ATRA, a difference in affinity for RA receptors might be involved in the difference in effective concentrations. According to a previous report, the structure, especially the H6–H7 loop, is involved in the interaction between several synthetic retinoids and RA receptors. Thus structural differences among the tested carotenoids and their different affinities for RA receptors might contribute to their suppressive effects on Th17 cell development.

In conclusion, our results suggest that oral administration of fucoxanthin inhibits Th17 cell development, probably through RA receptors, and works against Th17-dependent inflammatory diseases such as autoimmune diseases, inflammatory bowel diseases, and asthma. In regard to the safety of oral administration of fucoxanthin, no abnormal changes were observed in the liver, kidney, spleen, or gonadal tissues under oral administration of 1,000 mg/kg of body weight over 30 d. Since it is expected that the RA-like activity of fucoxanthin might affect the homeostasis of vitamin A metabolism, we intend to investigate the in vivo effects of oral administration of fucoxanthin on inflammatory diseases without side effects on vitamin A metabolism using mouse model such as experimental autoimmune encephalomyelitis or experimental colitis. We also intend to investigate the mechanisms of these different activities among fucoxanthin, fucoxanthinol, and other non-provitamin A carotenoids, focusing on structural features and the affinity for RA receptors.

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References