Feeding with Both β-Carotene and Supplemental α-Tocopherol Enhances Type 1 Helper T Cell Activity among Splenocytes Isolated from DO11.10 Mice

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β-Carotene and/or supplemental α-tocopherol were fed to DO11.10 mice to investigate their effect on the immune function of naive splenocytes. A high secretion of interleukin-12 and interferon-γ in response to the ex vivo primary antigen presentation occurred only when both were fed. This is consistent with the suppressed immunoglobulin E production under the similar condition described in our previous report.

Key words: β-carotene; α-tocopherol; antigen presentation; interleukin-12; interferon-γ

The prevalence of the type I allergic disorder has become a serious problem in developed countries. The IgE antibody specific to respective allergens plays an essential role in the type I allergic disorder. Epidemiological studies have shown that a high intake of fruits and vegetables reduces the risk of incidence of allergic diseases.1-3 These studies indicate that the consumption of fresh fruits and vegetables helps to suppress wheezing, allergic rhinoconjunctivitis, and allergic eczema in adults and children. Fruits and vegetables are rich in such antioxidants as vitamin C, vitamin E, flavonoids, and carotenoids. Forgarty et al. have reported that a higher intake of vitamin E suppressed the incidence of allergic disease by inhibiting allergen-specific IgE production.4 Therefore, the anti-oxidative action of dietary vitamin E may be responsible for the anti-allergic effect of fruits and vegetables. The immunological phenomena accompanying the intake of fruits or vegetables containing β-carotene, and the intake of β-carotene itself have already been investigated.5-8 We have previously reported the suppression of IgE production in BALB/c mice immunized with ovalbumin (OVA) by feeding both β-carotene and supplemental α-tocopherol,9 the representative molecule possessing vitamin E activity. With our system, feeding only either β-carotene or supplemental α-tocopherol resulted in little or no effect on the suppression of IgE production.

The production of IgE occurs under the relative predominance of type 2 helper T cells (Th2). Differentiation from naive helper T cells to Th2, which induces humoral immunity, or to Th1, which induces cellular immunity, is alternative. Skewing this differentiation to modulate the balance between Th1 and Th2 affects the immune status of an individual. Thus, to prevent an allergic disorder, enhancing Th1 activity is a method worth considering. To study the effect of fed β-carotene and/or supplemental α-tocopherol on Th1 activity, we employed DO11.10 mice,10 an OVA-specific T cell receptor transgenic strain of BALB/c mice, purchased from Jackson Laboratories (Bar Harbor, ME, USA) and bred in an institute for animal experimentation at our university. After feeding one of the test diets (Table 1) to DO11.10 mice for three weeks, the mice were anesthetized and then decapitated. Their spleens were extracted and dispersed under hygienic conditions. The secretion of cytokines from dispersed naive splenocytes incubated with or without OVA was first measured as an index of Th1 activity, because Th1 is a major source of this cytokine. Only when β-carotene and supplemental α-tocopherol were fed together, the secreted IFN-γ was significantly enhanced (Fig. 1). This did not conflict with our previous data indicating the suppression of IgE production by feeding β-carotene and supplemental α-

Abbreviations: OVA, ovalbumin; Th2, type 2 helper T cell; Th1, type 1 helper T cell; IFN, interferon; IL, interleukin; APC, antigen-presenting cell
tocopherol to BALB/c mice immunized with OVA. Although β-carotene is a pro-vitamin A, vitamin A activity was probably not involved in the enhanced IFN-γ secretion because retinol acetate did not enhance it. Moreover, another carotenoid, lycopene, did not have an evident effect such as that of β-carotene.

Interleukin (IL)-12 is a cytokine produced in activated antigen-presenting cells (APC) by binding with helper T cells via antigen-presentation, and secreted IL-12 plays an crucial role in the differentiation from naive helper T cells to Th1.11) The bioactive form of IL-12, *i.e.* IL-12 p70, is a hetero-dimer composed of the p40 subunit and p35 subunit. Only when both β-carotene and supplemental α-tocopherol were fed, the secretion of IL-12 p70 was enhanced (Fig. 2A). This is consistent with the result for IFN-γ secretion. Therefore, the difference in IFN-γ secretion by diet could be attributable to the difference of IL-12 p70 secretion from the antigen-presenting cells. As to secretion of the p40 subunit of IL-12, no significant difference among groups could be detected (Fig. 2B). The mice fed with β-carotene and supplemental α-tocopherol did not show any increase in the amount of the secreted p40 subunit, whereas the amount of secreted IL-12 p70 increased. This difference reveals that the secreted p40 subunit not included in the p70 hetero-dimer was less in those mice than in the control mice. It has been reported that the homo-dimer of the p40 subunit worked as a receptor antagonist against IL-12 p70.12) Over-expression of the p40 subunit would inhibit the activity of IL-12 p70 by forming its homo-dimer, although the subunit is indispensable to form the cytokine. Thus, in addition to the enhanced secretion of IL-12 p70, the suppression of secreted p40 not included in p70 might have contributed to the up-regulation of IL-12 activity. We have recently detected the suppressed synthesis of mRNA for the p40 subunit in lypopolysaccharide-stimulated RAW264 cells supplemented with β-carotene to the culture medium, but not with α-tocopherol.13) With our *ex vivo* antigen-presentation system, dietary β-carotene would, on the one hand, promote the production of IL-12 for some reason, *e.g.* enhanced antigen-presentation, and, on the other hand, inhibit the pathway for the production of the p40 subunit as detected in RAW264 cells. Details of this need to be demonstrated in future.

Sato *et al.* have reported the down-regulation of serum IgE level and inhibition of the type I allergic response in mice by feeding only β-carotene.14) They observed the cytokine secretion from isolated splenocytes of BALB/c mice that had been immunized with OVA, which was the secondary response to the responsible immunogen. Their results indicate that dietary β-carotene up-regulated the Th1-type response by itself. This is apparently inconsistent with our results, because we detected a significant difference only when β-carotene and supplemental α-tocopherol were both fed. Such inconsistency is probably attributable to the amount of α-tocopherol included in the control diet. Their control diet included more α-tocopherol, 11 mg (as vitamin E)/100 g of diet from the manufacturer’s data sheet, than ours (5 mg/100 g of diet, see the footnote to Table 1). Their results and ours enable us to suggest the effect of β-carotene on IL-12 secretion from the antigen-presenting cells, including macrophages, was the major

**Table 1.** Composition of the Diets Used in These Experiments

<table>
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<th></th>
<th>Ctrl</th>
<th>AT</th>
<th>BC</th>
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<th>AT+RA</th>
<th>AT+LY</th>
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<td></td>
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<td>4.7</td>
<td>4.7</td>
<td>4.65</td>
<td>4.69</td>
<td></td>
</tr>
</tbody>
</table>

*One gram of the vitamin mixture contained 0.005 g of α-tocopherol acetate, 500 IU of vitamin A, and other vitamins, but no carotenoids. **The amount of retinol acetate (0.01 mg/100 g of diet) was based on the equivalent vitamin A activity to that of β-carotene (0.05 mg/100 g).*

**Fig. 1.** Effect of β-Carotene or Related Food Components with α-Tocopherol on the IFN-γ Secretion from Splenocytes Stimulated with the Antigen.

DO11.10 mice were fed on a diet with added β-carotene (BC), retinol acetate (RA) or lycopene (LY), and/or supplemental α-tocopherol (AT) for 3 weeks, before their splenocytes were collected. See Table 1 for details about the amount of added nutrients and abbreviated group names. In this experiment, 3 × 10^6 dispersed splenocytes were treated with (unfilled column) or without (filled column) OVA (30 μg/ml) for two days. The secreted IFN-γ levels were measured by a commercially available ELISA system (Becton, Dickinson and Company, Japan; Tokyo, Japan). Data are expressed as the mean ± SD of 5 mice. Statistical analyses were carried out by one-way ANOVA followed by *post hoc* tests with the Bonferroni/Dunn method; *p < 0.05* compared with the value in the absence of added nutrients (Ctrl).
reason for modulation of the immune-system observed in our results. To accomplish this effect of \(\beta\)-carotene in vivo, a considerable level, i.e. more than that in our control diet, of \(\alpha\)-tocopherol may be required.

We found in this study that a diet with \(\beta\)-carotene and supplemental \(\alpha\)-tocopherol induced higher secretion of IFN-\(\gamma\) and IL-12 among murine naive splenocytes given antigenic stimulation, which we employed as a model for the primary response to immunization. As for the function of \(\beta\)-carotene, vitamin A activity could be excluded. Thus, another function, i.e. redox function, would be focused. Indeed, we detected up-regulation of glutathione in splenocytes of mice fed with \(\beta\)-carotene (unpublished data) as well as in RAW264 cells incubated with \(\beta\)-carotene.\(^{13}\) The intracellular redox status of macrophages based on glutathione has been demonstrated to be important to their activity for cytokine production; and reduced macrophages have more favorably induced Th1 cells than Th2 cells from naive helper T cells.\(^{15,16}\) We will next investigate the effect of dietary \(\beta\)-carotene with supplemental \(\alpha\)-tocopherol on enhancing Th1 activity, especially considering its redox effect on the antigen-presenting cells, to clarify the action mechanism involved in the synthesis and secretion of IL-12. Furthermore, we will investigate such Th2 activity as the secretion of IL-4, which could be interfered from the Th1 activity, under the same condition.

Acknowledgments

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References

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