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IMMUNOMODULATION IN HUMANS CAUSED BY
BETA-CAROTENE AND VITAMIN A

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ABSTRACT

Carotenoids and retinoids can stimulate some human immune responses. These include cytokine release with anti-tumor cell activity, increased natural killer cells and activated lymphocytes after both in vitro and in vivo treatment with beta carotene. Such stimulations seem only partly due to retinoids formed from carotenoid metabolism and may be due to effects caused by the structure of beta carotene. Changes in immune functions could explain in part the cancer resistance provided by high carotenoid or retinoid intakes in animals.

KEY WORDS: beta carotene, natural killer cells, activated lymphocytes, cancer.

INTRODUCTION

A variety of dietary components are possible inhibitors of human cancer initiation or promotion (1,2). Two related groups include: (a) retinoids, particularly retinol or preformed vitamin A, and (b) carotenoids, provitamin A compounds. The latter group includes beta carotene which can be converted into retinol in vivo and others, like canthaxanthin, which cannot (3). However, all have immunomodulatory effects at physiological levels which may affect cancer growth.

Recently, great emphasis has been given to understanding the mechanisms of cancer prevention mediated by vitamin A and related compounds. This review article provides an immunology perspective on the actions of vitamin A, including modulation of T-lymphocyte helper cells, macrophages, Langerhans cells, and natural killer cells. The direct effects of vitamin A and related compounds on cancer cells and their toxicity, metabolism and deficiency are extensively reviewed elsewhere and are not discussed here (1,2).

6To Whom Correspondence Should be Directed
Retinoids, Carotenoids, and Cancer. During the past decade there has been increasing research which has documented a relationship between dietary retinoids, carotenoids, and cancer incidence (1-4). Both retinoid and carotenoid intakes have been inversely correlated with the incidence of certain cancers in humans, such as: adenocarcinomas, buccal cavity cancer, basal cell carcinoma, and lung, esophageal, and bladder cancers (1,2). Vitamin A deficiency increases the risk of cancer in animals and enhances the sensitivity of epithelial tissues to carcinogenesis by chemicals, irradiation, and viruses (5). Conversely, high intakes of vitamin A and related compounds may have a beneficial effect on the reduction of cancer risk.

Several modes of action have been proposed to account for the inhibitory effects of vitamin A on carcinogenesis: (a) interference with the interaction of the carcinogens or their active metabolites and the target site, (b) alteration of the metabolic pathway of carcinogen activation via inhibition of certain mixed function oxidases (1), (c) lysosomal labilization and subsequent breakdown of premalignant cells (6), (d) induction of differentiation (7) or slowing of proliferation and (e) host immunomodulation with subsequent alteration of tumor growth (8,9).

Carotenoids, especially beta-carotene, appear to have a major inhibitory effect on cancer incidence. Epidemiological studies have demonstrated a positive correlation between beta-carotene intake and lower cancer risk (10-12). For example, serum beta-carotene levels were low in patients with gastrointestinal cancer in Israel, with cervical cancer in Japan, and with precancerous stomach lesions in Colombia (13). There may also be an association between low serum levels of beta-carotene and the risk of oral cancers and of squamous cell carcinomas of the lung (14,15). Thus, prospective trials are in progress to evaluate the prophylactic efficacy of beta-carotene in reducing overall cancer risk in humans (13) and animals (16).

The postulated anti-cancer mechanisms for carotenoids include the ones proposed for retinoids mentioned above (14). In addition, carotenoids can act as anti-oxidants by quenching free radicals (17,18). Modification of host immune defenses may also be an important activity of carotenoids which could play a role in their anti-cancer effects (17).

**IMMUNOMODULATORY EFFECTS**

T-lymphocytes. A variety of synthetic and naturally occurring retinoids stimulate cellular immune functions. Retinoic acid therapy caused enhanced cellular immune reactions and increased antigen recall reactions, while simultaneously improving the efficacy of dermatoses treatment (8). Retinoic acid at non-toxic concentrations increased the response of human T-lymphocytes (19) or thymocytes (20) to phyto-hemagglutinin (PHA), a T-cell mitogen, but not to concanavalin A (Con A) or pokeweed mitogen (PWM) (21). TPA-induced T-lymphocyte proliferation was also increased by retinoic acid given in vivo (22). We have recently shown that 13-cis retinoic acid increased the number of mature T-helper lymphocytes (CD4+) in vitro (23) after incubation of peripheral blood mononuclear cells (PBMC) for 72 hours at low, clinically achievable concentrations (10^-8M). In addition, more numbers of helper cells showed expression of transferrin and interleukin-2 (IL-2) receptors which indicated increased numbers of activated T-helper cells (Fig. 1). In vivo experiments in oral leukoplakia patients taking 13-cis retinoic acid 30mg/kg/day for three months showed a 50% increase in numbers of T-helper cells, IL-2 receptor expression, and mitogenic response to phyto-hemoagglutinin (unpublished data). We found increased activation of lymphocytes and macrophages by increased percentage of cells with markers for these functions.

Beta-carotene (180 mg/day) given orally for two weeks to normal human volunteers increased the frequency or percentage of lymphocytes with CD3+ (total T-cells) and CD4+ (T-helper cells), but not CD8+
(T-suppressor/cytotoxic) surface markers (24). We have shown in vitro that beta-carotene produced a significant increase in CD4+ cells (23) and a dramatic increase in the percentage of natural killer cells among PBMC (Fig. 1). The increase in the expression of IL2-receptors was associated with increased NK and T-helper cells. Production of interferon by T-lymphocytes was increased by beta-carotene (25).

Similar immunostimulatory effects have been observed in animals given carotenoids. Canthaxanthin, provided as a supplement in the diet of mice at 1%, reduced the number of T-suppressor cells which were increased by ultraviolet irradiation (see Figure 3). Canthaxanthin also increased the percentage of T-helper cells four-fold, and enhanced lymphoid cell expression of activation markers (IL2-receptors) after 2 months of dietary supplementation in mice (Fig. 2). As canthaxanthin is not metabolized into retinol, this change was due to the carotenoid structure, rather than increased retinol in tissues. Both beta-carotene and canthaxanthin showed improvement in the response of rat lymphocytes to the mitogens in vivo (17).

Thus the overall effect of retinoids and carotenoids on T-lymphocytes appears to be stimulatory with increased responsiveness to mitogens, and an increase in the percentage of helper cells (CD4+). However, the mechanisms involved, as well as the clinical relevance of these effects, remains to be determined. Some possible mechanisms involving T cell stimulation are described below.
Fig. 2. Activation of mouse spleen cells by canthaxanthin after ultraviolet (UV) irradiation exposure. Mice were fed with dietary canthaxanthin (1% of diet) prior to UV exposure. After 20 weeks, spleen cells were collected and stained with various monoclonal antibodies to analyze in flow cytometer. Values were mean percentages with standard deviation from three or more experiments containing 5-8 mice. (Th - T-helper cells, TSC - T-suppressor cells). The mice received 4.6 J/M²/s with 80% in wavelength of 280-340 nm. The dorsa of the mice were shaved and they got five 30 minute exposures per week for 15 weeks.

**B-lymphocytes.** High dietary intakes or deficiencies of retinoids and carotenoids may be expected to alter immune functions of both B and T-cells either directly or through their interactions. Retinoic acid ($10^{-7}$ M) in vitro increased the induction of antibody-producing human tonsillar B-lymphocytes sensitized to sheep red blood cells (SRBC), a T-cell dependent antigen (26). However, it inhibited B-cell transformation induced by Epstein-Barr virus, a T-independent antigen (19). While animal studies suggest that dietary retinoids enhance antibody production, similar data in humans is lacking.

**Natural Killer Cells.** In a mouse model, induction of cell-mediated cytotoxicity by NK cells to allogenic tumor cells was stimulated by low doses of retinoic acid while high doses were suppressive (27). In humans, NK cell activity was increased by retinoic acid (28) concomitant with suppressed tumor growth. Sidell et. al. (29) observed that NK cells collected from 4 of 10 donors showed a significant increase in cytotoxicity against K562 cells after exposure to retinoic acid. At high concentrations ($10^{-5}$ M), retinoic acid had inhibitory effects on spontaneous and interferon-induced cytotoxicity of NK cells after prolonged periods of incubation (21,25). In our laboratory, we
have recently found that both beta carotene (Fig. 1), and canthaxanthin (Fig. 2) produced an increase in the percentage of NK cells after human PBMC were incubated with them for 3 days at clinically achievable concentrations in vitro (23). However, the effect of carotenoids on NK cells in humans needs further evaluation.

Monocytes/Macrophages. Mononuclear cells, especially monocytes (blood) and macrophages (tissues), have critical roles in host defense mechanisms and immunoregulation.

Fig. 3. Cytotoxicity of peritoneal macrophages (PM) after mice were fed with various concentrations of retinyl palmitate (RP). Skin tumors developed in mice following the initiation (DMBA) and promotion (TPA) processes. During the treatment with RP, PM were collected after 21 weeks and the cytotoxicity was measured against B-16 melanoma cells (47). The values were mean percentages with standard deviation of three or more experiments. Each contained 7 mice.

Retinoids affected the development and differentiation of human monocytes/macrophages in vitro including monocytic cell lines (1,7). Retinoic acid increased the growth of granulocyte/macrophage precursor cells, suggesting possible involvement in the early stages of differentiation by increasing the sensitivity to colony stimulating factor (30). Retinoic acid induced an increase in the phagocytic activity and surface marker expression on immature monocyte/macrophage human cell lines (31). At physiological levels ($10^{-7}$M), retinoic acid produced an increase in interleukin 1 (IL-1) production from PBMC (32) and increased their cytotoxicity to tumor cells in vitro (33). We (34) and others (35) have shown that retinoic acid induced activation of macrophages in mice (Fig. 3).
Fig. 4. Cytotoxic activity of supernatants from murine macrophages cultured in vitro with lipopolysaccharides (LPS) for 48 hours. The change in the percent of MTT dye incorporated by L929 tumor cells was assessed after 72 hour incubation with culture supernatants. All the values represent the mean and standard deviation of more than three replicate experiments (37). Mice fed BC and treated with LPS in vitro to get cytokine release as described for human cells (37).

Beta-carotene also enhanced antigen presentation by monocytes. We have observed (37) that beta-carotene increased the ability of monocytes in vitro to produce a novel tumor cytotoxic factor (Fig. 4). It does not seem to be TNF, IL-1, IL-2, or interferon, and may play a role in host defense in vivo. Dietary beta carotene and canthaxanthin yielded mouse monocytes that released enhanced levels of cytotoxic activity in vitro for L-929 leukemia cells, without in vitro stimulation by carotenoids. In addition, the percentage of monocytes (Fig. 2) with markers of activation (Mac2) increased following consumption of a diet with 1% canthaxanthin for 2 months by mice (unpublished data). Recently, we and others have shown that beta-carotene protects against UV-B damage by preventing free radical action and by increasing phagocytosis (38) in vitro (37) (Fig. 5).

The above data suggests that retinoids and carotenoids might act by different pathways to stimulate monocytes. Retinoids appear to act at an early stage of the differentiation process and at early stages of the activation process by improved phagocytosis. On the other hand, carotenoids act at later stages of the activation process primarily as scavengers for oxygen radicals, enhancers of antigen presentation, and inducers for tumor cytotoxic factors.
**Fig. 5.** Alterations of phagocytosis after treatment with ultraviolet irradiation (UV) and beta-carotene (BC) on human monocytes in vitro as described previously (38). Purified human monocytes were incubated with beta-carotene after UV exposure. Phagocytosis was determined using $^{51}$Cr-labelled sheep red blood cells. Values are the mean percentages with standard deviations of three or more experiments containing several samples.

**Langerhans Cells.** Langerhans cells, antigen-presenting macrophage-like cells, have an important role in immune reactions in the skin. Ultraviolet irradiation decreased human Langerhans cell membrane markers such as ATPase staining, expression of OKT6 and HLA antigen (39) after vitamin A treatment for erythrokeratodermia variabilis (40). The surface markers were restored, including OKT6, HLA-Dr and ATPase staining after culturing for 48 hours in vitro (41). Retinol produced an increase in IL1 production and an increase in the density of OKT6, HLA-Dr and ATPase markers on human gingival Langerhans cells. Thus, retinoids seem to have immunostimulatory effects on Langerhans cells, which are important in antigen presentation to T-lymphocytes in the skin.

**DISCUSSION**

Interest in the potential mechanisms for the anti-cancer effect of retinoids and carotenoids has largely focused on their differentiating capabilities (1,2,4). Vitamin A has inhibitory capabilities against development of certain cancers (42,46), such as reducing the development of skin tumors due to chemical carcinogens (47,48). Vitamin A's marked immunomodulatory effects in animal systems may explain in part its significant
effects on cancer resistance (49,50). In this review we have attempted to highlight recent data on immunomodulation by vitamin A and related compounds (Table 1). These immune changes may play an important role in the anti-cancer effects of retinoids and carotenoids. Further laboratory and clinical studies are warranted in this regard to explain the relationship between immunomodulation and these compounds. We recognize that there are additional parameters which may influence immunomodulation including: (a) chronic deficiency problems; (b) the transportation and biosynthesis of these compounds through binding proteins (51), and (c) receptors for these compounds on various cells which function in cell modulation.

Fig. 6. Diagramatic representation of mechanisms for vitamin A actions on the components of the immune system.

Retinoids and carotenoids appear to have different effects on the immune system. Retinoids act on the differentiation processes of immune cells, increasing mitogenesis of lymphocytes and enhancing phagocytosis of monocytes/macrophages. Recent reports noted that retinoids activate protein kinase C (56), NK cells in vivo (57), and IL2 receptors activate human thymocytes (58). Carotenoids modify the release of some cytokine-like products after activation of lymphocytes and monocytes which may enhance immunosurveillance through activation of NK cells (Fig. 6). They also act as anti-oxidants reducing loss of immunological functions due to the immunotoxic effects of oxide radicals. Thus, it may be possible to exploit immunomodulatory effects of carotenoids for the treatment of specific diseases. For instance, beta-carotene increases NK cells and T-helper cell numbers. Restoring the number of these cells may be useful in acquired immunodeficiency syndromes such as (AIDS) where
Emphasis is placed on those potentially providing anti-cancer effects in pro-vitamin A compounds like beta carotene which may activate T-helper cells (TH) directly, or indirectly via interleukin 1 (IL1) produced by activated macrophages (Mo). Carotenoids seem to further extend the activation process to natural killer cells (NK) through elevation of one or more cytokines such as interleukin 2 (IL2), interferon-gamma (IFN-y), and tumor necrosis factor (TNF).

Clinical trials with carotenoids to stimulate activation of helper cells, macrophages and NK cells in AIDS patients would be of great interest due to their low toxicity. Thus immunomodulation may be an exciting tool to stimulate the cellular immune system and to understand the role of these dietary agents in cancer prevention.
TABLE 1

Immunomodulatory Effects of Vitamin A

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Compounds</th>
<th>Function or Expression of Markers</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-cells</strong></td>
<td>Retinoids</td>
<td>1. ( T_3^+ + T_6^- ) thymocyte maturation</td>
<td>increased</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Mitogenesis</td>
<td>increased</td>
<td>19,21,22</td>
</tr>
<tr>
<td></td>
<td>Carotenoids</td>
<td>1. CD4(^+) cells (helper)</td>
<td>increased</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Interferon production</td>
<td>increased</td>
<td>25</td>
</tr>
<tr>
<td><strong>B-cells</strong></td>
<td>Retinoids</td>
<td>1. ( \text{T-dependent antibody production} )</td>
<td>increased</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. ( \text{T-independent} )</td>
<td>inhibited</td>
<td>26</td>
</tr>
<tr>
<td><strong>Monocytes/Mo</strong></td>
<td>Retinoids</td>
<td>1. Differentiation</td>
<td>occurred</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Surface Markers</td>
<td>increased</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) Phagocytosis</td>
<td>increased</td>
<td>32,38,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Chemotaxis</td>
<td>increased</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Cytotoxicity</td>
<td>increased</td>
<td>33,34,35,38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Cytokins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) Interleukin-1</td>
<td>increased</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Interferon</td>
<td>increased</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Macrophage inhibitory factor</td>
<td>decreased</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) Lysozymes</td>
<td>increased</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Superoxide radicals</td>
<td>increased</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Carotenoids</td>
<td>1. HLA-Dr expression</td>
<td>increased</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Cytotoxicity</td>
<td>increased</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Phagocytosis</td>
<td>increased</td>
<td>38</td>
</tr>
<tr>
<td><strong>Langerhans Cells</strong></td>
<td>Retinoids</td>
<td>1. OKT6 expression</td>
<td>increased</td>
<td>40,41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. HLA-Dr expression</td>
<td>increased</td>
<td>40,41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. ATPase staining</td>
<td>increased</td>
<td>40,41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. IL-1 production</td>
<td>increased</td>
<td>41</td>
</tr>
<tr>
<td><strong>Natural Killer Cells</strong></td>
<td>Retinoids</td>
<td>1. Cytotoxicity</td>
<td>increased</td>
<td>27,28,29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(at low concentrations)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. IFN-induced cytotoxicity</td>
<td>inhibited</td>
<td>25,27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(at high concentrations)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2

Effects of dietary retinyl palmitate (RP, 120 units/gram diet) and canthaxanthin (CTX, 1% of diet) on the frequency of lymphocytes and macrophages (Mo) identified with surface markers and activation markers.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Percentages of Surface Markers on Spleenocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Helper cells (L3T4)</td>
</tr>
<tr>
<td>virus</td>
<td>23.0 ± 2.6</td>
</tr>
<tr>
<td>virus + RP</td>
<td>23.0 ± 2.0</td>
</tr>
<tr>
<td>virus + CTX</td>
<td>29.3 ± 2.5*</td>
</tr>
</tbody>
</table>

*Statistical analysis of student 't' test show P values less than 0.05. ND = not determined, IL2-R = Interleukin 2 receptors.

These markers were detected by flow cytometer after 8 week post LP-BM5 murine leukemia virus infection. Results were presented with mean values and standard deviations of three or more experiments containing 5 mice/group. Modified from Watson et al. (55).

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