Dietary β-Carotene Stimulates Cell-Mediated and Humoral Immune Response in Dogs

ABSTRACT The role of β-carotene on immune response in domestic dogs is not known. Female Beagle dogs were fed 0, 2, 20 or 50 mg β-carotene/d; blood was sampled at wk 0, 1, 2, 4 and 8 for analysis of the following: lymphoproliferation, leukocyte subpopulations and concentrations of interleukin-2 (IL-2), immunoglobulin (Ig)G and IgM. Delayed-type hypersensitivity (DTH) response was assessed at wk 0, 3 and 7. β-Carotene supplementation increased plasma β-carotene concentrations in a dose-dependent manner. Compared with unsupplemented dogs, those fed 20 or 50 mg of β-carotene had higher CD4+ cell numbers and CD4:CD8 ratio. However, there was no treatment difference in CD8+, CD21+ and major histocompatibility complex (MHC) class II+ cells. Plasma IgG, but not IgM concentration was higher in dogs fed β-carotene through- out the study period. The DTH response to phytohemagglutinin (PHA) and vaccine was heightened in β-carotene–supplemented dogs. β-Carotene feeding did not influence mitogen-induced lymphocyte proliferation or IL-2 production. Immune response was impaired in dogs classified as low β-carotene absorbers compared with similar dogs fed the same amount of β-carotene. Therefore, dietary β-carotene heightened cell-mediated and humoral immune responses in dogs. J. Nutr. 130: 1910–1913, 2000.

KEY WORDS: • β-carotene • immunity • canines

Studies have suggested a specific role for β-carotene in regulating immune function [see Chew (1995) for review]. Alexander et al. (1985) reported a 30% increase in the number of T helper (Th3) cells in human adults given β-carotene.

Mice supplemented with β-carotene showed an increased number of Th cells, increased expression of interleukin-2 (IL-2) receptors on natural killer cells (Prabhala et al. 1989), and enhanced proliferation and induction of cytotoxic T (Tc) cells (Seifert et al. 1981). Cattle fed β-carotene had increased mitogen-induced lymphocyte proliferation during the peripar- tum period, whereas preformed vitamin A did not produce a similar response (Michal et al. 1994). β-Carotene also en- hanced humoral immune response in mice (Jyonouchi et al. 1994 and 1995). Tomita et al. (1987) reported that the action of β-carotene was antigen specific. In contrast, nothing is known concerning the possible immunoregulatory action of β-carotene in dogs. In a previous study (Chew et al. 2000), we reported that domestic dogs can absorb β-carotene, and high concentrations are found in blood leukocytes. Our present objective was to study the immunomodulatory role of dietary β-carotene on cell-mediated and humoral immune responses in domestic dogs.

MATERIALS AND METHODS

Female Beagle dogs (4–5 mo old; 7.0 ± 0.8 kg body weight; Marshall Farms USA, North Rose, NY) were randomly assigned (n = 14/treatment) to daily supplementation with 0, 2, 20 or 50 mg β-carotene (10% cold water dissolvable; BASF, Ludwigshafen, Germany) for 8 wk. β-Carotene was incorporated into a commercial basal diet (The Iams, Lewisburg, OH) and fed two times daily (200 food/d). The diet composition was as follows (g/kg): 66.2 moisture, 262 protein, 74.5 ash, 160 fat, 14.8 Ca, 10.3 P and 437.3 nitrogen-free extract. All dogs were housed in 2 × 2 m² pens (2 dogs/pen) in a temperature- (20–22°C) and light-controlled (14 h light) facility. Body weight was recorded at wk 0, 4 and 8. The research protocol was approved by the Washington State University Institutional Animal Care and Use Committee.

Blood was collected by jugular venipuncture into heparinized evacuated tubes at wk 0, 1, 2, 4 and 8 and aliquots used for HPLC and immune function analysis.

HPLC. Plasma was extracted for analysis of β-carotene, retinol and α-tocopherol by HPLC as previously described (Park et al. 1998b). The identity of the eluted compound was confirmed by comparing its absorption spectrum with that of a corresponding standard. Examination of plasma β-carotene absorption profiles revealed that two dogs each from the 20 and 50 mg β-carotene dietary groups had plasma β-carotene concentrations that averaged 6–24% (low responders, LR) of that found in the remaining dogs (responders, R). Consequently, all statistical analyses on the effects of dietary β-carotene on plasma β-carotene and immune response were made with the LR dogs removed. Separate statistical analyses were made to study the possible difference between LR (n = 4) and R (n = 24) dogs.

Delayed-type hypersensitivity. Cutaneous delayed-type hyper- sensitivity (DTH) response was measured at wk 0, 3 and 7 as previously described (Kim et al. 2000a). All dogs were injected intradermally with 100 µL of one of the following: 1) saline (8.5 g/L) as a negative control, 2) an attenuated polyvalent vaccine containing canine distemper virus, adenovirus type-2, parainfluenza virus and parvovirus (undiluted; Vanguard 5, Smithkline Beacham, West Chester, PA), and 3) phytohemagglutinin (PHA, 0.5 g/L). Skin induration at 24, 48 and 72 h postinjection was expressed as the percentage of
increase in skin thickness compared with 0 h. All injections and skin measurements were made by the same person to reduce variations.

**Lymphoproliferation.** Proliferation response of peripheral blood mononuclear cells (PBMC) to PHA (8 and 40 mg/L final concentration), concanavalin A (Con A; 4 and 20 mg/L) and pokeweed mitogen (PWM; 0.4, 2, and 10 mg/L) was assessed at wk 0, 1, 2, 4 and 8 using whole-blood cultures (Kim et al. 2000a). This was done to mimic in vivo conditions.

**Leukocyte subset.** Subpopulations of CD5 (total T), CD4 (Th), CD8 (Tc), major histocompatibility complex (MHC) II (activated B cells), and CD21 (mature B cells) were quantitated by flow cytometry at wk 0, 2, 4, and 8 (Kim et al. 2000a).

**Interleukin-2 production.** The production of IL-2 by cultured PBMC supernatant was assessed using whole blood. Blood was diluted 1:2 with RPMI-1640 and triplicate cultures were stimulated with PHA (8 or 40 mg/L final concentration), concanavalin A (20 mg/L at wk 4) and pokeweed mitogen (PwM; 0.4, 2 and 10 mg/L) was assessed at wk 0, 2, 4 and 8 using whole-blood cultures (Kim et al. 2000a).

**Statistics.** Data were analyzed by split-plot ANOVA using the General Linear Model of SAS (1991). Differences among treatment means were compared by a protected Least Significant Difference test and considered significant at $P < 0.05$.

### RESULTS

There was no significant treatment difference in body weight (7.80 ± 0.04 kg) during the study. Plasma β-carotene was not detectable in all dogs before β-carotene supplementation but increased in a dose-dependent manner ($P < 0.05$) through wk 8 of β-carotene supplementation (Fig. 1). Maximal blood β-carotene concentrations occurred between wk 2 (20 mg β-carotene) and 4 (50 mg β-carotene). Plasma β-carotene in dogs fed 2 mg β-carotene was not significantly different from that of unsupplemented dogs. Plasma β-carotene in LR dogs averaged 6–24% ($P < 0.05$) of that observed in R dogs (Table 1).

Dietary β-carotene did not influence plasma retinol (6.4 ± 1.7 μmol/L) or α-tocopherol concentrations (37.5 ± 11 μmol/L).

**Delayed-type hypersensitivity.** Skin induration response to saline during all periods was low and not different among treatments. At wk 0, no dietary difference was observed in DTH response to vaccine and PHA. Skin response to both vaccine and PHA at wk 7 was higher ($P < 0.05$) at 24, 48 and 72 h postinoculation in fed 20 and 50 mg β-carotene (Fig. 2). The same trend ($P < 0.05$) was observed at wk 8. There was no difference between LR and R dogs in DTH response to PHA or vaccine at wk 3. However, at wk 7, DTH response to vaccine at 24 and 48 h postinoculation was 64 and 55% higher ($P < 0.05$), respectively, in R compared with LR dogs.

**Lymphoproliferation.** Dietary β-carotene did not influence PHA-, Con A- or PWM-stimulated PBMC proliferation at all time periods. The stimulation indices across all periods and dietary groups averaged 70 ± 5, 63 ± 4, 105 ± 6 in PBMC cultures stimulated with PHA (8 or 40 mg/L), Con A (4 or 20 mg/L), and PWM (0.4, 2 or 10 mg/L), respectively. Interestingly, PBMC proliferative response to Con A (20 mg/L at wk 2 and 4) and PWM (10 mg/L at wk 4) was lower ($P < 0.05$) in LR dogs than in R dogs (Table 1). Other concentrations of mitogens produced similar results.

### TABLE 1

Comparisons of β-carotene uptake and immune response in low responder (LR) and responder (R) dogs fed 20 or 50 mg β-carotene daily for 8 wk¹,²

<table>
<thead>
<tr>
<th></th>
<th>Period, wk</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Plasma β-carotene, μmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>0</td>
<td>0.012</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>0.050</td>
</tr>
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<td><strong>Plasma IgG, g/L</strong></td>
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<td></td>
</tr>
<tr>
<td>LR</td>
<td>6.9</td>
<td>7.2b</td>
</tr>
<tr>
<td>R</td>
<td>8.5</td>
<td>9.4a</td>
</tr>
<tr>
<td><strong>Lymphocyte proliferation, stimulation index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A (20 mg/L)</td>
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<td></td>
</tr>
<tr>
<td>LR</td>
<td>58</td>
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<td>R</td>
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<tr>
<td>R</td>
<td>63</td>
<td>57</td>
</tr>
</tbody>
</table>

¹ Means (n = 4 for LR; n = 24 for R) within a period with different superscripts differ significantly, $P < 0.05$.

² Abbreviations: IgG, immunoglobulin G; Con A, concanavalin A; PWM, pokeweed mitogen.
Values are means (SEM, n = 12–14). Means at a time with different superscripts differ significantly, P < 0.05.

Leukocyte subpopulations. The percentages of leukocyte subpopulations measured were similar before dietary β-carotene supplementation. However, dogs fed 20 (wk 8) or 50 (wk 4 and 8) mg β-carotene had higher (P < 0.05) percentages of CD4+ cells than unsupplemented dogs (Table 2). The ratio of CD4+/CD8 + cells (1.21 ± 0.15 vs. 0.85 ± 0.11) was higher (P < 0.05) at wk 4 in dogs fed 50 mg β-carotene compared with control. No treatment difference was observed in the population of CD8+, CD5+, CD21+, and MHC II+ cells. The percentages of CD4+ (28.4 ± 2.5% vs. 38.4 ± 3.1%; wk 4), CD21+ (6.26 ± 1.54% vs. 10.82 ± 2.48%; wk 2), and MHC class II (81.0 ± 3.9% vs. 91.3% ± 5.2%; wk 4) cells were lower (P < 0.05) in LR compared with R dogs.

IL-2 production. Production of IL-2 by whole-blood cultures and in plasma was not influenced by dietary β-carotene throughout the experiment. Concentrations were 17.2 ± 0.6 and 70 ± 4 mg/L, respectively. No significant difference was observed between LR and R dogs.

Immunoglobulin production. Plasma IgG concentration was higher (P < 0.05) at wk 1, 2, 4 and 8 in dogs fed 20 mg β-carotene compared with unsupplemented dogs (Table 2). Dogs fed 20 or 50 mg β-carotene also had higher concentrations of plasma IgG at wk 2. Major differences occurred during wk 2–4, showing a transient effect of β-carotene on IgG production. In contrast, there was no treatment difference in plasma IgM throughout the study, and concentrations were 1.62 ± 0.17 g/L. Plasma IgG but not IgM concentrations were lower (P < 0.05) at wk 1 and 2 in LR dogs compared with Rb dogs (Table 1).

DISCUSSION

This study demonstrates, for the first time, that dietary β-carotene enhanced both cell-mediated and humoral immune responses in domestic dogs. Dogs fed 20 or 50 mg β-carotene/d (corresponding to 2.5–6 mg β-carotene/kg body) had heightened DTH response to the vaccine (specific immune response) and to PHA (nonspecific immune response) as early as 3 wk after the initiation of β-carotene supplementation. Recently, we also showed enhanced DTH response to vaccine and PHA in dogs fed 5–20 mg lutein (Kim et al. 2000a) and to vaccine in cats fed 1–10 mg lutein/d (Kim et al. 2000b). The cutaneous response to mitogens is a useful clinical test in assessing in vivo T-lymphocyte function in dogs (Miyamoto et al. 1992). Taken together, these studies show that dietary β-carotene and lutein can enhance T cell immune response as assessed by the DTH test in Beagle dogs.

The heightened T cell response as measured by the DTH response is consistent with a higher percentage of CD4+ T cells in dogs fed 20 or 50 mg β-carotene compared with unsupplemented animals. The higher population of Th cells in this study is in agreement with others. For example, Alexander et al. (1985) reported higher numbers of total T and Th cells in human volunteers supplemented with 180 mg of β-carotene for 2 wk. We recently reported that dogs (Kim et al. 2000a) and cats (Kim et al. 2000b) fed lutein had higher percentages of blood lymphocytes expressing CD4+ cell surface markers. Mice supplemented with β-carotene similarly showed a higher number of Th cells (Prabhala et al. 1989).

Dogs fed β-carotene (especially those fed 20 mg) had higher concentrations of plasma IgG but not IgM. Greater IgG production was not accompanied by a higher percentage of CD21+ B cells. Changes in the population of CD21+ cells may not identify the true population of plasma cells; consequently, they may not reflect IgG production. Cats (Kim et al. 2000b) but not dogs (Kim et al. 2000a) fed lutein showed a higher population of B cells in peripheral blood. β-Carotene, lutein and astaxanthin stimulated in vivo antibody production in response to T cell–dependent antigens (Jyonouchi et al. 1994), and β-carotene and astaxanthin enhanced Th cell clone–mediated antibody response (Jyonouchi et al. 1995). Similarly, murine splenocytes incubated with astaxanthin,
β-carotene or canthaxanthin showed higher antibody production in vitro (Okai and Higashi-Okai 1996). As with cats fed lutein (Kim et al. 2000b), plasma IgG concentration was elevated in dogs fed β-carotene even without an antigenic challenge. However, a polyvalent vaccine and a mitogen were injected intradermally into all dogs during the DTH skin test. Whether these small antigenic challenges could prime memory B cells to secrete IgG is not known. Also, unsupplemented dogs challenged in the same way did not show a similar response in IgG production.

Cytokines such as IL-1, IL-2, tumor necrosis factor α, and interferon γ play important roles in DTH response. Therefore, the lack of influence of dietary β-carotene on IL-2 production by PBMC was unexpected. These results are similar to those observed in dogs (Kim et al. 2000a) and cats (Kim et al. 2000b) fed lutein and suggest the involvement of cytokines other than IL-2. In addition, dietary β-carotene did not influence mitogen-induced PBMC proliferation. This lack of mitogenic response is not consistent with the observed stimulation of the DTH and T-cell responses observed with dietary β-carotene.

The mechanism by which β-carotene enhanced DTH response and antibody production could not be explained from the present study. However, it is clear that Th cells are involved in both arms of the immune mechanism. Perhaps future studies specifically targeting changes in the production of other cytokines or changes in other cell surface markers may shed new light on the mechanism by which β-carotene stimulates these immune responses. Nevertheless, β-carotene has potent antioxidant activity. Therefore, it may serve to maintain the functional integrity of cell membranes and organelles from reactive oxygen species produced during cellular metabolism. Indeed, uptake of β-carotene by peripheral blood lymphocyte subcellular organelles has been demonstrated in dogs (Chew et al. 2000).

About 14% “low responder” dogs (4 of 28) were identified in this study. Similar low responders have been reported in humans (Borel et al. 1998) and calves (Poor et al. 1992). We report here, for the first time, that these canine low responders have lower immune status, including lower DTH response, lymphocyte mitogenesis, circulating subpopulations of Th, B and MHC II cells, and plasma IgG concentrations, compared with dogs that can absorb β-carotene. The impaired immune status of low responders compared with responders, even though both groups of dogs were fed the same amount of β-carotene, further illustrates the importance of adequate circulating concentrations of β-carotene in modulating immunity. In other words, not all animals given the same supplement will respond in the same manner. Similar comparisons on the immune response of poor carotenoid absorbers have not been reported previously.

In summary, dietary β-carotene is absorbed by domestic dogs and enhances cell-mediated and humoral immune responses.

**LITERATURE CITED**


