Reduction of Murine Cutaneous UVB-Induced Tumor-Infiltrating T Lymphocytes by Dietary Canthaxanthin

James A. Rybski, Thomas M. Grogan, Mikel Aickin, and Helen L. Gensler

Departments of Pathology, Family and Community Medicine (Cancer Center Division), and Radiation Oncology, University of Arizona College of Medicine, Tucson, Arizona, U.S.A.

The effect of dietary canthaxanthin, retinyl palmitate, or their combination on the tumor-infiltrating T-lymphocyte response (T-TIL) in de novo murine ultraviolet type B irradiation-induced tumors was investigated to elucidate potential mechanisms of action of these compounds. We found that dietary canthaxanthin greatly reduced the number of tumor-infiltrating helper/inducer, suppressor/cytotoxic, and interleukin-2 receptor-positive T lymphocytes and also observed a concomitant statistically significant increase in tumour incidence in canthaxanthin-fed animals. The addition of retinyl palmitate to the canthaxanthin diet ameliorated this negative effect on TIL and the development of skin tumors. We conclude that dietary retinyl palmitate and canthaxanthin can modulate the host T-cell immune response within a growing tumor and may affect tumorigenicity. *J Invest Dermatol 97:892–897, 1991*

Non-melanoma skin cancer induced by ultraviolet B (UVB) irradiation is a serious national health problem [1]. There are approximately 400,000 new cases reported in the United States each year [2], and the incidence rate is increasing [3].

Pioneering work by Kripke [4] and Daynes et al [5] in the murine system has shown that non-melanoma skin cancer induction by UVB irradiation is dependent upon an active host immunosuppressive response mediated by suppressor T cells. These suppressor T cells are specific for tumor-associated antigen [6]. They are capable of preventing the antitumor response following transfer of tumor cells into non-irradiated animals by suppressing the induction of UVB-tumor specific helper T cells [7,8]. These suppressor T cells are critical for tumor development in that most UVB-induced non-melanoma skin tumors are of the "regressor" phenotype, i.e., they will not grow when adoptively transferred into non-irradiated syngeneic hosts [9].

Vitamin A and its derivatives (retinoids) are immunomodulators [10] and in animal-model systems are active as chemopreventive agents of photocarcinogenesis in albino hairless mice [11,12]. Epidemiologic evidence suggests that, in humans, a high dietary intake of retinoids leads to a decrease in cancer [13]. It has been shown that dietary retinoids can reduce both post-surgical as well as burn-induced immunosuppression [14,15], and these compounds are being tested for the prevention and treatment of human cancer, particularly cutaneous malignancies [16]. Animal studies indicate that retinoids can 1) overcome neonatal tolerance, a process thought to be mediated by suppressor T cells [17]; 2) increase the activity of immune accessory cells [18]; and 3) activate helper T-cell activity [19]. However, clinical use of retinoids has been limited due to their potential toxicity and teratogenicity [16].

Beta-carotene (a pro-vitamin A) and canthaxanthin (a non-vitamin A precursor carotenoid) are dose-dependently effective in reducing photocarcinogenesis in albino hairless mouse studies [20]. In man, high carotenoid intake has been associated with reduction in prostatic carcinoma [21] without the reported toxicity associated with retinoids [22]. The carotenoid mechanism of action is as yet unknown but may be linked to either its antioxidant activity [23,24] and/or its immunomodulatory capability [25–27]. Bendich and Shapiro [26] reported increased rat T- and B-cell mitogenic responses following 20 weeks of either dietary beta-carotene or canthaxanthin. Tomita et al [27] demonstrated that dietary beta-carotene resulted in a T-cell dependent rejection of syngeneic 3-methylcholanthrene-induced fibrosarcoma cells. In vitro, beta-carotene stimulated human peripheral blood leukocytes to secrete a cytokine effective against a wide range of human neoplastic cell lines [25].

This study addressed the hypothesis that dietary retinyl palmitate and/or canthaxanthin modulate photocarcinogenesis by means of altering subpopulations of T-cells infiltrating primary UVBinduced tumors. To test this hypothesis we applied the technique of immunohistochemistry to maintain and quantitate the number and subtypes of immune cells infiltrating the de novo UVB-induced tumors. We also measured the rates of tumor development and tumor burden per mouse as end points of consequence.

MATERIALS AND METHODS

Animals and Treatments Specific pathogen-free, pigmented female C3H/HeN strain mice (Charles River Breeding Laborato-

Manuscript received January 16, 1991; accepted for publication July 22, 1991.

Presented in part at the 77th Annual Meeting of The United States and Canadian Academy of Pathology, San Francisco, California, March 5–10, 1989 [Lab Invest 60:82A (abstract), 1989].

Supported by the National Institutes of Health grant CA-27502, CA-17094.

Reprint requests to: Dr. James A. Rybski, Department of Pathology, Room 5207, The University of Arizona Health Sciences Center, Tucson, Arizona 85719.

Abbreviations:

AIN: American Institute of Nutrition
BD: basal diet
CTX: basal diet supplemented with 1% (w/w) canthaxanthin
IL2R: interleukin-2 receptor
IU: international units
RPC: basal diet supplemented with a combination diet of 1% CTX and 120 IU RP
TIL: tumor-infiltrating lymphocytes
T-TIL: tumor-infiltrating T lymphocytes
UVB: ultraviolet irradiation type B
120 RP: basal diet supplemented with 120 IU of retinyl palmitate/gm

0022-202X/91/$03.50

Table I. Monoclonal Anti-Murine Antibodies Used in the Immunohistochemical Analysis of De Novo UVB-Induced Tumors

Monoclonal Antibody	Hybridoma Clone	Specificity	Working Concentration	Source[a]
Anti-mu	Ig8	slgM positive B-cells	1:200 dilution of ascitic fluid	1
Anti-kappa	OX40	95% slg positive B-cells	1:200 dilution of ascitic fluid	1
Anti-Thy 1.2	30-H12	T-cells	2.5 μg/ml	2
Anti-L3T4	GK1.5	Helper-inducer T-cells	5 μg/ml	2
Anti-Lyt2	53-6.7	Suppressor-cytotoxic T-cells	125 μg/ml	2
Anti-IL2R	AMT-13	Interleukin-2 receptor positive cells	8 μg/ml	3
Anti-Mac-1	M1/70	Resident and tumoricidal macrophages	4 μg/ml	3
Anti-Mac-2	M3/38	Phagocytic macrophages	20 μg/ml	3
Anti-HSA	M1/75	Erythrocytes	4 μg/ml	3
Anti-IA^k	Not available	IA^k positive cells	1.25 μg/ml	2

[a] 1, Accurate Chemical and Scientific Corporation, Westbury, NY. 2, Becton-Dickinson Immunocytometry Systems, Mountain View, CA. 3, Boehringer-Mannheim Corporation, Indianapolis, IN.

ries, Wilmington, MA) were fed the American Institute of Nutrition (AIN) synthetic 76A diet, which contained 4 International Units (IU) of retinyl palmitate per gram of diet as the retinoid source (Dyets, Inc., Bethlehem, PA). This basal diet (BD) was supplemented with either 120 IU of retinyl palmitate/gm (120 RP), 1% (w/w) canthaxanthin (CTX), or a combination diet of 1% CTX + 120 IU RP (RPC). The CTX and RP were provided by Hoffmann-La Roche (Nutley, NJ). The animals were UVB irradiated using the method of Daynes et al [28] 30 min/d, 5 times/week for 24 weeks with a bank of six FS40 fluorescent sun lamps. The animals' dorsa were shaved weekly and they received a total dose of 9.9×10^5 J/m^2.

Experimental Design Animals were randomized and began dietary treatments at 5 weeks of age. After 18 weeks, each dietary group was then divided into two treatment arms; one arm began UVB-irradiation and the other served as the unirradiated control group. Samples of de novo induced UVB tumors were taken from three animals/group at 29 weeks after the first UVB-radiation treatment. All animals remained on their diets throughout the experiment. A total of 220 animals were placed into the UVB-irradiated arm and were subdivided as follows: BD, 50 animals; 120 RP, 55 animals; RPC, 60 animals; and CTX, 55 animals. None of the unirradiated, dietary-matched animals developed skin tumors during the course of the experiment.

Immunohistochemistry Tumor biopsies were snap-frozen in OCT compound (Miles Laboratories, Elkhart, IN) at −150°C in liquid nitrogen–quenched isopentane. The frozen blocks were stored at −80°C until multiple 4-μm serial sections were cut and stained with a panel of anti-murine monoclonal antibodies to examine the overall cellular response to the de novo tumor (Table I). In this report we have focused on the T-TIL response as exemplified by monoclonal antibodies to L3T4, Lyt-2, and the interleukin-2 receptor (IL2R). Antigen expression was visualized using biotin:avidin-horseradish peroxidase with diaminobenzidine as chromogen [29]. Slides were counterstained with methyl green and antigen-positive cells were enumerated in seven adjacent high-power fields of highest lymphocyte infiltration, yielding a total of 0.44 mm^2 analyzed per mouse. Three animals/group were studied and the T-TIL response was enumerated 4 times/animals.

Statistical Analysis The count data were analyzed using a mixed-effects extension of the basic Poisson regression model [30] with an allowance for extra-Poisson variability [31] or the Wilcoxon rank-sum test, as indicated in the figure legends. The immunohistochemical and tumor size measurements were conducted in a blind fashion. Tumor-free survival was calculated from the date of first UVB irradiation to the date of the first tumor development. The survival curves displayed in the figures were calculated using the Kaplan-Meier method [32]. The log-rank method was used for comparisons [33]. All tests were two-tailed.

RESULTS

We tested the hypothesis that dietary canthaxanthin and retinyl palmitate alter subpopulations of tumor-infiltrating T lymphocytes (T-TIL) in primary UVB-induced skin tumors. The subcutaneous malignancies found in these UVB-irradiated mice exhibited a pleomorphic, large cell appearance. In some instances the malignancies had a spindling aspect, suggesting a soft-tissue sarcoma. The autochthonous tumor burden per mouse at 27.5 weeks after the first ultraviolet irradiation is presented in Fig 1. Based upon analysis of variance, CTX and 120 RP resulted in tumor burdens significantly lower than that found in mice fed the basal diet ($p = 0.0081$ and 0.0135, respectively). Dietary supplementation with a combination

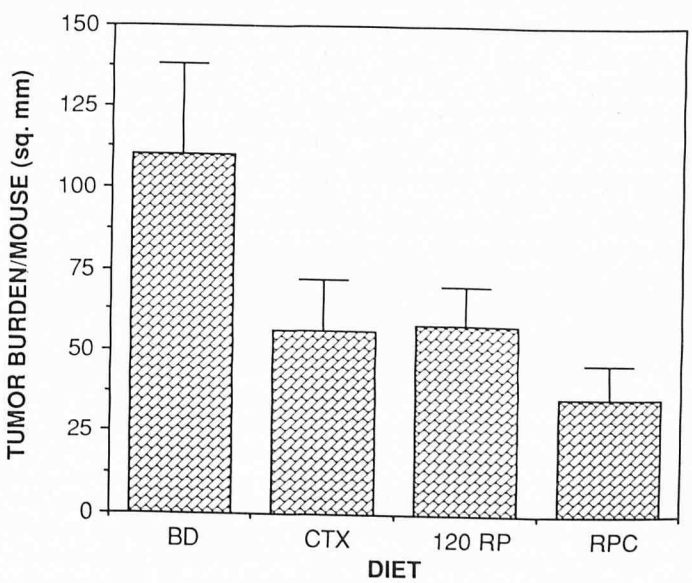
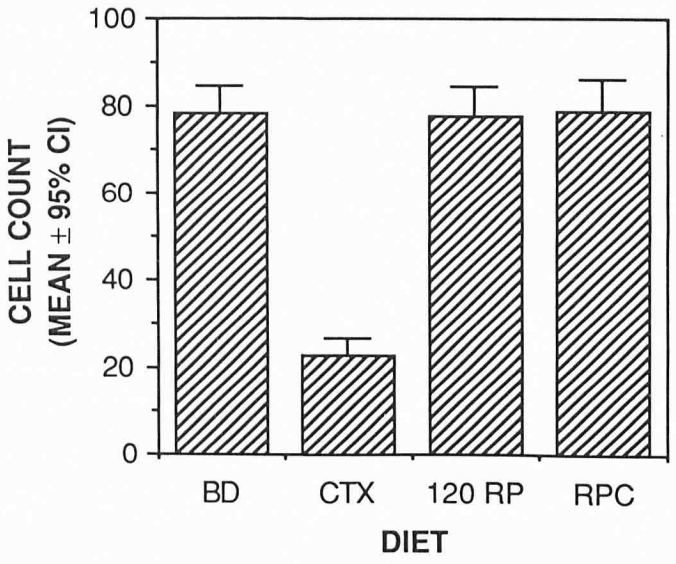


Figure 1. Autochthonous tumor burden per mouse in UVB-irradiated animals fed retinyl palmitate and/or canthaxanthin. Tumors were induced by 9.9×10^5 J/m^2 of UVB delivered over a 24-week period. The tumor area per mouse was calculated as the total tumor area (length × width of each tumor) per mouse at 27.5 weeks after the first ultraviolet radiation exposure. The data are expressed as the mean ± SEM. BD, basal diet consisting of AIN 76A supplemented with placebo beadlets; CTX, basal diet supplemented with 1% canthaxanthin; 120 RP, basal diet supplemented with 120 IU of retinyl palmitate per gram of diet; RPC, basal diet supplemented with 1% canthaxanthin plus 120 IU of retinyl palmitate per gram of diet.



SIMULTANEOUS TESTS OF MEAN DIFFERENCE AT 0.05 LEVEL

COMPARISON	P-VALUE
BD vs. CTX	0.0000*
BD vs. 120 RP	0.9282
BD vs. RPC	0.9442
CTX vs. 120 RP	0.0000*
CTX vs. RPC	0.0000*
120 RP vs. RPC	0.8806

Figure 2. Effect of dietary retinyl palmitate and/or canthaxanthin on the host UVB-tumor infiltrating L3T4-positive lymphocyte response. Mice were treated as described in the legend for Fig 1. Tumor biopsies from three animals per group were snap-frozen and 4-μm sections were stained with monoclonal anti-L3T4 antibody. Antigen-antibody interaction was visualized with biotin:avidin horseradish peroxidase methodology and DAB as chromogen. Antigen-positive cells were enumerated in seven high-power fields (40× objective) and analyzed with a mixed-effects extension of the Poisson regression model. Data are expressed as the mean cell count ± 95% confidence interval.

of these two micronutrients resulted in further reduction of tumor burden. These results indicate that the 120 RP and CTX were acting as chemomodulatory agents in these pigmented mice.

The number of L3T4-positive (helper/inducer phenotype) T cells within these primary UVB-induced tumors was dramatically reduced in animals fed 1% canthaxanthin (CTX) when compared with the three other tumor-bearing dietary groups ($p \ll 0.0001$, Fig 2). However, there was no difference in the number of L3T4-positive TIL in the mice fed 120 IU retinyl palmitate/gm of diet (120 RP), 1% canthaxanthin plus 120 IU retinyl palmitate (RPC), or the basal diet (BD) (Fig 2).

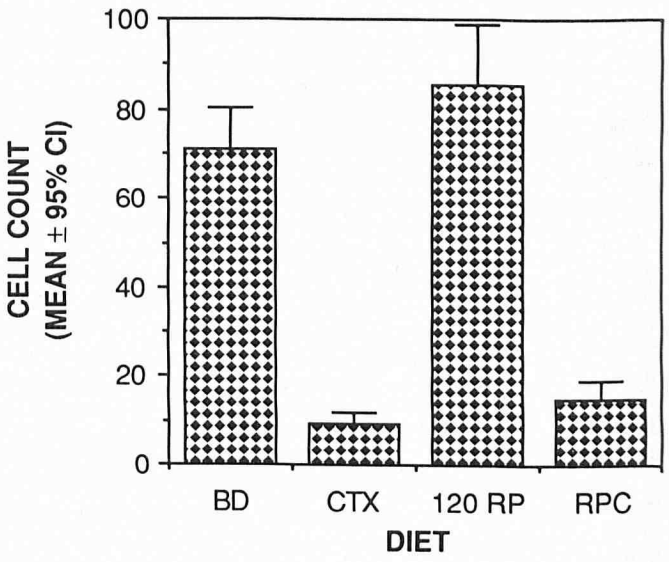
The Lyt-2-positive (suppressor/cytotoxic phenotype) TIL in the CTX group was also sharply reduced when ranked against the levels found in animals fed the BD ($p \ll 0.0001$) or 120 RP ($p \ll 0.0001$) (Fig 3). Those animals provided with the combination diet also had severely reduced Lyt-2-positive TIL compared to BD animals ($p \ll 0.0001$), although the reduction was less than in those animals receiving CTX alone ($p = 0.0122$). Interestingly, there was a marginal increase in the number of Lyt-2-positive TIL in those animals treated with 120 RP over values in the BD animals ($p = 0.0644$).

The number of IL2R-positive TIL in animals fed CTX was significantly lower than that found in all other diets ($p \ll 0.0001$, Fig 4). However, the IL2R-positive TIL response was greater in 120 RP-fed animals than in the BD animals ($p \ll 0.0001$), and those animals receiving RPC had a larger IL2R TIL response than CTX mice ($p \ll 0.0001$), but the RPC-induced response was somewhat reduced when compared to the BD animals ($p = 0.063$).

Collectively, these data suggest that the presence of dietary 120 RP tended to offset the inhibitory effect of CTX on T-TIL. This can be seen in the numerical parity of L3T4-positive TIL in animals fed RPC, 120 RP, and BD (Fig 2) and the increase in the number of Lyt-2 positive ($p = 0.0122$, CTX versus RPC; Fig 3) and IL2R-positive TIL ($p \ll 0.0001$, CTX versus RPC; Fig 4).

Tumor-free survival was significantly less in the CTX group than the BD animals (Fig 5*A*, $p = 0.016$), although there was a delay in the initial development of tumors in animals fed 1% canthaxanthin. Addition of 120 IU of retinyl palmitate to the 1% canthaxanthin diet ameliorated the negative impact of CTX on tumor-free survival (Fig 5*B*, $p = 0.231$ BD versus RPC and Fig 5*C*, $p = 0.045$ RPC versus CTX).

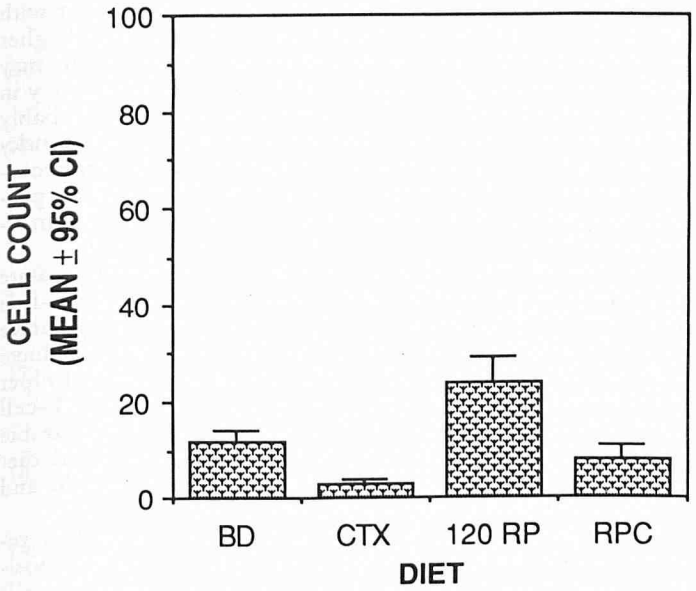
The combination of CTX or 120 RP with UVB irradiation did



SIMULTANEOUS TESTS OF MEAN DIFFERENCE AT 0.05 LEVEL

COMPARISON	P-VALUE
BD vs. CTX	0.0000*
BD vs. 120 RP	0.0644
BD vs. RPC	0.0000*
CTX vs. 120 RP	0.0000*
CTX vs. RPC	0.0122*
120 RP vs. RPC	0.0000*

Figure 3. Effect of dietary micronutrients on the host UVB-tumor infiltrating Lyt-2-positive lymphocyte response. Mouse treatment and symbols are described in the legend for Fig 1. Tumor biopsy, immunohistochemistry, cell enumeration, and statistical methods are as described in the legend for Fig 2 with the exception that anti-Lyt-2 antibody was used in the place of anti-L3T4.



SIMULTANEOUS TESTS OF MEAN DIFFERENCE AT 0.05 LEVEL

COMPARISON	P-VALUE
BD vs. CTX	0.0000*
BD vs. 120 RP	0.0000*
BD vs. RPC	0.0630
CTX vs. 120 RP	0.0000*
CTX vs. RPC	0.0000*
120 RP vs. RPC	0.0000*

Figure 4. Effect of dietary micronutrients on the host UVB-tumor infiltrating interleukin-2 receptor (IL2R)–positive lymphocyte response. Mouse treatment and symbols are described in the legend for Fig 1. Tumor biopsy, immunohistochemistry, cell enumeration, and statistical methods are as described in the legend for Fig 2 with the exception that anti-IL2R antibody was used in the place of anti-L3T4.

not selectively inhibit the growth of these animals (Fig 6). Although the irradiation procedure limited weight gain by all exposed animals, only those UVB-irradiated mice fed the combination diet had a significantly lower body weight than the BD mice, based upon analysis of variance, and this difference averaged only 1.5 g/mouse. Thus, the observed reduction of T-TIL and decreased tumor-free survival in the CTX group cannot be attributed to selective dietary toxicity or nutritional deficiency.

DISCUSSION

Dietary canthaxanthin (CTX) resulted in a profound depression of L3T4-positive and Lyt-2–positive T lymphocytes infiltrating primary UVB-induced tumors. There was also a sharp decline (relative to control levels) in cells positive for the interleukin-2 receptor (IL2R), a marker of T-cell activation. Additionally, those animals fed CTX had a delay in tumor development, but a poorer overall tumor-free survival than the BD or RPC groups. Thus, this single dietary component appears to have regulated T-cell infiltration into these autochthonous tumors and host susceptibility to tumor development. When 120 RP was added in combination with dietary CTX, the number of L3T4-positive and IL2R-positive cells in the tumors were at control levels and tumor-free survival was not sig-

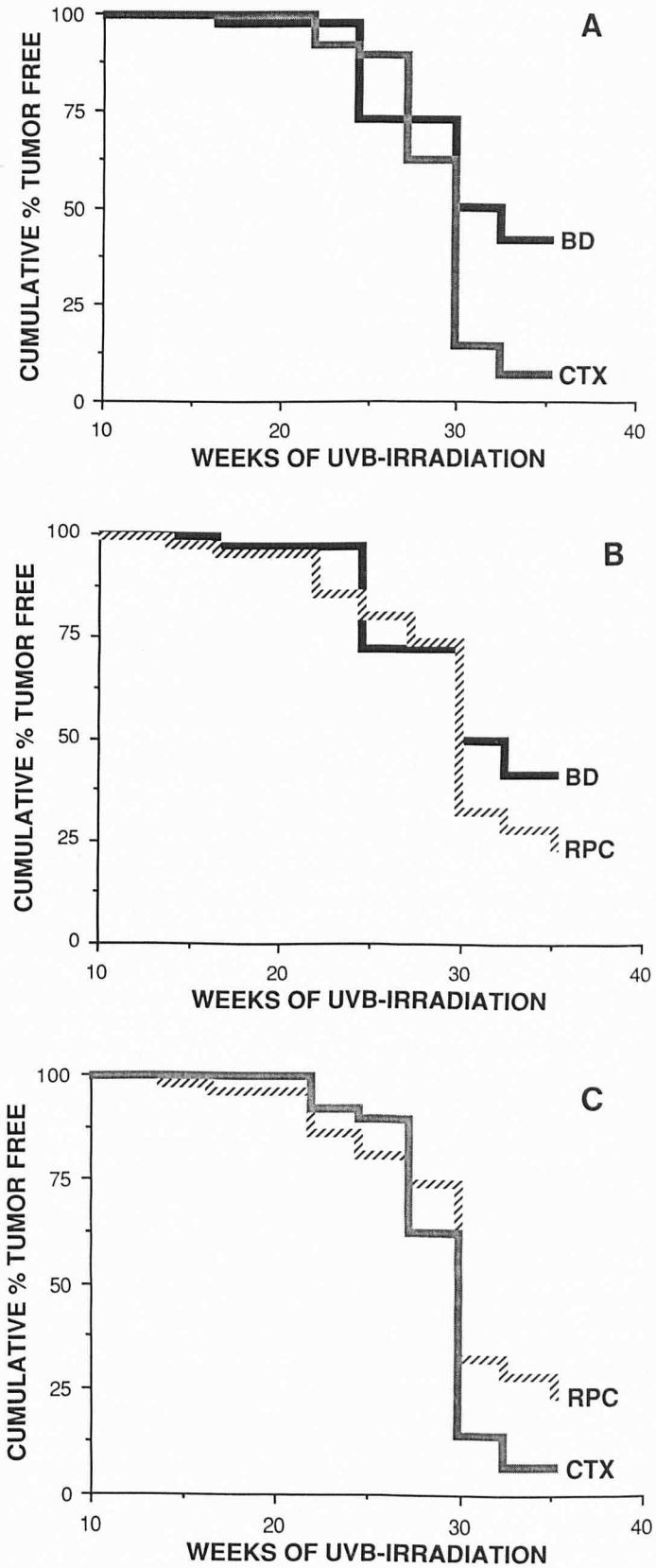


Figure 5. Correlation of tumor-free survival with dietary micronutrient treatment in UVB-irradiated mice. *A* compares 50 animals fed the basal diet (BD) with 55 canthaxanthin (CTX)-fed animals ($p = 0.016$, log-rank). *B* compares the BD group with 60 animals fed the combined diet (RPC) consisting of 120 IU retinyl palmitate/gm plus 1% canthaxanthin ($p = 0.231$, log-rank). *C* compares the RPC fed animals with the CTX group ($p = 0.045$, log-rank).

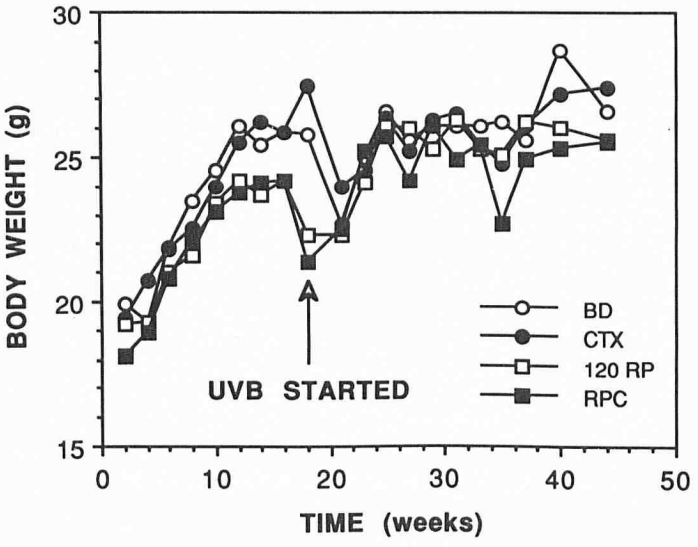


Figure 6. Effect of dietary retinyl palmitate and/or canthaxanthin on the body weight of UVB-irradiated mice. Animal treatment and symbols are described in the legend for Fig 1. Data are expressed as the mean of 20 mice per group.

nificantly different from the BD group, so that retinyl palmitate mitigated the canthaxanthin effect.

The mechanism by which canthaxanthin modulates T-cell infiltration into UVB-induced tumors is unknown. However, there have been reports of T-cell immunoregulation by carotenoids. Male Wistar rats fed canthaxanthin and beta-carotene had elevated splenic T- and B-cell mitogen-induced responses [26] and mice fed beta-carotene exhibited a heightened rejection of syngeneic fibrosarcoma cells mediated by Lyt-2-positive T-lymphocytes [27]. Alternatively, the function of antigen-presenting cells may have been influenced by the canthaxanthin diet, thereby affecting recruitment of T cells into the tumors. Such influences by carotenoids have been described [25]. More recently Mufti and Watson reported a statistically significant increase in the percentage of activated splenic marcrophages in C57BL/6 mice fed 1% canthaxanthin for 12 weeks [34]. Additionally, Schwartz et al showed that oral canthaxanthin increased the macrophage and T-cell infiltrate into 7,12-dimethylbenz(a)anthracene-induced squamous cell carcinomas in a hamster model [35].

If tumor containment was Lyt-2-positive T-lymphocyte dependent in the present study, one would predict that the CTX group would have had the largest tumor burden, but this was not the case. Philipps et al [36] have shown that the in vitro cytotoxic lymphocyte response to the regressor UV-tumor 1591 was Lyt-2-positive and selection for progressor variants of 1591 by these cytotoxic lymphocytes led to the sequential loss of immunodominant class I major histocompatibility complex-like molecules resulting in a highly metastatic progressor variant. Lill and Fortner [37] reported that regressing UVB tumors from unirradiated animals contained three-fold greater numbers of highly cytotoxic T lymphocytes than progressing tumors. These T cells were isolated directly from tumor fragments implanted into UVB-irradiated (immunosuppressed) or unirradiated (immunocompetent) animals. The present protocol, however, involved measurement of the hosts' tumor-infiltrating T-lymphocyte response in de novo UVB tumors as opposed to transfer of tumor fragments and would therefore depict the natural pathogenesis of these tumors.

It is intriguing to note that those animals fed the basal diet supplemented exclusively with CTX had an increased latency, as was also reported by Mathews-Roth [20]. However, at the thirty-fifth week after initiation of UVB irradiation, the CTX group had a poorer overall tumor-free survival than the other groups, in contrast with the results of Mathews-Roth and Krinsky [38,39]. This higher tumor incidence at the completion of the present experiment may be the in vivo consequence of the L3T4-positive cell deficiency in the CTX group. Our discordant tumor incidence results probably reflect differences in mouse strains as Mathews-Roth and Krinsky used an albino mouse model and a different UVB-induction protocol. The carotenoid effect appears to be one of inhibiting the promotional phase of carcinogenesis without altering overall tumor-free survival [40].

The second finding of this study was that dietary retinyl palmitate lessened the suppressive effect of canthaxanthin on both tumor-free survival and T-TIL, most notably on the number of L3T4-positive helper T lymphocytes. Studies by Romerdahl and Kripke [7,8] have shown that regressing murine UVB tumors contain a greater helper T-lymphocyte activity than progressing tumors, and helper T-cell activity was vital for tumor rejection. The presence of comparable numbers of helper T cells in the RPC group with the basal diet group may explain, in part, the reduction in tumor burden and improved tumor-free survival in the RPC group.

In summary, dietary supplementation with canthaxanthin resulted in a dramatic decrease, relative to control levels, in L3T4-positive, Lyt-2-positive, and interleukin-2 receptor-positive T cells infiltrating into autochthonous UVB-induced tumors in mice. Accompanying this reduction in T-TIL was a decrease in tumor-free survival. The addition of retinyl palmitate, along with dietary canthaxanthin, prevented both the canthaxanthin-induced reduction of L3T4-positive and interleukin-2 receptor-positive T cells in autochthonous UVB-induced tumors, and the canthaxanthin-induced decrease in tumor-free survival. Thus, the hypothesis that canthaxanthins' mechanism of action is to increase T-TIL was not substantiated by this study, whereas one antineoplastic mechanism of action of retinyl palmitate may indeed be an increase in T-TIL. A major role for nutrition is recognized in assessments of cancer risk factors [41,42], and it appears likely that dietary immunomodulation will prove to be a significant component of host susceptibility.

REFERENCES

1. Penn I: Ultraviolet light and skin cancer. Immunol Today 6:206-208, 1985
2. Scotto J, Fears TR, Fraumeni JF: Incidence of nonmelanoma skin cancer in the United States. NIH Publication No. 83:2433, 1983
3. Fears TR, Scotto J: Changes in skin cancer morbidity between 1971-1972 and 1977-1978. J Nat Cancer Inst 69:365-370, 1982
4. Kripke ML: Immunological unresponsiveness induced by ultraviolet radiation. Immunol Rev 80:87-102, 1984
5. Daynes RA, Burnham DK, DeWitt CW, Roberts LK, Krueger GG: The immunobiology of ultraviolet radiation carcinogenesis. Cancer Surv 4:51-99, 1985
6. Roberts LK: Characterization of a cloned ultraviolet radiation (UV)-induced suppressor T-cell line that is capable of inhibiting anti-UV tumor-immune responses. J Immunol 136:1908-1916, 1986
7. Romerdahl CA, Kripke ML: Regulation of the immune response against UV-induced skin cancers: specificity of helper cells and their susceptibility to UV-induced suppressor cells. J Immunol 137:3031-3035, 1986
8. Romerdahl CA, Kripke ML: Role of helper T-lymphocytes in rejection of UV-induced murine skin cancers. Cancer Res 48:2325-2328, 1988
9. Kripke ML: Immunoregulation of carcinogenesis: past, present and future. J Natl Canc Inst 80:722-727, 1988
10. Watson RR, Rybski JA: Immunological response modification by vitamin A and other retinoids. In: Chandra R (ed.). Nutrition and Immunology. Alan R. Liss, New York, 1988, pp 87-99
11. Connor MJ, Lowe NJ, Breeding JH, Chalet M: Inhibition of ultraviolet-B-skin carcinogenesis by all-trans-retinoic acid regimens that inhibit ornithine decarboxylase induction. Cancer Res 43:171-174, 1983
12. Epstein JH, Grekin DA: The effect of chronic applications of tritinoin

(retinoic acid) on acute responses of mammalian skin to UVB radiation in vivo. J Invest Dermatol 76:178–180, 1981

13. Mathews-Roth MM: Carotenoids and cancer prevention—experimental and epidemiological studies. Pure Appl Chem 57:717–722, 1985
14. Cohen BE, Gill G, Cullen PR, Morris PJ: Reversal of postoperative immunosuppression in man by vitamin A. Surg Gynecol Obstet 149:658–662, 1979
15. Fusi S, Kupper TS, Green DR, Ariyan S: Reversal of post burn immunosuppression by administration of vitamin A. Surgery 96:330–335, 1984
16. Gensler HL, Meyskens F: The role of retinoids in cutaneous cancer prevention. In: Dawson MI (ed.). Chemistry and Biology of Synthetic Retinoids. CRC Press, Boca Raton, FL (in press)
17. Malkovsky M, Medawar PB, Thatcher DR, Toy J, Hunt LS, Dore C: Acquired immunological tolerance of foreign cells is impaired by recombinant interleukin 2 or vitamin A acetate. Proc Natl Acad Sci USA 82:536–538, 1985
18. Katz DR, Drzymala M, Turton JA, Hicks RM, Hunt R, Palmer L, Malkovsky M: Regulation of accessory cell function by retinoids in murine immune responses. Br J Exp Pathol 68:343–350, 1987
19. Carman JA, Smith SM, Hayes CE: Characterization of a helper T lymphocyte defect in vitamin A-deficient mice. J Immunol 142:388–393, 1989
20. Mathews-Roth MM: Antitumor activity of β-carotene, canthaxanthin and phytoene. Oncology 39:33–37, 1982
21. Ohno Y, Yoshida O, Okada K, Yamabe H, Schroeder FH: Dietary β-carotene and cancer of the prostate: a case control study. Cancer Res 48:1331–1336, 1988
22. Mathews-Roth MM: Lack of genotoxicity with beta-carotene. Toxicol Lett 41:185–191, 1988
23. Burton GW, Ingold KU: Beta-carotene: an unusual type of lipid antioxidant. Science 224:569–573, 1984
24. Mathews-Roth MM, Wilson T, Fujimori E, Krinsky NI: Carotenoid chromophore length and protection against photosensitization. Photochem Photobiol 19:217–222, 1974
25. Abril EA, Rybski JA, Scuderi P, Watson RR: Beta-carotene stimulates human leukocytes to secrete a novel cytokine. J Leuk Biol 45:255–261, 1989
26. Bendich A, Shapiro S. Effect of β-carotene and canthaxanthin on the immune responses of the rat. J Nutr 116:2254–2262, 1986
27. Tomita Y, Himeno K, Nomota K, Endo H, Hirohata T: Augmentation of tumor immunity against syngeneic tumors in mice by β-carotene. J Nat Cancer Inst 78:679–681, 1987
28. Daynes RA, Schmitt MK, Roberts LK, Spellman CW: Phenotypic and physical characteristics of the lymphoid cells involved in the immunity of syngeneic UV-induced tumors. J Immunol 122:2458–2464, 1979
29. Grogan TM, Fielder K, Rangel C, Jolley CJ, Wirt DP, Hicks MJ, Miller TP, Brooks R, Greenberg B, Jones S: Peripheral T-cell lymphoma: aggressive disease with heterogeneous immunotypes. Am J Clin Pathol 83:279–288, 1985
30. Frome EL: The analysis of rates of Poisson regression models. Biometrics 39:665–674, 1983
31. Breslow NE: Extra-Poisson variation in log-linear models. Appl Stat 33:38–44, 1984
32. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958
33. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163–170, 1966
34. Mufti S, Watson RRW: Effect of chronic ethanol consumption and retroviral infection on carcinogenesis and its possible inhibition by an immunostimulant, canthaxanthin: definition of a murine model. Prog Clin Biol Res 325:283–304, 1990
35. Schwartz J, Sloane D, Shklar G: Prevention and inhibition of oral cancer in the hamster buccal pouch model associated with carotenoid immune enhancement. Tumor Biol 10:297–309, 1989
36. Philipps C, Strauss H, Wortzel R, Schreiber H: Unusual or aberrant MHC antigen expression on tumors. A novel MHC Class I molecule as a tumor specific antigen. Correlation between the antibody defined and CTL defined target structures. J Immunogenetics 13:93–99, 1986
37. Lill P, Fortner G: Identification and cytotoxic reactivity of inflammatory cells recovered from progressing or regressing syngeneic UV-induced murine tumors. J Immunol 121:1854–1860, 1978
38. Mathews-Roth MM, Krinsky NI: Carotenoid dose level and protection against UV-B induced skin tumors. Photochem Photobiol 42:35–38, 1985
39. Mathews-Roth MM, Krinsky NI: Carotenoids affect development of UV-B induced skin cancer. Photochem Photobiol 46:507–509, 1987
40. Krinsky N: Carotenoids and cancer in animal models. J Nutr 119:123–126, 1989
41. Doll R, Peto R: The Causes of Cancer. Oxford University Press, Oxford, 1981
42. Committee on Diet, Nutrition and Cancer: NCI Monograph, No. 2. National Academy Press, Washington, 1982