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Effects of lycopene, a carotenoid, on intrathymic T cell differentiation and peripheral CD4/CD8 ratio in a high mammary tumor strain of SHN retired mice

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We previously reported that lycopene, one of the carotenoids, significantly suppressed the development of spontaneous mammary tumors of mice and the contribution of lycopene to the maintenance of physical homeostasis was suggested. In this study, we investigated the effects of lycopene on the intrathymic and peripheral T cell subpopulation as immunoregulatory parameters. In tumorous control mice, inactivation of intrathymic T cell differentiation, an increase of the percentage of $CD4^+$ $CD8^-$ cells and a decrease of $CD4^+CD8^+$ cells were observed. On the other hand, in the lycopene-treated mice, the abnormal intrathymic T cell differentiation was recovered and brought to non-tumorous levels. The peripheral CD4/CD8 ratio was partially augmented by lycopene treatment which resulted from an increased $CD4^+$ subpopulation. These observations suggest that one of the immunomodulating roles of lycopene is to normalize the change of intrathymic T cell differentiation caused by tumorigenesis.

Key words: Carotenoid, lycopene, mammary tumor, mice, T cell.

Introduction

Recent studies indicated that β-carotene and carotenoids were effective in the prevention of several types of tumors.[1,2] We found that the administration of natural β-carotene-rich algae *Dunaliella bardawil* prevented spontaneous mammary tumorigenesis in mice.[3,4] Lycopene, one of the carotenoids, is considered to be superior to β-carotene in quenching the singlet oxygen[5] and further contributes to the same extent or even more than β-carotene to total carotenoids in human tissue.[6]

Previously, we found that chronic ingestion of lycopene markedly suppressed the development of spontaneous mammary tumors in mice and indicated the possibility of the contribution of lycopene to the maintenance of physical homeostasis.[7]

There is accumulating evidence that carotenoids can enhance the immune response, including nonspecific and specific immune functions and tumor immunity.[8] This tempted us to examine the effects of lycopene on intrathymic T cell differentiation and the peripheral T cell subpopulation as immunoregulatory parameters. This paper deals with this problem.

Materials and methods

Animals and treatments

Retired female mice of a high mammary tumor strain of SHN/Mei[9,10] maintained in our laboratory were used. Throughout the experiments, the animals were kept in teflon cages (15 × 27 × 12 cm) with wood shavings (three or four each), maintained in a windowless animal room, which was air conditioned (21–22°C and 55–75% relative humidity) and artificially illuminated (14 h of light from 5:00 a.m. to 7:00 p.m.) and provided with tap water *ad libitum*. Each mouse was checked for palpable size (≥3 mm) of mammary tumors every 7 days. Both tumorous and non-tumorous mice were divided into two groups, i.e. control and experimental. The control mice received AIN-76TM diet (Nihon-Nosan Kogyo KK, Yokohama, Japan). The experimental mice were given AIN-76TM diet supplemented with lycopene (Makhteshim Chemical Works, Beer-Sheva, Israel) at the concentration of 5.0×10^{-5}%. Each diet, which was packed *in vacuo* and kept at −20°C, was changed every day. After 10 days of treatment, mice were killed by decapitation under light ether anesthesia.

Preparation of lymphoid cell

At autopsy, blood was collected from the trunk and diluted twice with 10 U/ml of heparin/PBS(−). Thy-

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Table 1. Body weight and weights of thymus and spleen in each group (mean ± SEM)

Group	Body weight (g)	Thymus weight (mg/30 g body weight)	Spleen weight (mg/30 g body weight)
Non-tumorous			
control (6)	31.8 ± 0.7	30.3 ± 5.4	95.6 ± 7.0[a]
experimental (5)	30.1 ± 0.7	25.5 ± 4.4	93.9 ± 7.4[a]
Tumorous			
control (5)	31.6 ± 1.0	29.7 ± 1.9	108.9 ± 4.2[a]
experimental (5)	31.7 ± 0.8	26.2 ± 3.6	87.2 ± 6.6[b]

Number of estimates is given in parentheses.
[a,b]Values with different superscripts are different at $p < 0.05$.

mus and spleen were also immediately removed, weighed and pressed with slide glass in PBS(−). The cell suspension was passed through a #200 metal sieve and washed three times with PBS(−). Diluted blood and thymic and splenic cell suspensions were layered onto Ficoll-Conray solution (Immuno-Biological Laboratories, Fujioka, Japan) and centrifuged at 400 *g* for 30 min at room temperature. The interface layer was collected and washed three times with PBS(−).

Flow cytometric analysis

Cells were incubated with 1 μg/million cells of R-phycoerythrin (PE)-conjugated anti-mouse CD4 and fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD8a rat IgG monoclonal antibody (Phar-

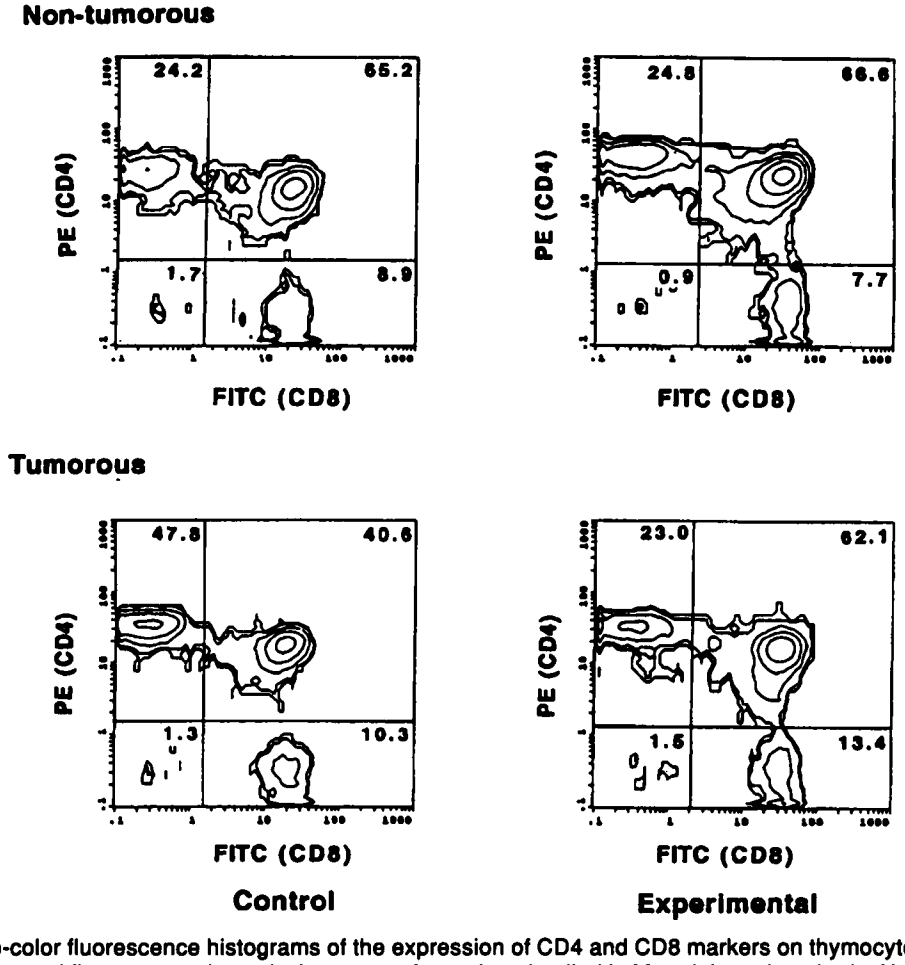

Figure 1. Two-color fluorescence histograms of the expression of CD4 and CD8 markers on thymocytes from mice of each group. Staining and flow cytometric analysis were performed as detailed in Materials and methods. Numbers in histograms are the percentage of each subpopulation.

Table 2. Percentage of thymocytes expressing surface antigenic markers in each group (mean ± SEM)

Group	$CD4^+CD8^-$	$CD4^+CD8^+$	$CD4^-CD8^-$	$CD4^-CD8^+$
Non-tumorous				
control (5)	27.7 ± 4.7^a	58.5 ± 7.4	3.2 ± 0.6^a	10.6 ± 2.1
experimental (5)	$35.2 \pm 8.9^{a,b}$	54.0 ± 11.9	1.2 ± 0.5^b	9.4 ± 2.4
Tumorous				
control (5)	48.7 ± 7.2^b	37.4 ± 8.9	$2.2 \pm 0.3^{a,b}$	11.8 ± 1.5
experimental (5)	26.1 ± 2.2^a	63.2 ± 2.1	$1.5 \pm 0.3^{a,b}$	9.4 ± 1.1

Number of estimates is given in parentheses.
^{a,b}Values with different superscripts are different at $p < 0.05$.

Table 3. Percentage of splenic lymphocytes expressing surface antigenic markers and CD4/CD8 ratio in spleen of each group (mean ± SEM)

Group	$CD4^+CD8^-$	$CD4^-CD8^+$	CD4/CD8
Non-tumorous			
control (6)	33.2 ± 1.4	14.7 ± 0.8	2.3 ± 0.1^a
experimental (5)	39.8 ± 3.6	14.0 ± 1.1	3.2 ± 0.3^b
Tumorous			
control (5)	38.6 ± 3.1	13.1 ± 1.0	$3.0 \pm 0.2^{a,b}$
experimental (5)	38.2 ± 3.1	12.6 ± 0.5	$3.1 \pm 0.4^{a,b}$

Number of estimates is given in parentheses.
^{a,b}Values with different superscripts are different at $p < 0.05$.

Table 4. Percentage of peripheral blood lymphocytes expressing surface antigenic markers and CD4/CD8 ratio in each group (mean ± SEM)

Group	$CD4^+CD8^-$	$CD4^-CD8^+$	CD4/CD8
Non-tumorous			
control (6)	55.9 ± 1.4	22.9 ± 1.8	2.5 ± 0.2
experimental (5)	63.3 ± 1.6	21.9 ± 2.0	3.0 ± 0.3
Tumorous			
control (5)	58.0 ± 2.5	22.3 ± 1.8	2.7 ± 0.3
experimental (5)	64.8 ± 1.9	22.2 ± 1.6	3.0 ± 0.3

Number of estimates is given in parentheses.

Mingen, San Diego, CA) for 1 h at 4°C. Stained cells were washed three times with PBS(−) and analyzed by an EPICS Elite flow cytometer (Coulter Cytometry, Hialeah, FL). A fluorescence histogram of at least 5000 counts was collected each sample.

Statistics

Data in each parameter were analyzed by the multiple range test to evaluate the statistical significance of difference.

Results

Lycopene showed little effect on body weight and weights of thymus and spleen in non-tumorous mice. However, in tumorous mice, spleen weight of the experimental group was significantly smaller than that of the control (Table 1).

As shown in Figure 1 and Table 2, there were little differences in the intrathymic T cell subpopulations between the control and the experimental groups of non-tumorous mice, except the $CD4^-CD8^-$ proportion, which was significantly higher in the former. In tumorous mice, the percentages of $CD4^+$ $CD8^-$ cells and $CD4^+CD8^+$ cells in the experimental group were apparently lower and higher, respectively, than those in the control. In general, the proportions of the intrathymic T cell subpopulation of the tumorous mice were maintained at the levels of non-tumorous mice by lycopene treatment.

In non-tumorous mice, the CD4/CD8 ratio in splenic lymphocyte was significantly higher in the experimental group than in the control. The ratio was increased in the control of the tumorous animals; however, it was little changed in the experimental group (Table 3).

The CD4/CD8 ratio in the peripheral blood lymphocytes increased in the experimental group more than in the control, in the non-tumorous mice, but the difference was not significant (Table 4).

Discussion

It is well known that carotenoids enhance the immune function. These include T and B lymphocyte proliferation, the induction of specific effector cells capable of killing tumor cells, and the secretion of factors required for communication between immunologically competent cells.[8] Lycopene, one of the carotenoids, has a marked anti-mammary tumor potential[7] and thus we examined the effect of lycopene on T cell differentiation.

Inactivation of intrathymic T cell differentiation was observed by malignancies.[11,12] The studies using the mice transplanted hepatoma MH134 cells[11] and mammary adenocarcinoma D1-DMBA-3 cells[12] revealed an increase and a decrease of

$CD4^+CD8^-$ cells and $CD4^+CD8^+$ cells, respectively. Quite similarly, in the present study, $CD4^+CD8^-$ and $CD4^+CD8^+$ cells were elevated and decreased, respectively, in mice developing spontaneous mammary tumors. On the other hand, lycopene treatment for 10 days recovered the proportions of $CD4^+CD8^-$ and $CD4^+CD8^+$ cells to those in the thymus of non-tumorous mice.

The peripheral CD4/CD8 ratio increased in the lycopene-treated non-tumorous mice, whereas it did not in the tumorous mice. In all groups, the proportions of $CD8^+$ cells were constant and thus an increase of the CD4/CD8 ratio is due to an increase in the proportion of the $CD4^+$ cells. It has been reported that β-carotene supplementation increased the number of T helper $(CD4)^+$ cells.[13] CD4 antigen is expressed on $CD8^-$ cells in the peripheral tissue and is commonly used as a convenient marker for helper T cells.[14] Helper $CD4^+$ T cells are known to release multiple cytokines which may be essential for the functional activation of human tumor-reactive effector cells.[15].

All observations suggest that lycopene enhances the immunoresponse by increasing helper T cells and that one of the immunomodulating actions of lycopene is to normalize the change of intrathymic T cell differentiation caused by tumorigenesis.

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