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Effect of Vitamin E as an Immunopotentiation Agent and Its Influence on Tumor Growth in Mice

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Introduction

Since the discovery of vitamin E[4], its physiological function has been thought to be due either to an antioxidant role[19] or to a possible role in stabilizing cell membranes[12]. Other mechanisms such as the regulation of Prostaglandin synthesis by vitamin E have been suggested[9].

Recently, it has been reported that vitamin E, supplemented by diet, enhances humoral immune responses to sheep red blood cells[20], increases resistance to bacterial infections[8] or stimulates phagocytosis[2]. In regard to cellular immunity, Tanaka et al.[18] have suggested that vitamin E enhanced helper T cell activity through the assay of antibody titer in mice, and Sheffy[16] has reported that lymphoproliferative responses were depressed in cells from vitamin E deficient dogs. Furthermore, Lim et al.[11] have reported that a significant activation of antibody-dependent cellular cytotoxicity (ADCC) was shown in mice receiving high vitamin E diet.

But all of these reports do not mention the appropriate dosage and serum tocopherol level which manifest the immunopotentiative effect. We studied the relation between immune responses and serum vitamin E levels in detail and reported[22] that lymphoproliferative responses to PHA, Con A and LPS were significantly enhanced after 5 to 20 IU/kg/day of vitamin E were injected intraperitoneally (i.p.) daily for 14 days and doses exceeding 80 IU/kg/day were toxic in BALB/c mice.

In the present study, we have reported that spleen cells prepared from normal or Meth-A tumor bearing BALB/c mice, receiving 14 daily i.p. injection of 15 IU/kg/day of vitamin E, acquired cytotoxicity to the tumor by the method of in vivo Winn assay, and vitamin E enhanced secondary response to the tumor. These results strongly suggest that vitamin E stimulates the helper and cytotoxic functions of T lymphocytes under the appropriate administration. Furthermore the effects of vitamin E on Meth-A tumor growth were studied in BALB/c mice, and resulted in inhibition of the tumor growth and prolongation of mean survival days.

Key Words: Vitamin E, Immunopotentiatin agent, Winn assay.
索引語：ビタミンE，免疫賦活剤，Winn 中和テスト.
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Abbreviations used in this paper: PHA: Phytohaemoagglutinin, Con A: Concanavalin A, LPS: Escherichia coli lipopolysaccharide, PG: Prostaglandin, i.p.: intraperitoneally, s.c.: subcutaneously.

Materials and Method

Animals: 7 to 10-week old male inbred BALB/c mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, and they were fed by commercial solid feed which contains 5 mg of vitamin E/100 g.

Chemicals: [dl]α-tocopherol as vitamin E and HCO 60, solvent of [dl]α-tocopherol, as placebo were used. Both were obtained from the Eisai Pharmaceutical Corp., Tokyo. Each was diluted in saline solution to the desired concentration and 0.5 ml was injected into the intraperitoneal cavity (i.p.).

Serem tocopherol level: Blood was drawn from the heart and the serum tocopherol level was, measured from pooled serum in each group by the method of KATO as previously described[10]. Briefly, 0.5 ml of serum was added to 0.5 ml of distilled water, then 1.0 ml of ethanol, containing 7 μg of tocol as an internal standard, was added and the supernatant was dried under nitrogen gas flow at 40°C in a water bath. The residue was redissolved in 50 μl of n-hexan and 20 μl was injected into the column (Nucleosil-5NH_2). Tocopherol was detected with Hitachi 204 fluorescence spectro photometer. (Ex. 298 nm. Em. 325 nm.)

Spleen Cell Preparation: The mice were sacrificed by cervical dislocation and their spleens were removed aseptically. After measuring the spleen weight, single cell suspensions were severally prepared by gently pressing each spleen through a #100 mesh stainless steel screen. Erythrocytes were lysed with Tris-buffered ammonium chloride, and then the spleen cells were centrifuged and washed three times with Hanks' balanced solution. The single cells adjusted to 5×10^6/ml were resuspended in RPMI 1640 containing 10% heat-inactivated fetal calf serum and 0.1 mg/ml Gentamycin (Shionogi Pharm. Co. Ltd. Tokyo).

Lymphoproliferative Assay to Mitogens: Cultures were set up in triplicate in Nunclon microtiter plates (Nunc, Denmark). 5×10^5 spleen cells/0.1 ml and 0.1 ml of RPMI 1640 supplemented with 20% heat inactivated fetal calf serum were placed in each well. Purified Phytohaemoagglutinin (PHA 0.13 μg/0.01 ml/well: Wellcome, USA) and Concanavalin A (Con A 0.33 μg/0.01 ml/well: Wako Junyaku, Tokyo) were used to assess T-cell responses. Escherichia coli lipopolysaccharide (LPS 3.3 μg/0.01 ml/well: Difco, USA) was used to assess B-cell proliferation. Spleen cells were incubated for 64 hr at 37°C in 5% CO_2 in a humidified atmosphere. 16 hr prior to culture termination, 0.5 μCi of tritiated thymidine (^{3}H-TdR: New England Nuclear, Boston: specific activity 21.8 Ci/mM) was added to each well. After culture, the cells were harvested on a glass fiber filter using an automatic multiple cell harvester (LM 101 LABO MACH: Labo Science Co. Tokyo) and the incorporation of ^{3}H-TdR was determined by a liquid scintillation counter (Isocap 300. Nuclear Chicago Co.). We calculated the mean counts per minute (cpm) of the triplicate cultures, and expressed Δ cpm as follows.

Δ cpm=mean cpm of mitogen stimulated culture of the triplicate—mean cpm of unstimulated culture of the triplicate.

We determined the lymphoproliferative assay for each spleen of each mouse respectively, and the mean Δ cpm$\pm$standard deviation was calculated from the Δ cpm in each experimental group.

Tumor: Meth-A, methylcholanthrene induced fibrosarcoma of BALB/c origin, was maintained by intra-peritoneal transplantation in adult BALB/c mice. Tumor cells were washed three times and adjusted to desired numbers in RPMI-1640 before implantation. Tumor cells were implanted in the right footpad. The thickness of footpad was measured by calipers and tumor growth was calculated as follows.

tumor growth=the thickness of footpad implanted tumor—the thickness of the opposite footpad.

WINN Assay: The ability of spleen cells to inhibit tumor growth in vivo was assessed by a modification of the WINN assay[21]. Meth-A tumor cells and spleen cells of BALB/c mice were suspended together at a concentration of 5×10^5/ml tumor cells and 1×10^8/ml spleen cells in RPMI-1640 containing 5% heat-inactivated fetal calf serum. The effector-to-target ratio was thus 200: 1, which had been established in preliminary experiments to be optimal. After 30 minutes preincubation at 37°C in water bath, the cell mixtures were washed one time and resuspended in RPMI-1640. Then, they were implanted in the back s.c. of BALB/c mice in volumes of 0.2 ml. Thus 5×10^4 tumor cells mixed with 1×10^7 spleen cells were implanted into each mouse. Two tumor diameters were measured by calipers and tumor growth rates were calculated as follows.

tumor growth rates=$\sqrt{1\times m}$

1: longest diameter m: shortest diameter

Statistics: The results were evaluated by Student's t-test.

Results

Effect of Vitamin E on Lymphoproliferative Response.

The various doses of vitamin E, i.e., 5, 10, 20, 40, or 80 IU/kg/day, placebo equivalent to 20 IU/kg/day of vitamin E or 0.5 ml/day of saline solution were injected i.p. daily for 14 days after which the mice were sacrificed and the lymphoproliferative assay was carried out. Each group consisted of 4 BALB/c mice. The lymphorpoliferative responses, especially to PHA and LPS, were significantly enhanced in the groups treated with 5 to 20 IU/kg/day of vitamin E and the serum tocopherol levels were elevated to about twice the control in these condition (Table 1). On the other hand, this immunopotentiating effect disappeared in the group treated with 40 IU/kg/day of vitamin E, and the mitogen responses were contrarily suppressed in the group trcated with 80 IU/kg/day of vitamin E. The response to Con A showed the similar pattern though not significant. The placebo did not affect the mitogen responses as compared with the control group treated with 0.5 ml/day of saline solution. The results are represented in Table 1.

Effect of Vitamin E on Tumor Neutralization—Non-tumor Bearing Mice

The effect of vitamin E on tumor neutralization by normal spleen cells was studied by the method of WINN assay. 15 BALB/c mice were divided into 3 groups and 15 IU/kg/day of vitamin E, its solvent equivalent or 0.5 ml/day of saline solution was injected i.p. daily for 14 days

Table 1. Effect of Vitamin E on Lymphoproliferative Responses

	mitogen response (mean Δcpm$\pm$S.D.)			serum tocopherol level (μg/ml)[b]	spleen weight index[e]
	PHA	Con-A	LPS		
control[a]	10222 $\pm$8325	19838 $\pm$16046	27010 $\pm$6337	3.55	4.65
placebo[b]	8662 $\pm$2376	20561 $\pm$3432	27323 $\pm$6277	3.48	4.80
5E[c]	28614* $\pm$4565	42226 $\pm$14801	50517*** $\pm$2136	5.39	4.62
10E	39717** $\pm$7814	52241* $\pm$6373	40309* $\pm$5380	6.29	4.94
20E	37719** $\pm$10338	44911 $\pm$5226	42399** $\pm$3620	7.29	4.63
40E	15809 $\pm$13199	27842 $\pm$13331	28909 $\pm$3048	9.93	4.68
80E	3584** $\pm$1377	20580 $\pm$1561	17101** $\pm$3714	21.91	6.37

a) control: 0.5 ml/day of saline solution.
b) placebo: solvent equivalent to 20 IU/kg/0.5 ml/day of [dl]-α-tocopherol.
c) E: IU/kg/0.5 ml/day of [dl]-α-tocopherol.
d) Blood was drawn from heart and the serum tocopherol level was measured from pooled serum in each group by the method of Kato.
e) spleen weight index was calculated as follows: $\frac{\text{mean spleen weight (mg)}}{\text{mean body weight (g)}}$

Various doses of vitamin E, solvent or saline solution were inoculated i.p. daily from day 1 to day 14, and lymphoproliferiative assay, serum tocopherol level and spleen weight index were studied on day 15. Each group consisted of 4 BALB/c mice.
*$p<0.1$ **$p<0.05$ ***$p<0.01$ (vs. control group)

respectively. After these treatment, the mice were sacrificed and spleen cells were prepared from the pooled spleens of each group consisting of 5 BALB/c mice. 5×10^4 Meth-A cells mixed with 1×10^7 spleen cells prepared from each group were implanted s.c. in respective 10 normal BALB/c mice and tumor growth was measured. Tumor growth was significantly inhibited in the group using spleen cells treated with vitamin E as compared with the other two groups which showed similar tumor growth curves ($p>0.05$). Each group consisted of 10 BALB/c mice and the results are represented in Figure 1.

Effect of Vitamin E on Tumor Neutralization—Tumor Bearing Mice

Similar experiments using tumor-bearing mice were studied. 10 BALB/c mice, implanted with 1×10^5/0.02 ml of Meth-A tumor cells in the right footpad, were divided into two groups at random. From the same day of tumor implantation, one group received 14 daily i.p. injection of 15 IU/kg/day of vitamin E, and the other its solvent equivalent. These mice were sacrificed on the 15th day and spleens were removed aseptically. The tumor thickness at sacrifice time was 2.81 ± 0.33 mm in the placebo group and 2.44 ± 0.39 mm in the vitamin E group ($p<0.1$). Spleen cells were prepared from these two groups respectively, and 5×10^4 tumor cells mixed with 1×10^7 spleen cells were implanted into back s.c. of each group consisting of 10 BALB/c mice respectively. 5×10^4/0.2 ml of Meth-A tumor cells only were implanted similarly into 5 BALB/c mice as a control group. As a result, the mean survival time after the tumor implantation was longest in

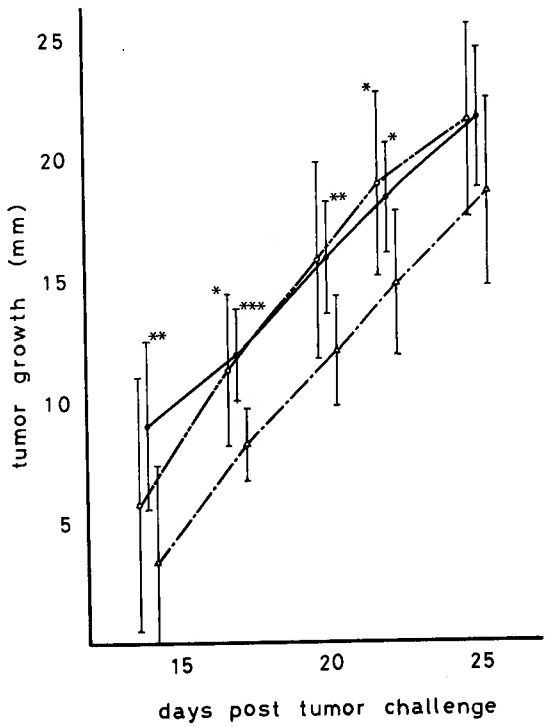

Fig. 1. Tumor Growth Curve after Winn Assay.
Effector cells are spleen cells prepared from non-tumor bearing BALB/c mice which received 14 daily i.p. injection of 15 IU/kg/day of vitamin E (— — —), its slovent equivalent (— — —), or 0.5 ml/day of saline solution (——). Target cells are Meth-A tumor cells. ETratio is 200: 1.5×10^4 Meth-A cells mixed with 1×10^7 spleen cells were implanted in back s.c. of 10 BALB/c mice respectively, and tumor growth was measured.
*$p<0.05$ **$p<0.02$ *$p<0.01$
(v.s. Vitamin E group) $n=10$

the group using spleen cells treated with vitamin E, i.e., 48.4 ± 14.1 days, and furthermore two of 10 mice rejected the tumor in this group (Fig. 2). On the other hand, it was shortest in the placebo group, i.e. 34.1 ± 7.1 days ($p<0.02$ v.s. vitamin E group) and 37.0 ± 4.2 days in the control group. The survival percentage rates are shown in Figure 2.

Effect of Vitamin E on Secondary Response against Tumor

The effect of vitamin E on secondary response for tumor growth was evaluated by in vivo experiments. 20 BALB/c mice were implanted with 6×10^4/0.02 ml of Meth-A tumor cells in the right footpad on day 0 and divided into two groups at random. One group received daily i.p. injection of 15 IU/kg/day of vitamin E from day 7 to day 28, and the other its solvent equivalent in the same manner. The tumors were completely removed by the right leg amputation on day 14. The tumor growth at amputation was 1.49 ± 0.51 mm in the placebo group and 1.58 ± 0.56 mm in the vitamin E group. As a control group, non-tumor bearing 10 BALB/c mice received 0.5 ml/day of saline solution i.p. from day 7 to day 28, and underwent amputation of the right leg on day 14. All these 30 mice were rechallenged with 1×10^5/0.02 ml of Meth-A in the

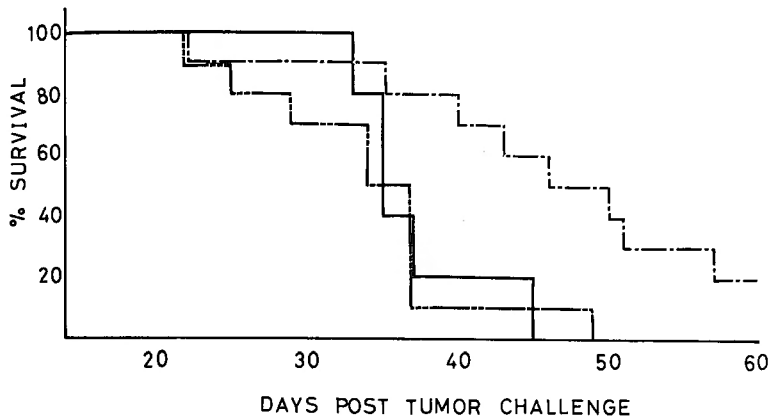

Fig. 2. Survival Percentage Rate after Winn Assay.
The effect of vitamin E on tumor neutralization test was studied by using spleen cells from tumor bearing mice. After implantation of 1×10^5/0.02 ml of Meth-A tumor, BALB/c mice received 14 daily i.p. injection of 15 IU/kg/day of vitamin E (— — —) or its solvent equivalent (— — — —). On the next day, spleens were removed and used as effector cells. Target cells were Meth-A tumor cells and ET ration was 200: 1.5×10^4 Meth-A cells mixed with 1×10^7 spleen cells were implanted in back s.c. of 10 BALB/c mice respectively. As a control group (——), 5×10^4/0.2 ml of Meth-A cells only were implanted similarly.

left footpad on day 21. As a result, 7 of 10 mice rejected the rechallenged Meth-A tumor in the vitamin E group (Fig. 3). On the other hand, 4 of 10 rejected it in the placebo group and no tumor rejection was observed in the control group. 40 days survivors were 9 of 10 mice in the vitamin

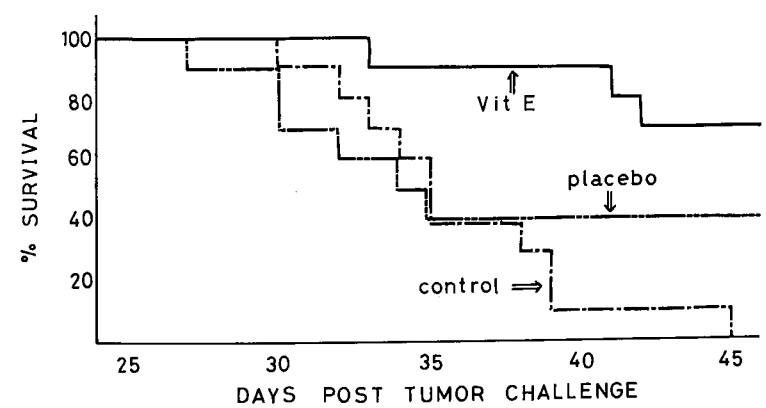

Fig. 8. Survival Percentage Rate after Tumor Rechallenge.
20 BALB/c mice were implanted with 6×10^4/0.02 ml of Meth-A tumor cells in the right footpad on day O, and divided into two groups. 15 IU/kg/day of vitamin E (——) or its solvent equivalent (— — —) was injected i.p. daily from day 7 to day 28. The tumors were completely removed by the right leg amputation on day 14. As a control group (— — —), non tumor bearing 10 BALB/c mice received daily i.p. injection of 0.5 ml/day of saline solution from day 7 to day 28, and underwent amputation of the right leg on day 14. All these 30 mice were rechallenged with 1×10^5/0.02 ml of Meth-A in the left footpad on day 21. 40 days survivors were 9 of 10 mice in the vitamin E group, 4 of 10 mi the placebo group ($p<0.02$ v.s. vitamin E group) and only 1 in the control group.

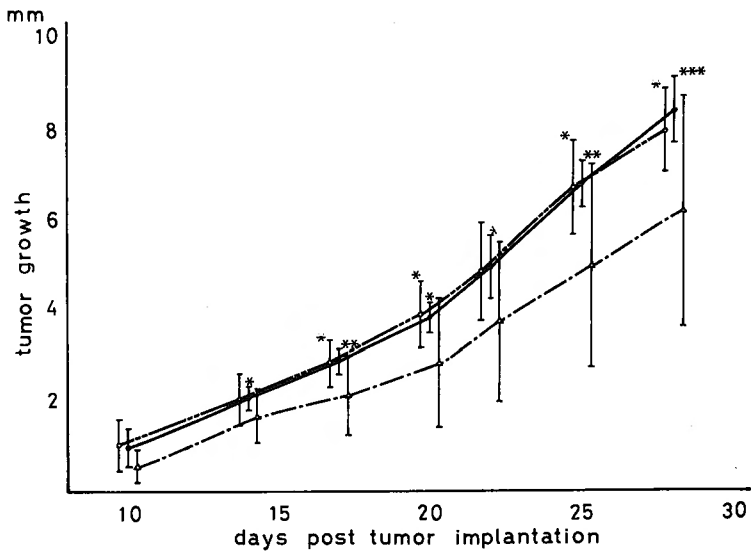

Fig. 4. Effect of Vitamin E on Tumor Growth.
2×10^5/0.02 ml of Meth-A tumor cells were implanted in the right footpad and 20 IU/kg/day of vitamin E (— - —), its solvent equivalent (— - - —) or saline solution (——) was injected i.p. daily from the day of tumor implantation until death.
*p<0.1 **p<0.05 ***p<0.01
v.s. Vitamin E group n=10

E group, 4 of 10 in the placebo group (p<0.02 v.s. vitamin E group) and only 1 in the control group. The survival percentage rates are shown in Figure 3.

Effect of Vitamin E on Tumor Growth

From these results it is expected that vitamin E might inhibit the growing of primary tumor, so that we studied the effect of vitamin E on tumor growth in vivo. 30 BALB/c mice, implanted

Table 2. mean survival times ± standard deviation (days) after tumor implantation[a)]

	Group 1	Group 2	Group 3	Group 4[e)]
vitamin E[b)] group	35.6 ±3.2	40.9 ±5.2	40.0 ±5.1	34.3 ±3.1
placebo[c)] group	34.8 ±3.1	34.5* ±3.9	32.1*** ±3.8	32.4 ±3.3
control[d)] group	33.6 ±2.3	33.6*** ±2.3	33.6*** ±2.3	33.6 ±2.3

*p<0.05 ***p<0.01 n=8

a) 1×10^5/0.02 ml of Meth-A tumor cells were implanted in the right footpad on day 0.
b) 15 IU/kg/day of vitamin E.
c) Solvent equivalent to 15 IU/kg/day of vitamin E.
d) Nothing was injected i.p.
e) Vitamin E or placebo was injected i.p. daily as experimental schedule.
Group 1: from day −14 to day 0
Group 2: from day −7 to day 7
Group 3: from day 0 to day 14
Group 4: from day 7 to day 21

with 2×10^5/0.02 ml of Meth-A tumor cells in the right footpad, were divided into three groups at random. 20 IU/kg/day of vitamin E, its solvent equivalent or 0.5 ml/day of saline solution were injected i.p. daily respectively from the day of tumor implantation until death. The tumor growth was significantly inhibited in the vitamin E group compared with the placebo and the control groups ($p<0.05$ Fig. 4).

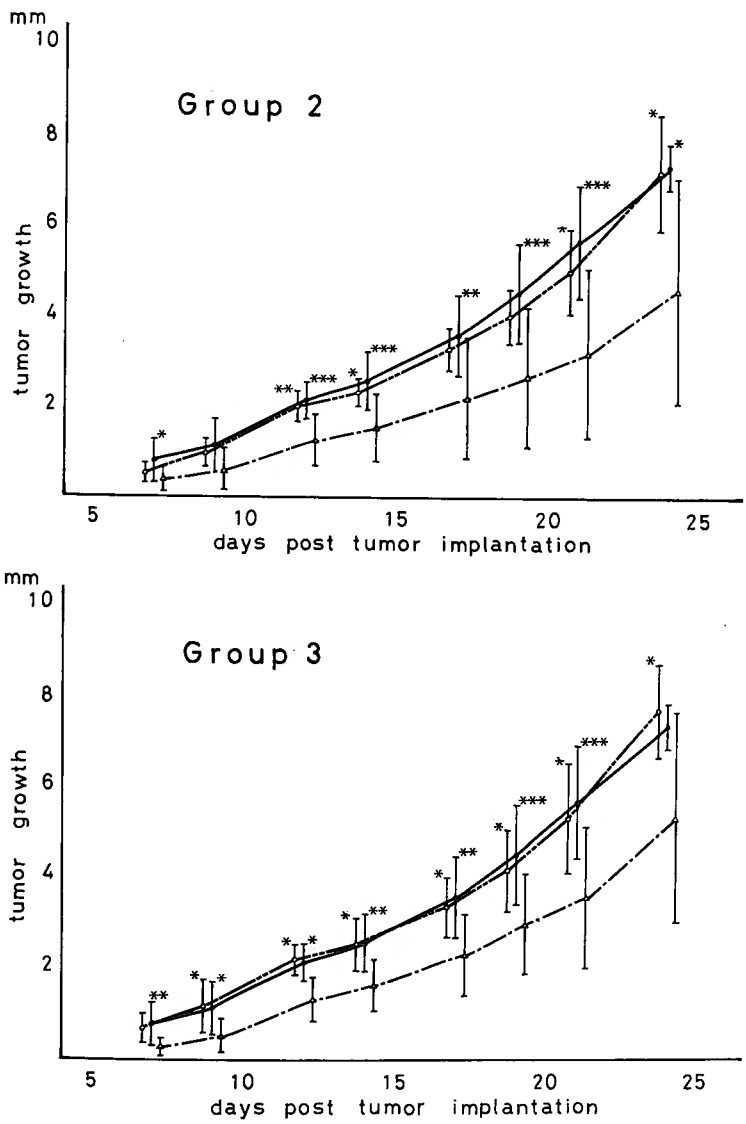

Fig. 5. Effect of Vitamin E on Tumor Growth.
1×10^5/0.02 ml of Meth-A tumor cells were implanted in the right footpad. 15 IU/kg/day of vitamin E (———) or its solvent equivalent (— — —) was injected i.p. daily for 14 days as experimental schedule. Nothing was injected in the control group (——)
*$p<0.05$ **$p<0.02$ ***$p<0.01$
v.s. Vitamin E group n=8

Subsequently, we prepared 4 treatment schedules in order to study the relation between the periods or timing of vitamin E administration and the inhibition effect on tumor growth. Mice were implanted with 1×10^5/0.02 ml of Meth-A in the right footpad on day 0, and received daily i.p. injection of 15 IU/kg/day of vitamin E or its solvent equivalent according to following schedule, i.e., from day −14 to day 0 in Group 1, from day −7 to day 7 in Group 2, from day 0 to day 14 in Group 3, and from day 7 to day 21 in Group 4. Each group consisted of 16 BALB/c mice and half received vitamin E and the remainder placebo. As a control group, 8 BALB/c mice were implanted with 1×10^5/0.02 ml of Meth-A in the right footpad on day 0 and no i.p. injection was received. As a result, the tumor growth was inhibited ($p<0.05$ or 0.02) and mean survival days were lengthened ($p<0.05$ or 0.01) in Group 2 and Group 3, but no effect of vitamin E was recognized in Group 1 and Group 4 (Fig. 5, Table 2). That is, it was necessary to manifest the effect of vitamin E that the serum tocopherol level was kept at about twice the control for a while following tumor implantation. No effect on tumor growth appeared when vitamin E was injected solely before the implantation or after tumor growth commenced.

Discussion

There have been some studies concerning the effects of vitamin E on carcinogenesis[7] and toxicities induced by radiation[14] or anticancer agents, especially, cardiotoxicity resulted from Adriamycin[15]. However, no investigator reported the direct effect of vitamin E on tumor growth.

In general, the physiologic role of vitamin E is considered to be its ability as an antioxidant, free radical scavenger. If vitamin E participates in permiability and stability of cell membrane, as established in erythrocytes[17], the same function may be expected to lymphocyte membrane in the capacity of a immunopotentiator. Recently, it has been reported that vitamin E enhanced humoral immune response to antigenic stimulation[20] and resistibility to bacterial infection[8]. Regarding cellular immunity, TANAKA et al.[18] have suggested that vitamin E stimulated the helper activity of T-lymphocyte through the assay of antibody-titer in mice and LIM et al.[11] have reported that significant activation of antibody dependent celluler cytotoxicity (ADCC) was shown in mice recieving high vitamin E diet. Our results that mitogen responses of spleen cells are enhanced by the daily i.p. injection of 5 to 20 IU/kg/day of vitamin E and depressed by 80 IU/kg/day reveal its function as stimulator of cellular immunity under the appropriate administration.

In addition, we reported[23] that vitamin E protected against the immunosuppression and loss of spleen weight induced by Adriamycin (ADR), Mitomycin C (MMC) or 5-Fluorouracil (5 FU) in mice.

It is said that ADR and MMC are quinon-containing drugs and function as free radical carriers through the interaction with microsomes[1], and that 5FU does not participate in the generation of free radicals. From these results, we speculated that vitamin E might function not only as a free radical scavenger but also as a membrane stabilizer.

On the hypothesis that vitamin E might activate the helper and secondarily cytotoxic function of T-lymphocyte, because it stimulates lymphoproliferative responses to both T and B cell mitogens, antitumor activities of spleen cells treated with vitamin E were evaluated through in

vivo WINN assays. Spleen cells prepared from normal BALB/c mice receiving 14 daily i.p. injections of 15 IU/kg/day of vitamin E showed tumor neutralizing effect against Meth-A tumor. Although the activity is weak and can not attain tumor rejection, it is noticeable that tumor neutralizing effect is induced by vitamin E even if on the condition of non-tumor bearing. Furthermore, vitamin E administration also augumented the antitumor activity of spleen cells harvested from tumor bearing mice and brought on tumor rejections in two of 10 mice and significant prolongation of mean survival time.

Here, two probabilities are suggested, i.e., vitamin E activates helper T lymphocyte primarily and cytotoxic T secondarily, or vitamin E stimulates both helper and cytotoxic T cells. From the tumor inhibition effect induced by spleen cells of non-tumor-bearing mice treated with only vitamin E, we speculated that non-specifically activated helper T cells might interact with and educate the cytotoxic T cells in recipient mice. Anyhow, vitamin E is considered to be activator of helper and cytotoxic T lymphocytes consequently.

Similar effect was observed in rechallenged Meth-A tumor. On the condition that vitamin E was administrated for the period of pre tumor removal and post tumor rechallenge, seven of 10 mice rejected the tumor. Vitamin E was effective on the induction of immunized mice against Meth-A tumor.

From these results, the therapeutic effects of vitamin E on tumor bearing mice is expected. According to some therapeutic schedule of vitamin E, we examined its appropriate administration, especially on the relation with tumor load. Three necessary conditions were revealed for the appearance of antitumor effects induced by vitamin E;

1) maintenance of serum tocopherol levels at about twice the normal mice
2) coexistence of vitamin E and tumor
3) little or residual tumor load

These results strongly suggest that vitamin E is cllinically effective for the residual cancer cells after surgical operation.

On the other hand, prostaglandin (PG) may participate in this phenomenon, because it has been reported that PG E_2 induced suppressor T cells[6] and that indomethacin, a blocker of PG synthesis, has a positive effect on tumor growth inhibition[13]. The first step of PGE_2 biosynthesis is peroxidation of arachidonic acid, and it is recognized that vitamin E inhibits this process. It is not clear whether this antitumor effect is due to the direct effect of vitamin E or to the PG paticipation. Further examinations are necessary to elucidate this problem.

Vitamin E is generally considered to be relatively non-toxic at high dosage in spite of being a fat soluble vitamin[3]. It is the appropriate dosage of vitamin E to manifest the favorable effects on immune responses and tumor growthes that increase the serum tocopherol level to about two fold of normal, i.e., 300 to 600 mg/day in adult man as peroral administration. This doses are fairly non-toxic because 800 IU of vitamin E ingested daily for three years has been observed to have no ill effects in man[5]. We have applied vitamin E clinically in cancer therapy and obtained good preliminary results similar to this experimental results.

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和文抄録

免疫賦活剤としてのビタミンEの効果および実験腫瘍発育に対するその影響

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ビタミンEを細胞性免疫の立場から，BAL B/C マウス脾細胞を用いて検討した結果，PHA, Con A, LPS に対するリンパ球幼若化能が，ビタミンE 5～20 IU/kg/day, 14日間の投与で有意に亢進した．このことは，至適投与条件下において，ビタミンEに免疫賦活剤としての効果があることを強く示唆するものである．そこで腫瘍発育に対するビタミンEの影響を BALB/C-Meth A 腫瘍の系を用いて検討し以下の結果を得た．

1) 非担癌 BAL B/C マウス脾細胞と Meth-A 腫瘍細胞を effector to target ratio 200:1 に調整した後，BAL B/C マウス背部皮下に移植して Winn assay を行うと，ビタミンE投与をうけた脾細胞は，明らかに腫瘍中和能を示した．

2) 担ガンマウス脾細胞を用いた同様の実験でも，ビタミンE投与脾細胞には腫瘍中和能を認めた．

3) 右足蹠に meth-A を移植し生着させてから患肢切断を行い，腫瘍を完全に除去した後，左足蹠に Meth-A を再移植し，その生着率に対するビタミンEの効果をみると，ビタミンE投与群では，有意に再生着が阻止された．

4) ビタミンE単独で Meth A 腫瘍の発育を有意に抑制し生存日数の延長を認めた．この腫瘍増殖抑制効果の発現には，腫瘍量が少ない時期に血中のビタミンEが至適濃度に保たれていることが必要であり，腫瘍生着後の投与，あるいは前投与のみでは無効であった．