Cantaurus, Vol. 3, 2-6, May 1995

The Effect of Vitamin E on Splenic and Hepatic Natural (NK) Cell Activity in the Rat

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ABSTRACT

This experiment examines the effect of dietary and injected α-tocopherol on the tumor killing activity of NK cells of the liver and spleen. Vitamin E appears to be required for immune function and may also enhance immunity, as shown by its effect on B and T cell proliferation, antibody production, and macrophage activity. NK cells (a type of large granular lymphocyte) play a vital role in the response against tumor cells and virally-infected cells. Rats either received [dl] α-tocopherol acetate in the form of a dietary supplement of 500 I.U./kg diet for 14 d or were given 3 subcutaneous injections of 10 I.U./kg body weight. Cell samples were isolated by density gradient centrifugation and tested for activity by the ^{51}Cr-release assay against radiolabeled YAC-1 tumor cells. Additional samples were incubated with cytokine-containing media to measure the effect of vitamin E on stimulated NK cells. Results indicate that dietary vitamin E may have slightly increased NK activity. Groups receiving injections had suppressed NK activity, possibly due to stress effects. Those rats, however, who received α-tocopherol injections were less suppressed than the control injection rats. Vitamin E has been shown to reduce tumor metastasis in mice, but this research does not support the notion that this occurs by way of a pronounced increase in NK activity.

INTRODUCTION

The compound α-tocopherol (Vitamin E) has been shown to affect immune function in many ways. Research involving Vitamin E-deficient diets indicates that it is necessary for immune function. Eskew *et al.* (1985) reported that rats fed diets deficient in vitamin E showed decreased lymphocyte blastogenesis in response to mitogens. Gerebremichael *et al.* (1984) reported that a vitamin E-deficient diet impaired the accessory cell function of macrophages. Their ability to present antigens provides a mechanism by which the humoral response may also be affected (Boswell 1980; Uranue 1980). Investigators who fed animals diets supplemented with Vitamin E at various levels have found it to be immunoenhancing at large, but not excessive levels (Yasanuga, *et al.* 1982). Meydani *et al.* (1990) found that an 800 mg dose of α-tocopherol increased antibody levels, mitogen-stimulated lymphocyte proliferation, and interleukin 2 (IL-2) levels in elderly humans. Moriguchi *et al.* (1990) also demonstrated that high dietary vitamin E increased lymphocyte blastogenesis and increased phagocytic activity of alveolar macrophages. Tanaka *et al.* (1979) provided evidence for increased helper T cell activity with vitamin E supplementation.

A critical component of the immune system involves those cells which exhibit natural killer (NK) cell activity. NK cells have a variety of functions including immunosurveillance of tumors, lysis of virally-infected and bacterial cells, and regulation of lymphohematopoiesis (Gorelik *et al.*, 1982; Kiessling *et al.*,1975) NK cell activity may be regulated by IL-2 and interferon-γ produced by T cells and IL-1 and IL-12 produced by macrophages. This activity has been shown to be suppressed by prostaglandins, specifically PGE_2, which is a product of macrophages (Bray & Brahmi, 1986; Ohnishi *et al.*, 1991; Humes *et al.*, 1977; Baxevanis *et al.*, 1993; Brunda *et al.*, 1980). A subpopulation of NK cells found in the liver have been characterized (Vanderkerken *et al.*, 1990; Bouwens & Wisse, 1987). These "pit cells" are large granular lymphocytes (LGL) with azurophilic granules, and may be highly activated.

It has been reported that dietary levels of Vitamin E have been shown to reduce the amount of prostaglandin in the bloodstream (Meydani *et al.*, 1988) A reduction in the onset and development of tumors with α-tocopherol supplementation has been reported (Kurek & Corwin, 1982). Because NK cells are involved in fighting malignancies, the effect of α-tocopherol may be in the reduction of immunosuppressive prostaglandins. However, stimulation of macrophage activity has also been observed with doses of α-tocopherol.

The effect of dietary α-tocopherol on NK activity in the blood and spleen has been studied (Meydani *et al.*, 1988). The investigators found no significant changes in tumor cell killing. They proposed that the use of corn oil with high amounts of ω6 polyunsaturated fatty acids (PUFA) and fish oil with a high amount of ω3 PUFA may have an effect on NK function. Linoleic and eicosapentanoic acids serve as metabolic precursors of immunosuppressive prostaglandins and leukotrienes. Sesame oil (42% PUFA) was used for the following experiment.

This experiment examines the effect of both dietary and parenterally-administered doses of [dl] α-tocopherol acetate on the NK cell activity of cells from the spleen and liver of rats. It also examines the effect of the

same treatments on activated cell cultures from these animals. A ^{51}Cr-release assay with radiolabeled YAC-1 tumor cells was used to determine NK activity.

MATERIALS AND METHODS

Animals and Treatments

Twenty four pathogen-free male Sprague Dawley rats, 43-45 d old, were obtained from Harlan Sprague Dawley, Indiannapolis, IN. The rats were individually caged under a 12 h reverse light cycle. Six rats were placed in each of four treatment groups: [1] control diet (CD); [2] vitamin E diet (ED); [3] control injection (CI); and [4] vitamin E injection (EI). The rats in the CD, CI, and EI groups received Purina Rodent Chow (Harlan Teklad) and water ad libitum. The ED group rats received Purina Rodent Chow supplemented with 500 mg/kg food [dl] α-tocopherol acetate. The rats were provided excess amounts every day and the amount eaten was determined by weight. The CI rats were given three 1 mL subcutaneous injections of sterile sesame oil with 3 days between each injection and the last falling 3 d before the experiment. The EI rats were given injections in the same manner, with 10 I.U./kg body wt. [dl] α-tocopherol acetate dissolved in the sesame oil.

Cell Isolation

Half of the rats (3 from each group) were sacrificed on day 14 and the other half were sacrificed on day 16. The rats were injected with 0.2 mL heparin through the portal vein and exsanguinated. The supradiaphragmatic vena cava was clamped off and a cannula was inserted through the hepatic vein. The livers were perfused with approx. 50 mL Hanks' Balanced Salt Solution (HBSS) and 0.1 % ethylenediaminetetraacetic acid (EDTA), using a 60 mL syringe. Applying force, approximately 13 mL of the effluate was collected into a test tube. The spleens were removed and single-cell suspensions were prepared in complete RPMI media (10% fetal bovine serum (FBS), 1% l-glutamine, 2.5% HEPES buffer, and 0.1 % gentamicin) by grinding between frosted-tip glass slides. Large cells and debris were allowed to settle out and the supernatant was removed. Lymphocytes were isolated from the liver samples by density gradient centrifugation, using Accu-paque separation media (Accurate Chemical & Scientific Corp.). Red cells were lysed with Hematall LA-Hgb reagent (Fisher Scientific) and total white cells were counted on a Celltrak 2 (Nova Biochemical, Waltham, MA) cell counter.

^{51}Cr-release Assay for NK activity

The assay for NK activity was run as described by Reynolds *et al.* (1981). Briefly, YAC-1 tumor cells (targets) were maintained in a 37°C, 5% CO_2 incubator with complete media (CM). On the assay day, target cells were incubated with $Na_2{}^{51}CrO_4$ for 1 h. They were washed once, incubated for an additional 30 min., and washed twice in CM. The samples were plated with the YAC-1 cells in triplicate on 96-well microtiter plates and incubated for 4.5 h. Effector:target ratios were adjusted to 100:1, 50:1, 25:1 and 12.5:1 for the spleen samples and 50:1, 25:1, 12.5:1 and 6.25:1 for the liver samples, with controls for spontaneous and maximun release. A second set of samples was plated in the same manner, but included 50 μL/well of 40% supernatant from mitogen-stimulated T cells in 60% CM. The spleen samples were incubated for 18 h and the liver samples for 16 h. The ^{51}Cr-release NK assay was then run. A volume of 1.5 mL of Cytoscint scintillation cocktail was added to each of the vials containing cell samples. Liver samples were placed on a Rackbeta 1214 (Wallack LKB, Gathersburg, MD) scintillation counter and spleen samples were placed on a Gammatrac 1191 (TM Analytic Inc., Grove Village, IL) scintillation counter. Results were given in counts per minute (c.p.m.).

Cell Preparation for Microscopy

Slides were prepared with a Cytospin 2 (Shandon Inc. Pittsburg, PA). 50 μL spleen cells in 400 μL phosphate buffered saline (PBS) w/ 1% bovine serum albumin (BSA) and 100 μL liver cells in 300 μL PBS w/ 1% BSA were spun @ 700 r.p.m. for 5 min. The cells were fixed in Leukostat fixative (Fisher Scientific), dryed, and stained w/ Wright-Geisma Sure-Stain (Fisher). The percentage of LGL was determined for each sample and adjusted to provide more accurate E:T ratios.

Data Analysis

The killing activity of cell samples were calculated as % cytotoxicity and Lytic Units (LU). Percent cytotoxicity is defined by the equation:

$$\frac{\text{experimental c.p.m. - spontaneous c.p.m.}}{\text{maximum c.p.m. - spontaneous c.p.m.}}$$

LUs were calculated using a computer program based on the equations of Pross and Maroun (1984) and were based on the number of lymphocytes per 10^7 effectors required to lyse 20% of the targets.

Means and standard deviations were calculated for every sample at all 4 E:T ratios. One-way analyses of variance were run for the % cytotoxicity results of non-activated spleen samples, activated spleen and liver samples, and for the LU results of both activated and non-activated spleen and liver samples.

RESULTS AND DISCUSSION

Results for the spleen cell samples indicate that dietary vitamin E may have slightly increased NK cell

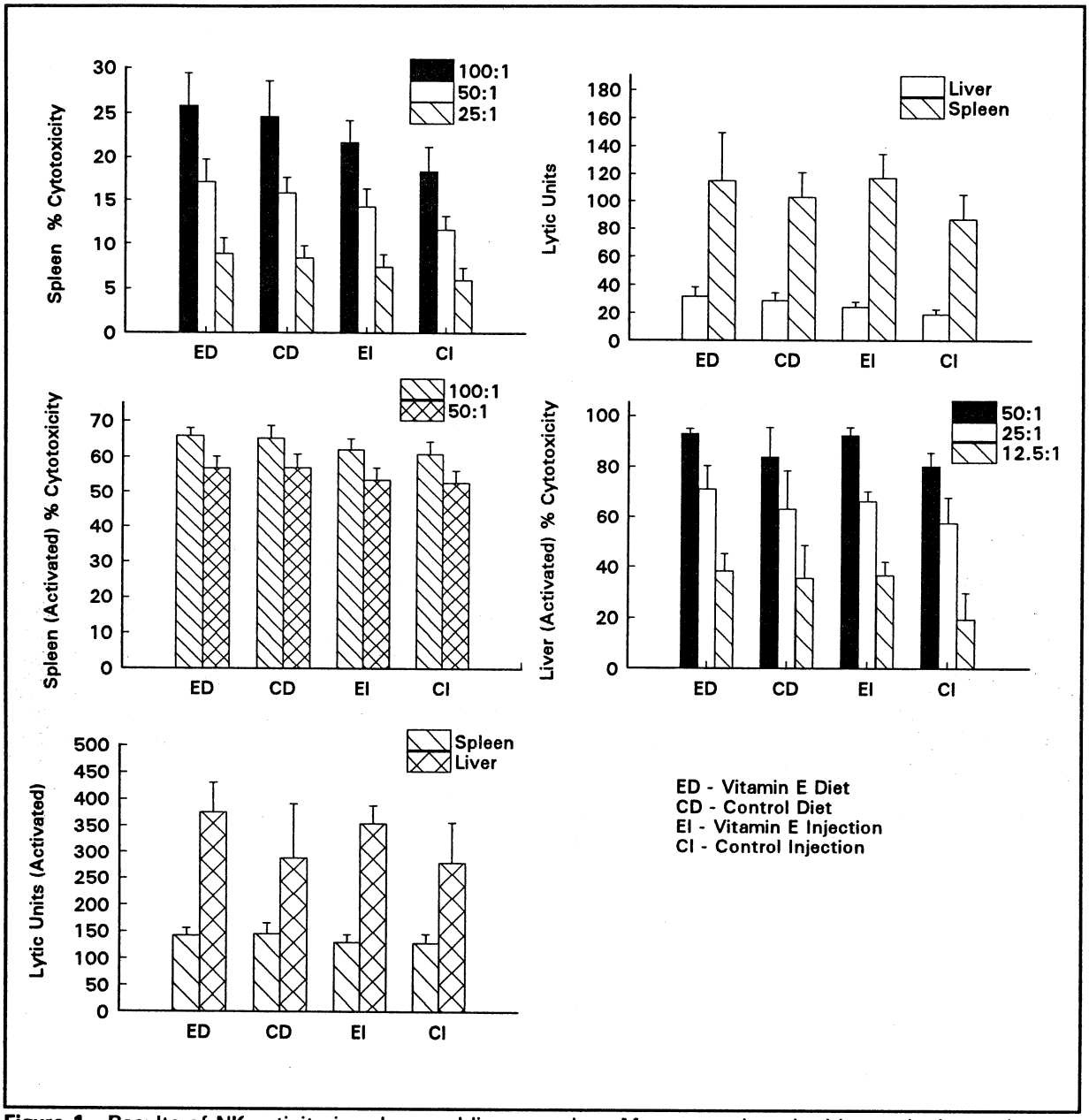

Figure 1. Results of NK activity in spleen and liver samples. Means are plotted with standard error bars.

activity (Fig. 1a), but that activity was somewhat suppressed in the injection groups. Also,the rats who received vitamin E injections had slightly higher NK activity than the rats of the control injection groups. This is evident in both the activated and non-activated samples (Figures 1a, 1b, 1c, and 1e).

Results for the liver cell samples indicated that both the dietary and injected vitamin E group rats had higher NK activity than the two control groups (Figures 1b, 1d, and 1e). Furthermore, the injections didn't appear to have a suppressive effect. This was again seen in both the activated and the non-activated samples. The one-way ANOVA showed no statistical significance ($p < 0.05$) in any of these tests, due to a great deal of variation within groups.

It has been reported that physically aversive stimuli reduce splenic NK cell activity (Cunnick, *et al.* 1988). The injections in this experiment could have provided aversive stimuli, possibly explaining the suppressed activity in the spleen cells from the injection group rats. If so, it appears that vitamin E may slightly reduce this stress effect.

Although these results provide some correlation between supplemented vitamin E and enhanced NK cell

activity, they do not support the notion that vitamin E has a pronounced effect on NK cell activity. Also, the idea that the previously reported reduction in tumor onset and growth with vitamin E supplementation may occur by way of increased NK activity is not supported by this research.

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ACKNOWLEDGEMENTS

The author would like to thank Dr. Joan Cunnick of Iowa State University for her advice and the use of her facilities. The help of Dr. Jonathan Frye, Prof. N.A. Dutrow, Erik Harmon, Shiu Huey Chou, and Kelly Nordyke Messingham was also appreciated.