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Systemic treatment with *n*-6 polyunsaturated fatty acids attenuates EL4 thymoma growth and metastasis through enhancing specific and non-specific anti-tumor cytolytic activities and production of TH1 cytokines

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Abstract

Recently, there has been a great interest in the effects of different types of *n*-6 polyunsaturated acids (*n*-6 PUFAs) upon the immune system and cancer development. However, the effects of *n*-6 PUFAs are still controversial and as yet undefined. The present study aimed to investigate the anti-tumor effects of *n*-6 PUFAs against EL4 thymoma and the associated immune mechanisms. To this, sesame oil, a vegetable oil enriched with *n*-6 PUFAs, or free linoleic acid (LA) were administered intraperitoneally into C57BL/6 mice before and after challenge with EL4 lymphoma cells. Treatment with either sesame oil or LA attenuated the growth and metastasis of EL4 lymphoma. The anti-tumor effect of LA was superior to that of sesame oil, and associated with an increase in the survival rate of the tumor-bearing mice. In addition, both sesame oil and LA showed dose-dependent anti-lymphoma growth in vitro. Treatment with LA generated significant increases in the anti-lymphoma cytolytic and cytostatic activities of T cells and macrophages, respectively, and enhanced production of IL-2 and IFN-γ while decreased production of IL-4, IL-6 and IL-10. In summation, the results suggest that *n*-6 PUFAs, represented by LA, can attenuate EL4 lymphoma growth and metastasis through enhancing the specific and non-specific anti-tumor cytolytic activities and production of TH1 cytokines. These findings might be of great importance for a proper design of systemic nourishment with PUFAs emulsions for cancer patients.

Keywords: *n*-6 Polyunsaturated fatty acids; Sesame oil; Linoleic acid; EL4 thymoma; Macrophages; T cells; CTL; Cytostasis; Cytokines

1. Introduction

The fatty acid composition of inflammatory and immune cells is sensitive to change according to the

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fatty acid composition of the diet. In particular, the proportion of different types of polyunsaturated fatty acids (PUFAs) in these cells is readily changed, and this provides a link between dietary PUFA intake, inflammation, and immunity [1]. In recent years there has been a great interest in the effects of *n*-3 and *n*-6 PUFAs upon the immune system and cancer development [2]. The *n*-6 PUFAs arachidonic acid (ARA) gives rise to the eicosanoid family of inflammatory mediators, including prostaglandins, leukotrienes and related metabolites. ARA is formed from linoleic acid (LA; 18:2 *n*-6), a major component of vegetable oils such as sesame, corn, sunflower, soybean, and safflower oils [3]. These *n*-6 PUFAs have been found to regulate activities of inflammatory cells, the production of cytokines and the various balances within the immune system [1,4–12]. However, several reports have also shown that feeding diets rich in *n*-6 PUFAs had no effect on these responses [13,14]. Such discrepancies are most probably due to a variety of effects, including differences in the amount of fat in the diet and the duration of feeding, the exact comparison being made (i.e. with a low-fat diet or with another type of high-fat diet).

n-6 PUFAs not only modulate the immune response, but also alter the growth and metastasis of different tumor models [15,16]; however, their anti-tumor effect is still controversial. For instance, *n*-6 PUFAs have been reported to increase the incidence, growth and metastasis of certain tumors such as mammary, prostate and colon cancers [17–21] and to decrease the metastasis of other tumors such as glioma, Lewis lung carcinoma, neuroblastoma, Jurkat and HL-60 leukemic cells, and RDM4 lymphoma [22–28]. Yet the immunomodulatory and anti-tumor mechanisms of these *n*-6 PUFAs are not fully understood. There is a concern that long-term consumption of large amounts of LA might increase cancer risk. In this regard, a study has reviewed both the epidemiologic and experimental literature on LA intake and cancer risk and performed meta-analyses of risk estimates from case-control and prospective cohort studies [29]. Such study concluded that animal experiments indicated that a minimum amount of LA is required to promote growth of artificially induced tumors in rodents; but above this threshold, LA did not appear to have a specific tumor-promoting effect.

In a previous study, we investigated the effect of sesame oil, a source of *n*-6 PUFAs, alone or in combination with EPA and DHA, *n*-3 PUFAs, on both the immune cells and the growth of murine melanoma [30]. We found in that study that treating mice with *n*-3 PUFAs simultaneously with sesame oil increases the total number of leukocytes in the thymus and spleen, and coincided with a higher percentage of $CD4^+$ and $CD8^+$ T cells in both organs [30]. Such effect was associated with a decrease in the growth of B16 melanoma. In the present study, we aimed to analyze the mechanism of anti-EL4 thymoma effect of two different sources of *n*-6 PUFA, sesame oil, which contains 43% LA, and the free LA. We found that these sources of *n*-6 PUFA induce significant anti-tumor effects toward EL4 thymoma by both direct and indirect effects. The indirect effect is mediated by increasing the non-specific tumoricidal activity of macrophages (Mϕ) and the specific cytotoxicity of T cells. Most importantly, the anti-tumor effect of LA was associated with skewing the immune response toward TH1-type cytokines.

2. Material and methods

2.1. Mice

Female C57Bl/6 mice, obtained from The Jackson Laboratory (Bar Harbor, ME), were maintained under specific pathogen-free condition on a 12 h light–dark cycle, and were provided with sterile food and water ad libitum. Mice were used for experiments at 8 weeks of age with an average of 20 g at the time of studies.

2.2. Treatment of mice with n-6 PUFA

Sesame oil (Takehaya, Tokyo, Japan) is enriched with the *n*-6 PUFA LA (43.1%), 38.2% oleic acid, 7.7% palamatic and 4.4% stearic saturated fatty acids. Emulsions of LA (Sigma) and sesame oil were prepared as described previously [31]. Free LA and sesame oil were dissolved in ethanol and diluted as emulsions in phosphate buffered saline (PBS) such that the final ethanol concentration in the preparations was no more than 0.2%. After preparing stock dilutions, the resultant emulsions were vortexed for 5 s and immediately pipetted into wells, in the case of in