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Short Communication

Dietary *n*-3 polyunsaturated fatty acids enhance metastatic dissemination of murine T lymphoma cells

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Epidemiological investigation and animal studies have shown that dietary *n*-3 PUFA prevent the development and progression of certain types of cancer. However, conflicting results have been reported by the few studies that focused on the effect of dietary *n*-3 PUFA on the development of metastases. In the present study, we investigated the metastatic dissemination of murine T lymphoma lines with different metastatic potential transplanted into mice fed a fish oil diet, compared with mice fed a maize oil diet. Transplantation of highly metastatic S11 cells into animals fed a fish oil diet induced a large lymphomatoid infiltration in the spleen, associated with an eight-fold increase in spleen weight, compared with normal animals on the same diet. In contrast, only a limited increase in spleen weight was found in animals transplanted with S11 cells while fed a maize oil diet. No significant increase in spleen weight was found in animals transplanted with low-metastatic 164T2 cells regardless of whether they were fed a fish oil or a maize oil diet. At the end of experiment, an overt cachexia was shown by animals fed a fish oil diet transplanted with S11 cells, but not by those transplanted with 164T2 cells. The particularly high pro-metastatic effect of dietary *n*-3 PUFA on S11 cells rules out the generalisation that dietary *n*-3 PUFA inhibit tumour growth and progression.

Dietary *n*-3 PUFA: Metastases: Murine lymphoma lines: Tumour cachexia

Epidemiological studies show that the risk of cancer is lower in populations whose diet is rich in the long-chain *n*-3 fatty acids[1–3] EPA and DHA. The recognition of a protective effect of *n*-3 PUFA on tumour development is further supported by animal studies showing that feeding *n*-3 PUFA-rich diets inhibited spontaneous and chemically induced carcinogenesis[4–6], and growth of mammary[7–9], colon[10] and liver[11] carcinomas in rats and mice. There are studies showing that the formation of experimental metastases is also affected by feeding host animals a diet rich in *n*-3 PUFA[12–21]. The results reported by these studies, however, are rather contradictory. For example, liver metastases from rat colon carcinoma were found to be promoted by Griffini *et al.*[15], but inhibited by Gutt *et al.*[19], in animals fed a fish oil diet. In addition, colon carcinoma cells enriched in *n*-3 PUFA by a passage in mice treated with *n*-3 PUFA reproduced a lower number of lung colonies when injected into mice fed a standard diet[13]. In our laboratory F10-SR cells, a highly metastatic melanoma line isolated from B16-F10 cells, were found to have reduced lung-colonising potential after transplantation into mice fed a fish oil diet[21]. The lung-colonising potential of B16-F10 cells was also reduced after their previous exposure to growth media supplemented with EPA[12]. On the other hand, a greater number of lung colonies was reproduced by B16 melanoma cells transplanted into animals treated with subcutaneous administration of *n*-3 PUFA before and after transplantation of tumour cells[14].

In order to expand our knowledge regarding the effects of dietary *n*-3 PUFA on metastatic diffusion, in the present study we sought to investigate to what extent feeding host animals a diet containing 5 % fish oil affects the metastatic diffusion of murine lymphoma lines with a different metastatic potential derived from a primary T-cell lymphoma[22]. In this context, use of this system of T lymphoma lines might contribute to clarify if the different biological characteristics of these lines are relevant to their response to dietary *n*-3 PUFA.

Materials and methods

Cell lines and culture conditions

The lymphoma lines used in the present study were kindly donated by Dr Y. St-Pierre (INRS-Institut Armand-Frappier, University of Québec, Canada). These were: (1) a low-metastatic 164T2 lymphoma line isolated from a primary T-cell lymphoma induced by radiation in C57Bl/6 mice (164T2 cells); (2) a highly metastatic variant (S11 cells), isolated through eleven *in vitro–in vivo* serial passages[22].

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When transplanted into syngeneic mice, S11 cells were reported to reproduce tumours with a higher incidence and growth rate compared with the parental 164T2 cells, leading to different survival spans of the animals transplanted with the two cell lines[22].

164T2 and S11 cells were grown at 37°C in a 5 % CO_2 humidified atmosphere in 5 ml Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Grand Island, NY, USA) containing 10 mM-HEPES, 2 mM-glutamine and supplemented with 15 % fetal calf serum (Gibco). Cells were propagated every 3 d by seeding 2×10^6 cells in 25 cm^2 tissue culture flasks (Sarstedt, Newton, NC, USA).

Animals and dietary treatments

Diets used in the present study were: a diet containing 5 % maize oil (maize oil diet), particularly rich in linoleic acid, and a diet containing 5 % fish oil (fish oil diet), particularly rich in EPA and DHA. The composition of these diets has been reported previously[21]. Newly weaned female mice (C57Bl/6 strain) (Charles River Laboratories, Inc., Wilmington, MA, USA) were placed on the maize oil diet for 1 week, then divided in two groups, each of twelve animals: one group continued to be fed the maize oil diet, while the other group was switched to the fish oil diet. The degree of incorporation of *n*-3 PUFA in animal tissues was determined by measuring the EPA + DHA:arachidonic acid ratio as reported previously[21]. The weight of injected and normal (not injected) animals, and food consumption were assessed three times per week from the beginning of the dietary treatment to the end of the experiment. Normal animals fed maize oil or fish oil diets exhibited a similar growth rate during the entire period of the experiment.

Determination of EPA + DHA:arachidonic acid ratio in liver lipids

The EPA + DHA:arachidonic acid ratio was determined by the GLC analysis of the methyl ester derivatives of the fatty acids isolated from the liver phosphatidylcholine, according to a procedure reported previously[21].

In animals fed the maize oil or fish oil diets, the maximum level of EPA + DHA:arachidonic acid ratios (0·2 and 3·7, respectively) was reached at week 5 of dietary treatment.

Determination of metastatic potential of lymphoma lines

Both 164T2 and S11 cells were suspended in Roswell Park Memorial Institute (RPMI) at $5{\cdot}0 \times 10^6$ cells/ml, and 0·2 ml of these suspensions were injected into the lateral tail veins of animals fed the maize oil or fish oil diets for 5 weeks. At 3 weeks after tumour cell injection, animals were killed by cervical translocation under diethyl ether anaesthesia, and spleens were weighed and examined histologically. The differences in spleen weights between normal animals and animals transplanted with 164T2 or S11 cells, both fed the fish oil and maize oil diets, were used as a quantitative index of the effect of dietary *n*-3 PUFA on the dissemination of the T lymphoma lines. In all the experimental animals, the measurement of spleen weight was performed 3 weeks after tumour cell injection.

Statistical analysis

The statistical significance of the differences was determined by Student's *t* test.

Results

As shown in Fig. 1, after 10 d following tumour cell injection, a reduction of body weight was seen in animals fed the fish oil diet and transplanted with S11 cells (Fig. 1(a)), but not in those transplanted with 164T2 cells (Fig. 1(b)). At the end of the experiment, the body weight of animals fed the fish oil diet and transplanted with S11 cells was 15 % lower than that of non-injected animals on the same diet. During the entire period of observation, no change of body weight was found in animals fed the maize oil diet and transplanted with either S11 or 164T2 lymphoma cells.

As shown in Fig. 2(a), compared with normal animals fed the fish oil diet, animals transplanted with S11 cells, while fed the same diet, exhibited an eight-fold increase of the spleen's weight ($P<0{\cdot}01$). The two-fold increase of the spleen's weight observed in the transplanted animals fed the maize oil diet was of a limited statistical significance. Spleen weight was scarcely modified in animals transplanted with 164T2 cells while fed the fish oil diet, and unchanged in the transplanted animals fed the maize oil diet.

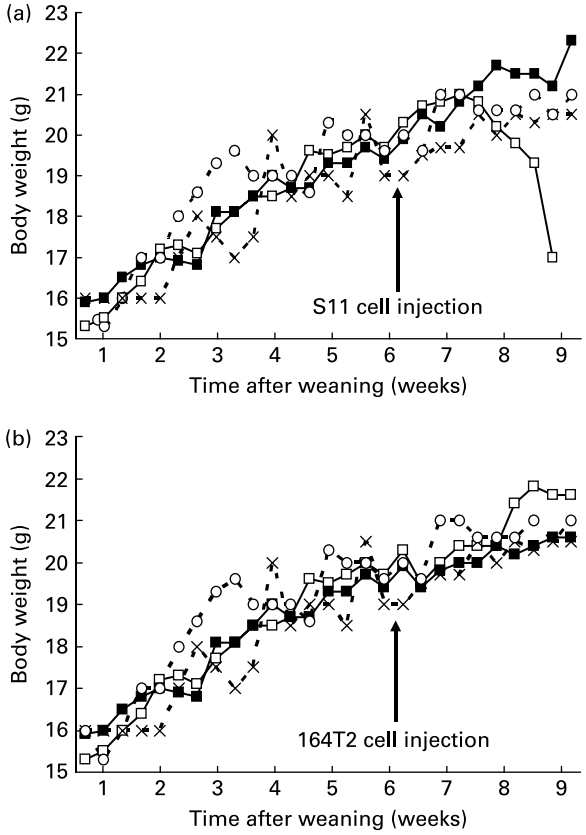

Fig. 1. Change of the growth rate of mice fed a 5 % fish oil (–□–) or a 5 % maize oil (–■–) diet, after transplantation of S11 (a) or 164T2 (b) lymphoma cells at the 6th week (↑) from the beginning of dietary treatment. Normal animals fed the maize oil (– × –) or fish oil (–○–) diet were used as controls. Each value represents the average of the weight of five animals.

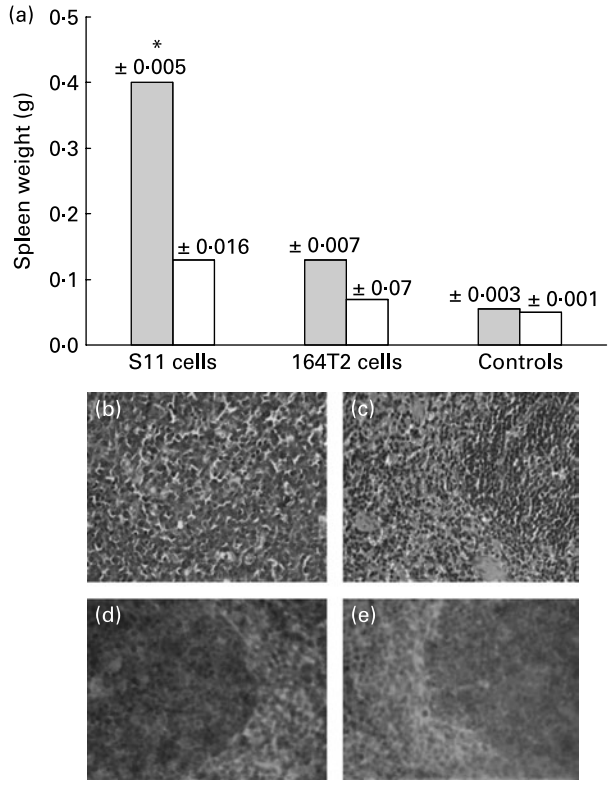

Fig. 2. Effect of feeding a 5% fish oil (▨) or a 5% maize oil (□) diet on the spleen weights (means and SD of five animals) in animals transplanted with S11 or 164T2 cells (a). The spleen weights (means and SD of five animals) in normal animals fed the maize oil or fish oil diet were used for comparison. Fig. 2(b–e) also shows the histological images (40×) of the spleens taken from the experimental animals. Images (b) and (c) are relative to the spleens of animals transplanted with S11 cells while fed the fish oil or maize oil diets, respectively; images (d) and (e) are relative to the spleens of animals transplanted with 164T2 cells while fed the fish oil or maize oil diets, respectively. Lymphomatoid infiltration is evident mainly in image (b). * Mean value was significantly different from that of normal animals fed the fish oil diet ($P<0·01$).

The examination of the histological images relative to the spleens of animals used in the experimental protocol (Figs. 2(b–e)) revealed that a lymphomatoid infiltration in the spleen was clearly evident only in the animals on the fish oil diet transplanted with S11 cells (see Fig. 2(b)).

Discussion

In spite of several reports about the protective activity of dietary *n*-3 PUFA on the incidence of certain tumours[3,23–25], the role of *n*-3 PUFA in the development of human tumours is still debated[23,26,27]. Animal studies, mainly addressing murine breast and colon cancers[12,13,18], point more strongly to an inhibitory activity of *n*-3 PUFA on tumour growth. However, studies on the influence of dietary *n*-3 PUFA on cancer metastasis, performed in a rather limited number of experimental models, produced contradictory results[12–21], possibly due to differences in the histogenesis of tumours as well as in the experimental protocols used in these studies.

In view of the lack of information on the influence of dietary *n*-3 PUFA on the malignant properties of lymphocyte-derived tumours, in the present study we investigated to what extent growth in animals fed a fish oil diet might influence the invasive properties of a system of T lymphoma lines with a different metastatic potential, the S11 and 164T2 cells, using comparison with animals fed a maize oil diet and transplanted with S11 and 164T2 cells. The infiltration of S11 cells in the spleen of fish oil diet-fed animals was much higher than that found in the spleen of S11 cell-transplanted animals fed the maize oil diet. This effect points to a pro-metastatic activity of *n*-3 PUFA on a highly metastatic T lymphoma line, a finding in contrast to the anti-metastatic effect of dietary *n*-3 PUFA reported for other models[12,13,16–19,21,28]. Dissemination of the low-metastatic 164T2 cells in the spleen of animals fed the fish oil diet, as revealed on the basis of the spleen weight, was of a limited magnitude, and not statistically significant. The different responses of S11 and 164T2 cells to dietary *n*-3 PUFA, as shown in the present study, highlight the specific biological characteristics of the two cell lines. The greater growth response of S11 cells to dietary *n*-3 PUFA accounts for the manifest cachexia that was observed in animals transplanted with S11 cells, but not in those transplanted with 164T2 cells.

On the whole, the stimulatory effect of dietary *n*-3 PUFA on the S11 lymphoma line rules out the generalisation that dietary *n*-3 PUFA inhibit tumour growth and progression[28]. As an explanation of the pro-metastatic effect of dietary *n*-3 PUFA on a highly metastatic T lymphoma line, we hypothesise that this effect is due to a reduction of the immunological control over lymphoma cells, a possibility supported by the recognition that diets rich in *n*-3 PUFA have immunosuppressive effects *in vivo* by inhibiting the cellular components of natural and acquired immunity[29]. It is worth noting that a high resistance to lymphokine-activated killer-mediated lysis was acquired by T lymphoma RDM4 cells following transplantation into animals fed a fish oil diet[30]. It is also possible that the pro-metastatic activity of dietary *n*-3 PUFA observed in the present study might be related to an increased rate of purine uptake induced by *n*-3 PUFA[31], as suggested by Griffini *et al.*[15] and by Klieverik *et al.*[20] to explain their finding that dietary *n*-3 PUFA promoted the formation of colon carcinoma metastases in the liver.

The different effect of dietary *n*-3 PUFA on lymphoma lines compared with other types of malignant cells leads us to conclude that some characteristics related to tumour histogenesis modulate the responses to dietary *n*-3 PUFA. This observation should be taken into consideration before introducing dietary protocols based on the use of high doses of *n*-3 PUFA for chemoprevention and chemosuppression of tumours.

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S. R. and A. M. designed the study and prepared the paper. A. M. and N. K. contributed to the successful execution of experimental work. L. C. and G. M. contributed to the lipid analysis.

None of the authors has conflicts of interests with respect to the present study.

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