Clin. Exp. Metastasis, 1997, **15**, 410–417

Influence of lipid diets on the number of metastases and ganglioside content of H59 variant tumors

Josée Coulombe, Guy Pelletier, Pierre Tremblay*, Ginette Mercier* and Daniel Oth*

*Centre de Recherche en Rhumatologie et Immunologie, Centre de Recherche du Centre Hospitalier de l'Université Laval, Sainte-Foy, Québec, Canada; *Institut Armand Frappier, Laval-des-rapides, Québec, Canada*

(Received 16 January 1996; accepted in revised form 7 January 1997)

We investigated the influence of the fatty acid composition of the diet on the number of hepatic metastases and the ganglioside profile of the primary tumor and metastases. C57BL/6 female mice were fed different diets containing either no fats (TEK) or 8% of fish oil (POL), linseed oil (LIN), safflower oil (SAF) or beef tallow (BT) and were injected subcutaneously in the dorsum with H59 cells, a variant of the Lewis lung carcinoma (3LLc) that metastasizes preferentially to the liver. The ω3 polyunsaturated fatty acid (PUFA)-rich diets (LIN and POL) elicited more metastases than the ω6 PUFA-rich (SAF), fat-free (TEK), or saturated fats (BT) diets. However, dietary fat did not influence the ganglioside composition of either the primary tumors or the metastases, at least in the glucidic part. However, comparison of diets with low (TEK, SAF, and BT) and high (LIN and POL) number of metastases showed that the levels of G3 (which could be a second band of GM2) were greater in metastases of the latter group. This study showed that the H59 hepatic metastases contained more GM2 than the s.c. tumors, irrespective of diet or the number of metastases produced. The small differences in the ganglioside profiles observed in this study could have resulted from the limitations of the HPTLC method. A detailed analysis of the lipid chains, as well as glycolipids other than gangliosides, could give more information on changes resulting from different lipid diets.

Keywords: gangliosides, lipid diets, metastases

Introduction

Membrane compositions in fatty acids [1] and polar glycosphingolipids (GLS), especially the gangliosides [2], are critical to the capacity of tumor cells of different sublines to form metastases in a defined target organ [2]. In the case of the H59 variant of the Lewis lung carcinoma, significant differences exist between the ganglioside makeup of the transplanted primary tumor and that of the metastases [3].

Address correspondence to: G. Pelletier, Centre de Recherche en Rhumatologie et Immunologie, Room T1–49 2705 Blvd Laurier, Sainte-Foy, Quèbec, Canada G1V 4G2. Tel: 418 654 2772; Fax: 418 654 2765.

Gangliosides, a class of biologically active cell-surface molecules, are expressed in high concentrations on the plasma membranes of tumor cells. Their chemical structure consists of a carbohydrate portion attached to a lipid composed of a long-chain base and a fatty acid (ceramide) [4]. Hakomori and Kannagi [5] reported that a link may exist between the sugars of the polar moiety and the hydrophobic ceramide moiety, resulting in a certain dependence of ganglioside expression on the fatty acid composition. Stewart and Boggs [6] demonstrated that the structure of the fatty acid chain, namely, its length and/or hydroxylation, can change the interactions between glycolipids.

The importance of the ceramid portion that anchors the ganglioside molecule in the cell membrane is suggested by studies showing that some glycosphingolipid metabolic products may modulate intracellular signal transduction as second messengers [7–9]. More recently, it was shown that the ceramide composition could predict tumor ganglioside immunosuppressive activity, i.e. molecules with short fatty acyl chains are more active than those with longer chains [4].

It has long been known that dietary fats can influence the membrane lipid composition of an *in vivo* growing tumor [10]. It has also been observed that the frequency of metastases given by a subcutaneously transplanted experimental tumor, such as the 3LL carcinoma, can be manipulated by dietary lipids [11]. In the present study, a subline of the Lewis lung carcinoma, the hepatotropic metastasizing 3LLc-H59 cell line was used. The H59 variant model represents an aspect of the metastatic process which could happen in human tumors especially in uveal melanoma which metastasizes mostly to the liver [12]. We asked two questions: (1) Does the fatty acid composition of the diet affect the number of hepatic metastases? and (2) Does diet influence the ganglioside profile of the H59 primary tumors and metastases?

Materials and methods

Cell cultures

The tumor line used was the H59 variant of the 3LLc Lewis lung carcinoma [13], given to us by Dr P. Brodt (McGill University, Montreal, Canada). This variant is preferentially metastatic to the liver.

The H59 cells were cultured in RPMI medium supplemented with 10% fetal calf serum (FCS) and penicillin/streptomycin (Gibco, Ottawa, Canada) under 5% CO_2 in air at 37°C. Confluent cells were harvested with Hanks' balanced salt solution (HBSS) (Sigma, St Louis, MO) containing 0.3% disodium ethylenediamine tetraacetate (Sigma), centrifuged at 150 ***g*** for 10 min, and resuspended in fresh HBSS at a concentration of 5×10^6 cells/ml. Cell viability was assessed by Trypan Blue (Gibco, Ottawa, Canada) exclusion as described [14].

Tumor and experimental animals

Eight-week-old C57BL/6 female mice were purchased from Charles River Inc. (St. Constant, Quebec, Canada). Mice (17–21 per group) were fed their respective experimental diets for at least 4 weeks, then were subcutaneously grafted with H59 variant cells (2×10^5) into the dorsum. This time period was chosen because we wanted the diet to influence not only the tumor and metastases, but the organism as a whole, which implies immune and 'natural' defense mechanisms on which dietary lipids exert some control after lipid metabolism has been equilibrated [15]. Growing tumors were observed twice a week and surgically removed with the mice under anesthesia (Somnotol, 0.5 mg/10 g of body weight; MTC Pharmaceutics Ltd, Cambridge, Ontario, Canada) when the tumor diameter reached 1.0–2.0 cm, which occurred about 3 weeks after grafting, regardless of the diet. The mice were fed their respective diets for an additional 13–20 days and then killed. No recurrent tumor growth was observed at the grafting site. Hepatic metastases were visually counted. Those judged to be sizeable (> 1 mm) were removed, weighed and stored in individual plastic tubes at –20°C until ganglioside determinations were performed. The results presented here are a combination of three experiments performed in identical conditions.

Experimental diets

The diets consisted of 'basal mix for adjusted fat' (casein, 232.14 g/kg; DL-methionin, 3.57 g/kg; sucrose, 527.38 g/kg; maltodextrine, 119.04 g/kg; cellulose fiber, 59.52 g/kg; Teklad 'vitamin mix', 11.9 g/kg; Teklad 'minerals mix', 41.67 g/kg; calcium carbonate, 4.76 g/kg) (Teklad, Madison, WI), to which a proportion of 8% (by weight) of various fats was added. Fats consisted of fish oil (Polepa) (POL group) from Efamol Research Inc. (Kentville, Nova Scotia, Canada), linseed oil (LIN group), safflower oil (SAF group), or beef tallow (BT group) from ICN (Montreal, Quebec, Canada). In a 'no fats' group, sucrose (8% by weight) was added to Teklad basal mix (TEK group). The fatty acid composition of the different fats used, as determined by Efamol Research Inc., has been published [16]. Cholesterol content was similar in all diets after adjustment as described by Oth *et al.* [17]. Fish, linseed and safflower oils were stored under nitrogen and refrigerated. New batches of food were prepared every second week and frozen. Mice were fed daily with freshly thawed food and uneaten food was removed.

Gangliosides extraction and purification

Gangliosides from both s.c. tumors and metastases were extracted according to the method of Ledeen and Yu [18] that we modified to use methylene chloride instead of chloroform, considering that it does not change the efficiency of the extraction (unpublished results). Briefly, the tissues were homogenized and sonicated, and the lipids were extracted with

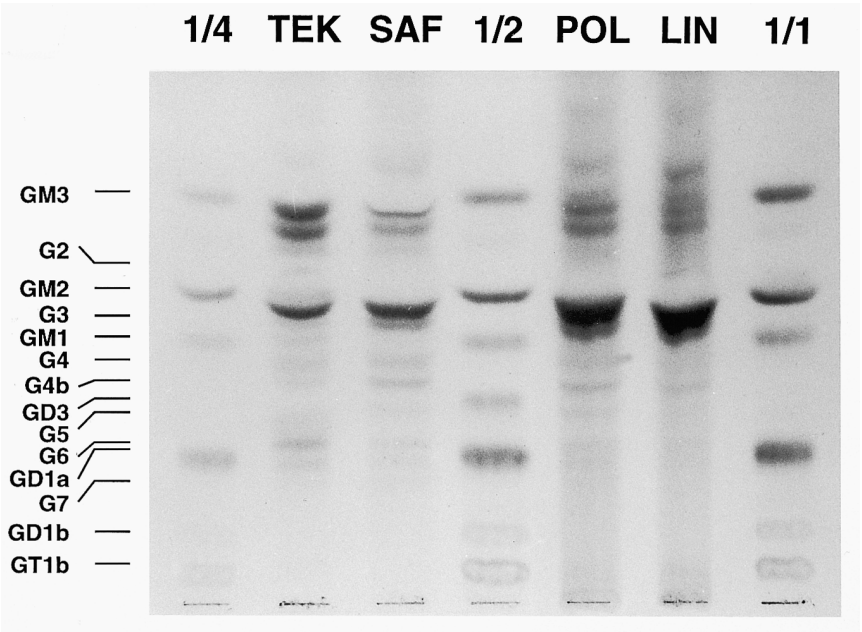

Figure 1. HPTLC silica gel chromatogram of liver metastases grown in mice fed TEK, SAF, POL, and LIN diets. 1/4, 1/2, and 1/1 are half dilutions of a standard solution containing gangliosides GM3, GM2, GM1, GD3, GD1a, GD1b, and GT1b. G2 to G7 are unidentified gangliosides found in the samples. No significant difference was found relating to the diet mice were fed. All metastases contained GM2 as the major ganglioside followed by GM3, G3, and others in low amounts.

methylene chloride:methanol solutions (30 ml/0.1 g of tissue) at successive ratios of 2:1, 1:1, and 1:2 (vol/vol) and dried. The dry residue was then dissolved in di-isopropylether:butanol 60:40 (vol/vol), and the gangliosides were extracted with 1 mM NaCl (2 ml/0.1 g) according to the method of Ladisch and Gillard [19]. This extract was then purified as described by Senn *et al.* [20] with a C18 cartridge (Sep Pack) (J. T. Baker, Phillipsburg, NJ) and chromatographed on DEAE Sephadex A-25 (Pharmacia, Baie d'Urfé, Canada). Salt was removed by Sep Pack C18. During the purification, we also used methylene chloride instead of chloroform.

High performance thin layer chromatography (HPTLC)

Extracts equivalent to 0.1 g of tissue were applied on a silica gel plate (Merck, Darmstadt, Germany) and compared with half dilutions of a standard solution containing bovine brain GM3, GM2, GM1, GD3, GD1a, GD1b, and GT1b (Calbiochem). Gangliosides migrated for 45 min in chloroform: methanol:$CaCl_2$ 0.22% (11:9:2). The plate was then heated for 20 min at 110°C and sprayed with HCl resorcinol to reveal ganglioside-associated sialic acid, which appeared as violet spots on the plate. The density of all violet spots was evaluated using an image analyzer (Amersham RAS, Aylesbury, England) as reported previously [21]. The results are expressed as a percentage of ganglioside-associated sialic acid, with 100% representing the whole ganglioside-associated sialic acid of the sampled tissue. The Rf order of all gangliosides analysed is represented in Figure 1.

Statistics

The Kruskall–Wallis test was used to compare the effect of the different diets on the number of metastases obtained (Table 1) and on the ganglioside profile of their s.c. tumors and metastases (Table 2). We used the chi-square test to compare the percentage of mice that developed metastases using the different diets. Comparison of the ganglioside profiles of s.c. tumors and their corresponding metastases (Table 2), in a given diet, was achieved by the Mann–Whitney test, also used to analyse the

Table 1. Number of liver metastases per tumor-bearing mouse

Diet	X[a]	F[b]
POL[c]	23.2 ± 20.9	18/21
LIN[c]	17.9 ± 18.4	14/17
TEK[d]	5.8 ± 7.2	12/21
SAF[d]	5.5 ± 11.7	11/20
BT[d]	5.3 ± 6.6	11/20

[a]Mean value ± S.D.
[b]Number of mice with metastases/total number of mice used for the experiment (*n*).
According to the Kruskall–Wallis test, all diets compared together are significantly different with a *P*-value of 0.001.
[c]POL and LIN were not significantly different, $P = 0.48$.
[d]TEK, SAF and BT were not significantly different, $P = 0.68$.

Table 2. Ganglioside patterns of subcutaneous tumors and liver metastases from different diets (% of total ganglioside-associated sialic acid)

Gangliosides	Subcutaneous tumors					Metastases			
	LIN ($n = 5$)	POL ($n = 4$)	SAF ($n = 5$)	TEK ($n = 5$)	BT ($n = 5$)	LIN ($n = 5$)	POL ($n = 7$)	SAF ($n = 3$)	TEK ($n = 4$)
GM3	76.1 ± 5.1	64.3 ± 8.5	70.3 ± 7.5	68.5 ± 7.7	65.4 ± 6.1	26.0 ± 4.6	33.4 ± 6.4	23.4 ± 2.4	21.6 ± 10.5
G2	1.8 ± 1.2	2.6 ± 1.5	3.0 ± 1.5	1.3 ± 1.0	1.8 ± 1.1	1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5
GM2	4.1 ± 1.1	7.2 ± 1.8	5.5 ± 1.1	4.1 ± 0.8	4.8 ± 1.1	47.3 ± 5.2	44.0 ± 8.0	52.7 ± 10.2	63.8 ± 14.8
G3	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.7	0.6 ± 0.6	18.1 ± 3.0	11.6 ± 2.3	8.0 ± 4.1	6.2 ± 2.9
GM1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6
G4	5.0 ± 1.3	9.4 ± 1.7	8.7 ± 1.9	8.3 ± 1.0	9.1 ± 1.4	2.6 ± 0.7	3.2 ± 1.0	3.7 ± 0.6	1.4 ± 0.6
G4b	6.1 ± 0.9	8.9 ± 1.9	7.0 ± 2.3	7.8 ± 1.7	8.3 ± 1.4	2.2 ± 0.9	3.3 ± 1.0	3.8 ± 1.0	0.6 ± 0.6
GD3	1.9 ± 0.9	3.5 ± 1.8	2.6 ± 2.0	1.7 ± 1.0	2.6 ± 1.2	0.0 ± 0.0	0.4 ± 0.4	0.9 ± 0.9	0.0 ± 0.0
G6	1.7 ± 0.8	3.0 ± 3.0	1.2 ± 0.8	2.7 ± 1.0	3.1 ± 1.0	1.2 ± 0.6	1.6 ± 0.8	2.0 ± 1.5	2.2 ± 1.4
GD1a	1.2 ± 0.8	0.0 ± 0.0	0.7 ± 0.7	1.5 ± 0.9	1.8 ± 0.8	1.2 ± 0.6	1.8 ± 0.8	2.5 ± 0.7	1.7 ± 1.0
G7	0.2 ± 0.2	0.0 ± 0.0	0.6 ± 0.6	2.0 ± 0.8	2.0 ± 1.1	0.5 ± 0.4	0.4 ± 0.3	3.0 ± 1.0	0.8 ± 0.8
Others	1.2 ± 1.2	0.9 ± 1.8	0.3 ± 0.3	1.3 ± 0.9	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Data are mean values ± S.E.M.

Table 3. Ganglioside patterns of subcutaneous tumors and metastases from diets with high and low numbers of metastases (% total ganglioside-associated sialic acid)

Gangliosides	Subcutaneous tumors		Metastases	
	LIN + POL ($n = 9$)	TEK + SAF + BT ($n = 15$)	LIN + POL ($n = 12$)	TEK + SAF ($n = 7$)
GM3	70.9 ± 4.9	68.8 ± 3.8	30.3 ± 4.2	22.4 ± 5.7
G2	2.2 ± 0.9	2.1 ± 0.7	0.4 ± 0.4	0.3 ± 0.3
GM2	5.5 ± 1.1	4.8 ± 0.6	45.4 ± 5.0	59.1 ± 9.1
G3*	0.3 ± 0.3	0.5 ± 0.3	14.3 ± 2.0	7.0 ± 2.2
GM1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
G4	7.0 ± 1.3	8.5 ± 0.8	3.0 ± 0.6	2.4 ± 0.6
G4b	7.3 ± 1.0	7.5 ± 1.0	2.8 ± 0.7	2.0 ± 0.8
GD3	2.6 ± 0.9	2.1 ± 0.8	0.3 ± 0.3	0.4 ± 0.4
G6	2.3 ± 0.8	2.3 ± 0.6	1.5 ± 0.5	2.1 ± 1.0
GD1a	0.7 ± 0.5	1.2 ± 0.4	1.6 ± 0.5	2.0 ± 0.6
G7	0.1 ± 0.1	1.4 ± 0.5	0.4 ± 0.3	1.7 ± 0.7
Others	1.1 ± 0.7	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

Data are mean values ± S.E.M.
*Metastases from LIN + POL had a significantly higher ($P = 0.03$) G3 value than those from TEK + SAF.

difference between metastases or s.c. tumors from diets of high and low metastatic potential (Table 3).

Results

Dietary fat and metastatic potential

According to the number of visible metastases found in the liver (Table 1), dietary fat can influence metastatic potential. From the five diets used in this experiment, two highly significantly different groups emerged ($P = 0.001$). The BT, SAF, and TEK diets all had similar low numbers of liver metastases and constituted the first group. The second group included the LIN and POL diets, which had similarly more metastases. Of all the diets, BT produced metastases with the smallest size.

Ganglioside profile of livers from non-tumor-bearing mice fed different diets

Results from the ganglioside pattern analysis of livers from normal (not tumor-transplanted) mice indicated no significant difference relative to the diet the mice were fed (data not shown). All livers contained mainly GM2 (63.4–76.7%) and GM3 (13.7–17.5%), with other gangliosides (G2, GM1, GD3 and G5) found only in small amounts.

Ganglioside profiles of s.c. tumors

Comparison of subcutaneous tumors grown in mice submitted to the different diets showed similar ganglioside patterns (Table 2). None was significantly different from the others. The predominant ganglioside was GM3 (around 70% of the total ganglioside-associated sialic acid), followed by G4, G4b, and GM2. The other gangliosides were found in very small amounts.

Ganglioside profiles of metastases

Comparison of the ganglioside profiles of liver metastases grown in mice fed different diets showed no significant differences (Table 2). In all liver metastases, the predominant ganglioside was GM2 (44.0–63.8%), followed by GM3 (21.6–33.4%), G3 (6.2–18.1%) and others in low amounts.

Comparison of ganglioside profiles of s.c. tumors and metastases

Comparison was made between the ganglioside profiles of liver metastases and those of the corresponding s.c. tumors grown in mice that were fed the LIN, POL, SAF and TEK diets (Table 2). Such a comparison could not be made with the BT diet because of the small number and low weight of the metastases that were obtained. For each diet, the ganglioside profile of the metastases had significantly more GM2 and less GM3 than its corresponding s.c. tumor ($P \leq 0.04$).

In mice fed the LIN, POL and SAF diets, the metastases had more G3 than their s.c. tumors ($P \leq 0.05$), whereas mice fed the LIN and TEK diets had metastases with less G4b than the s.c. tumors ($P \leq 0.01$). Mice fed the LIN and POL diets had less GD3 in metastases ($P \leq 0.05$); mice fed the POL, SAF and TEK diets had comparable lower values of G4 in metastatic cells ($P \leq 0.03$). The POL diet produced metastases with a smaller G2 content than the s.c. tumors ($p = 0.05$), and the SAF diet had a higher metastatic content of G7 ($P = 0.04$).

Ganglioside profile and high or low metastatic potential

To determine whether a relationship exists between the ganglioside profile and the metastatic potential, data relative to the LIN and POL diets, which constituted the group with the high number of metastases, were combined and treated as a single category. The same treatment was applied to data relative to the diets with a lower metastatic potential: BT, SAF, and TEK. Comparison of their s.c. tumor patterns showed no significant difference between the two groups (Table 3). However, the metastases of the high metastatic potential group (LIN and POL) had significantly more G3 ($P = 0.03$) than the other group.

Discussion

These results indicate that the quality of dietary fats representing 8% of food intake can strongly influence the number of liver metastases of 3LL-H59 cancer cells. It must be noted that the diets did not influence the growth rate of the primary tumors (results not shown). The two ω3 polyunsaturated fatty acid (PUFA)-rich diets (LIN and POL) produced more metastases than the ω6 PUFA-rich diet (SAF), the saturated fats diet (BT), and the fat-free diet (TEK). In the case of BT-fed mice, not only was the number of metastases small, but the weight of individual metastases was often very low and did not permit an accurate determination of the ganglioside profile. This observation extends that of Young and Young [22], who observed an elevated lung metastasizing capacity of the 3LL tumor grafted onto fish-oil-fed recipients. It differs, however, from observations made by others, in the case of experimental mammary tumors, where ω3 PUFA-rich diets were not associated with an increase in the number of metastases, compared with ω6 PUFA-rich diets [23, 24]. Whereas dietary ω3 PUFA accelerates the growth rate of virus-induced [25] or transplanted [17] lymphomas compared with ω6 PUFA-rich diets, the reverse was observed if the same comparison was made with experimental mammary tumors [26, 27]. Menhaden oil, which is rich in ω3 fatty acids, also exerts an inhibitory action on colon chemical carcinogenesis [28].

The metastatic phenomenon is a complex one and its connection with dietary PUFA may be on several levels (reviewed in [29]). Among them, use of PUFA as a precursor of eicosanoids of various types [30] seems to be a key factor. More particularly, many authors have focused on the role of prostaglandins and leukotrienes [29]. Thus, because of metabolic competition between PUFA derived from different precursors, syntheses of different series of eicosanoids will result directly from the initial proportions of ω6 and ω3 series of PUFA contained in the diet [31]. Prostaglandins, which can be secreted by both tumor and host cells, play a role in tumor dissemination in several experimental systems, including 3LL [32]. Whether these actions result principally from the activity of the tumor cells [33], angiogenic factors [31], or the host's natural killer cell defense mechanism [34] is still unclear.

Another level of connection between PUFA and the metastasizing process would be a modification of the glycolipids on the cell surface, namely, the gangliosides, which are supposed to be implicated in interactions such as cellular recognition [35], adhesion [36] and cell motility [37]. In the present work, however, dietary fat had no strong influence on the ganglioside composition of primary tumors or metastases, nor did it modify the ganglioside profile of the livers of non-tumor-bearing control mice. Whatever the diet, the mean percentages of GM3 and GM2 in the liver were all within relatively narrow ranges: 13.7–17.5% and 63.4–76.7%, respectively. The HPTLC permits observation of modification(s) of the ganglioside profile related to the glucidic part and, for the same carbohydrate portion, important differences in the lipid chain. However, slight differences in the lipid chain could exist that were not detected because they overcame the precision degree of the method. As the length and/or hydroxylation of the lipid chain can change the interactions between carbohydrates [6] that are implicated in cellular recognition and adhesion, it is possible that the same carbohydrate portions, detected in the present work, act differently, depending on the nature of the lipid part. This would explain the different metastatic potentials of cells with similar ganglioside patterns as determined by the methods we used here.

We confirmed in this study that the ganglioside profile of 3LLc-H59 liver metastases differs from that of the subcutaneously growing primary tumor: GM2 replaces GM3 as the predominant ganglioside [3]. However, variations between the s.c. tumor and its corresponding metastases did not have the same importance in all diet groups. It seems that if all the liver metastases had a higher proportion of GM2, how they achieve this biochemically would differ with the metastatic potential of the diet the mice were fed. LIN and POL, the high metastatic potential diets, had less GD3 ($P = 0.05$) in metastases than in s.c. tumors, which was not observed with the diets producing a low number of metastases. This variation may be related to the higher G3 value ($P = 0.02$) found in the metastases of diets with high metastatic potential (LIN and POL: ω3 polyunsaturated). Also, the increase of GM2 in metastases of this group was highly significant ($0.008 \leq P \leq 0.009$) but it was only significant ($0.01 \leq P \leq 0.03$) for the diets with low metastatic potential (TEK and SAF). Because of its Rf value, which is just below that of GM2, so near that in some cases it was very hard to dissociate them for densitometry analysis, G3 is strongly suspected to be a lipid-chain-modified GM2, which would imply an association between the lipid chain structure of the GM2 molecule and the liver-colonizing capacity of metastatic cells.

The results of the present study indicate that the ganglioside composition of H59 cells had some constant characteristics over modifications of the lipids furnished by the different diets. They also suggest that a particular ganglioside composition of cells in the s.c. tumor and/or metastasis is necessary to their survival so that only cells having the right pattern for a given environment can survive and divide. This scenario would confirm the gangliosides as being part of the specific characteristics needed to permit the growth of a particular cell into a given environment, or organ, as proposed in Paget's seed and soil theory [38]. The H59 hepatic metastasis, constituted of cells properly adapted to their new environment, may be homogeneous in its ganglioside composition, at least for certain gangliosides such as GM2 and GM3, suggesting that cells with a different ganglioside profile or that are unable to effect the correct modulation either die, destroyed by the immune system, or escape to other metastatic sites.

Gangliosides are only one part of the glycolipid family that may be involved in the metastatic process. Other acidic glycolipids, like sulfatides or neutral glycolipids, should be investigated to determine whether diet could influence their expression on the cell surface. Also, a more detailed study of the lipidic portion could shed light on the exact modifications caused by diet and help to explain their implication in the metastatic process. This study indicates that dietary PUFA influences the metastatic potential of H59 cells and agrees with the possible relationship between GM2 and liver-specific colonization, suggesting that the nature of the lipid chain of this ganglioside could modulate the interaction.

Acknowledgements

This project was supported by a grant from the Cancer Research Society Inc., Canada. J. Coulombe was recipient of a scolarship from the Fonds pour la Formation des Chercheurs et l'Aide à la Recherche (FCAR). The authors thank Mrs D. Castillaw for editorial assistance, and Mrs S. Toussaint and Mrs N. Plourde for technical assistance.

References

1. Calorini L, Fallani A, Tombaccini, *et al.* 1989, Lipid characteristics of RSV-transformed Balb/c 3T3 cell lines with different spontaneous metastatic potentials. *Lipids*, **24**, 685–90.

2. Hanisch F-G, Sölter J, Jansen V, Lochner J, Peter-Katalinic J and Uhlenbruck G, 1990, Glycosphingolipid expression on murine L1-fibrosarcoma cells: analysis of clonal *in vivo* and *in vitro* selected sublines with different lung colonization potential. *Br J Cancer*, **61**, 813–20.
3. Coulombe J and Pelletier G, 1993, Ganglioside and organ-specific metastatic colonization. *Int J Cancer*, **53**, 104–9.
4. Ladisch S, Li R and Olson E, 1994, Ceramide structure predicts tumor ganglioside immunosuppressive activity. *Proc Natl Acad Sci USA*, **91**, 1974–8.
5. Hakomori S-I and Kannagi R, 1983, Glycosphingolipids as tumor-associated and differentiation markers. *J Natl Cancer Inst*, **71**, 231–41.
6. Stewart RJ and Boggs JM, 1993, A carbohydrate–carbohydrate interaction between galactosylceramide containing liposomes and cerebroside sulfate-containing liposomes: Dependence on the glycolipid ceramide composition. *Biochemistry*, **32**, 10666–74.
7. Hannun YA and Bell RM, 1987, Lysosphingolipids inhibit protein kinase C: implication for the sphingolipidoses. *Science*, **235**, 670–4.
8. Zhang H, Desai NN, Olivera A, Seki T, Brooker G and Spiegel S, 1991, Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. *J Cell Biol*, **114**, 155–67.
9. Okazaki T, Bell RM and Hannun YA, 1989, Sphingomyelin turnover induced by vitamin D3 in HL-60 cells. Role in cell differentiation. *J Biol Chem*, **264**, 19076–80.
10. Awad AB and Spector AA, 1976, Modification of the fatty acid composition of Ehrlich ascites tumor cell plasma membranes. *Biochim Biophys Acta*, **426**, 723–31.
11. Scholar EM, Violi LAD, Newland J, Bresnick E and Birt D, 1989, The effect of dietary fat on metastasis of the Lewis lung carcinoma and the Balb/c mammary carcinoma. *Nutr Cancer*, **12**, 109–19.
12. Char DH, 1978, Metastatic choroidal melanoma. *Am J Ophthalmology*, **85**, 76–80.
13. Brodt P, 1986, Characterization of two highly metastatic variants of Lewis lung carcinoma with different organ specificities. *Cancer Res*, **46**, 2242–8.
14. Patterson MK Jr, 1979, Measurement of growth and viability of cells in culture. In: Jakoby WB and Pastan IH, eds. *Methods in Enzymology*, Vol. 58. New York: Academic Press, pp. 141–52.
15. Erickson KL, 1986, Dietary fat modulation of immune response. *Int J Immunopharmac*, **8**, 529–43.
16. Benquet C, Krystyniak K, Savard R and Guertin F, 1994, Modulation of exercise-induced immunosuppression by dietary polyunsaturated fatty acids in mice. *J Toxicol Envir Hlth*, **43**, 225–37.
17. Oth D, Mercier G, Tremblay P, *et al.* 1990, Modulation of CD4 expression on lymphoma cells transplanted to mice fed (*n*–3) polyunsaturated fatty acids. *Biochim Biophys Acta*, **1027**, 47–52.
18. Ledeen RW and Yu RK, 1982, Gangliosides: structure, isolation, and analysis. In: Neufeld EF and Ginsburg V, eds. *Methods in Enzymology*, Vol. 83. New York: Academic Press, pp. 139–91.
19. Ladish S and Gillard B, 1985, A solvent partition for microscale ganglioside purification. *Anal Biochem*, **146**, 220–31.
20. Senn H-J, Orth M, Fitzke E, Wieland H and Gerok W, 1989, Gangliosides in normal human serum. Concentration, pattern and transport by lipoproteins. *Eur J Biochem*, **181**, 657–62.
21. Soulières D, Rousseau A, Deschênes J, Tremblay M, Tardif M and Pelletier G, 1991, Characterization of gangliosides in human uveal melanoma cells. *Int J Cancer*, **49**, 498–503.
22. Young MRI and Young ME, 1989, Effects of fish oil and corn oil diets on prostaglandin-dependent and myelopoiesis-associated immune suppressor mechanisms of mice bearing metastatic lung carcinoma tumors. *Cancer Res*, **49**, 1931–6.
23. Kort WJ, Weijma IM, Stehmann TEM, Vergroesen AJ and Westbroek DL, 1987, Diets rich in fish oil cannot control tumor cell metastasis. *Ann Nutr Metab*, **31**, 342–8.
24. Adams LR, Trout JR and Karmali RA, 1990, Effect of *n*-3 fatty acids on spontaneous and experimental metastasis of rat mammary tumour 13762. *Br J Cancer*, **61**, 290–1.
25. Potworowski E, Bischoff P and Oth D, 1992, Prolongation of survival in retrovirally induced T cell lymphoma by dietary ω6 fatty acid. *Nutr Cancer*, **17**, 217–21.
26. Karmali RA, Marsh J and Fuchs C, 1984, Effect of Omega-3 fatty acids on growth of a rat mammary tumor. *J Natl Cancer Inst*, **73**, 457–61.
27. Gabor H and Abraham S, 1986, Effect of dietary Menhaden oil on tumor cell loss and the accumulation of mass of a transplantable mammary adenocarcinoma in Balb/c mice. *J Natl Cancer Inst*, **76**, 1223–9.
28. Reddy BS, Burill C and Rigotty J, 1991, Effect of diets high in ω-3 and ω-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. *Cancer Res*, **51**, 487–91.
29. Erickson KL and Hubbard NE, 1990, Dietary fat and tumor metastasis. *Nutr Rev*, **48**, 7–13.
30. Crawford MA, 1983, Background to essential fatty acids and their prostanoid derivatives. *Br Med Bull*, **39**, 210–13.
31. Ormerod LD, Garsd A, Abelson MB and Kenyon KR, 1990, Effects of altering the eicosanoid precursor pool on neovascularization and inflammation in the alkali-burned rabbit cornea. *Am J Pathol*, **137**, 1243–52.
32. Young MRI, Young ME, Lozano Y, Coogan M and Bagash JM, 1991, Regulation of protein kinase A activation and prostaglandin E2-stimulated migration of Lewis Lung carcinoma clones. *Int J Cancer*, **49**, 150–5.
33. Young MRI, Newby M and Meunier J, 1985, Relationship between morphology, dissemination, migration and Prostaglandin E2 secretion by cloned variants of Lewis lung carcinoma. *Cancer Res*, **45**, 3918–23.
34. Vaillier D, Daculsi R, Gualde N and Bezian JH, 1992, Effect of LTB4 on the inhibition of natural cytotoxic activity by PGE2. *Cell Immunol*, **139**, 248–58.
35. Kojima N and Hakomori S-I, 1990, Specific interaction between gangliotriaosylceramide (Gg3) and sialosyllactosylceramide (GM3) as a basis for specific cellular recognition between lymphoma and melanoma cells. *J Biol Chem*, **264**, 20159–62.
36. Cheresh DA and Klier FG, 1986, Disialoganglioside GD2 distributes preferentially into substrate-associated microprocesses on human melanoma cells during

their attachment to fibronectin. *J Cell Biol*, **102**, 1887–97.
37. Kojima N and Hakomori S-I, 1991, Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid–glycolipid interaction. *J Biol Chem*, **266**, 17552–8.
38. Paget S, 1889, The distribution of secondary growths in cancer of the breast. *Lancet*, **1**, 571–3.