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γ-Glutamyltranspeptidase in Murine Lymphomas*

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Abstract—*Five murine lymphoma cell lines were assayed for the content and activity of γ-glutamyltranspeptidase* (GTT). *All lines* [*S49; L–12; 230–23–8* (*C57 Black*); *2M3 and RA3–2C2*] *contained detectable amounts of GGT. The specific activities of GGT were low and ranged between* 1.2 *and* 2.3 mU/mg *protein in cells growth* in vitro. *A highly malignant variant of the* S49 *line was also grown* in vivo *in BALB/c mice. This subline invariably produces both solid and ascitic tumors with infiltrations into the pancreas, liver and spleen. GGT levels in the tumor cells were low and independent of tumor type (solid, ascitic), location, passage number or inoculum size. Infiltrations of S49 tumor cells in liver and spleen were invariably GGT – negative as judged by histochemical examination. GGT activities in suspension cultures prepared from solid. as well as ascitic tumors were low. Occasional high GGT activity of solid tumors was due to the presence of pancreas cells in them. The only host tissue significantly responding to the presence of tumors by elevated GGT levels was the liver. Compilation of data from this study and those of others clearly indicates that low GGT level is a typical property of tumors originating in the immune system.*

INTRODUCTION

THE ENZYME γ-glutamyltranspeptidase (GGT, EC 2.3.2.2) is a plasma-membrane enzyme present in a number of cell types. Several physiological processes have been suggested to involve GGT, e.g. transport of amino acids and peptides, metabolism of glutathione and prostaglandins and detoxification of xenobiotics [1–6].

The work of numerous laboratories has established elevated levels of GGT as a useful early marker for hyperplastic and neoplastic foci developing during experimental hepatocarcinogenesis in rats and hamsters [7–14]. Elevated levels of GGT have been associated with other tumor types in other species as well, e.g. mouse skin [15], human colon [16, 17], human lung [18], mouse mammary gland [19], hamster buccal pouch [20] and thryoid [21]; the highest increase of GGT activity was found in the liver [22]. Increases of GGT were also observed in uninvolved tissues of tumor-bearing rats [23].

In spite of the good correlation of elevated GGT levels with the (pre)neoplastic state of the cells, it does not seem that high specific activity of this enzyme is a prerequisite for tumor development or maintenance [13, 24–29]. However, it has been suggested that GGT-rich cells may have a selective advantage over GGT-poor (deficient) ones due to (i) increased resistant to the acute toxicity of various carcinogens [16, 30–35]; (ii) increased supply of cysteine from glutathione metabolism, or (iii) enhanced transport of neutral amino acids by the γ-glutamyl cycle [28, 31]. The first hypothesis enjoys the widest experimental support (see references above), whereas the second and third ones seem less likely, since levels of GGT are inversely proportional to neutral amino acid transport in porcine renal epithelial cells [36], and, except for that of glutamine, had no effect on neutral amino acid transport in human lymphoid lines [37]. Further, the lack of correlation between the specificity of various amino acid transport systems and the ability of those amino acids to serve as γ-glutamyl acceptors [38] argues against GGT as a major component of amino acid transport, at least in tissues other than the kidney.

While GGT has been studied most extensively in tumorous tissues of epithelial origin, less is known about the association of this enzyme with tumors

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originating from non-epithelial tissues: to the best of our knowledge, only two reports deal specifically with GGT activity in human and murine cancers originating in the immune system [27, 37]. We have therefore extended the studies of the association of GGT with various murine lymphomas, and focused the investigation on a highly tumorigenic subline [39] of the S49 mouse lymphoma [40], in order to test whether tumors proliferating in different locations within the same animal manifest differences in GGT levels. The work presented here shows that (i) similarly to human tumors originating in the immune system and several murine lymphomas [27], GGT is not elevated in the five mouse lymphomas studied here; (ii) there is no effect of the tumor location on GGT activities; and (iii) the only 'involved' (see below) tissue of tumor-bearing mice demonstrating elevated levels of GGT is the liver.

MATERIALS AND METHODS

Cells and tumors

Mouse lymphoma, T–25 *subline* [39] *of the* S49 *cell line* [40]. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum, penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were maintained at 37°C in a humidifed atmosphere containing 5% CO_2. Viability was measured using trypan blue exclusion.

Tumors. The T–25 subline [39] of S49 cells (1–2 × 10^7) were inoculated i.p. into 8- to 10-week-old syngeneic male BALB/c mice. Mice were obtained from the breeding colony of the Hebrew University, Jerusalem.

*Mouse lymphoma lines L*12, 230–23–8(*C*57 *black*) 2*M*3 *and RA*3–2*C*2. Lines L–12, 230–23–8 (C57 black) and 2M3 are mouse lymphoma lines. Line RA3–2C2 is a hybridoma between mouse and rat. The cells were grown routinely in RPMI medium containing 10% fetal calf serum. These lines were kindly provided by Dr A Gonen, Biotechnology General, Rehovot, Israel.

Rat embryo hepatocyte cultures

Pregnant female Sprague–Dawley rats were used to obtain and maintain monolayers of fetal rat hepatocytes. Hepatocytes were prepared from rat fetus livers weighing 200–250 mg each as previously described [41]. Cultures were grown according to Neuman *et al.* [42] and were practically free of fibroblasts during the experimental time period (up to 8 days).

Tissue preparation for the GGT assay

Normal and tumor-bearing mice were killed by decapitation. Organs, tissues and solid tumors were dissected and isolated from surrounding tissues, washed in 50 mM Tris–HCl, 250 mM sucrose buffer, pH 7.4, and were homogenized in a Teflon–glass homogenizer with 3 vol of the same buffer. Tissue and tumor homogenates were quickly frozen in acetone–dry ice and were stored at −20°C until used. Storage was for up to 7 weeks, during which there was no significant decrease in GGT activity.

Ascitic lymphoma cells were aspirated with the ascitic fluid from the abdominal cavity. Cells were washed thrice with Tris buffer, concentrated by centrifugation and homogenized as described above. Lymphoma cells growing in suspension cultures were washed thrice in Tris buffer and homogenized as described above. Rat hepatocytes were scraped off plates with a rubber policeman, washed and homogenized as described above.

Spectrophotometric assay of GGT

GGT was assayed spectrophotometrically by a modification of the method of Orlowsky and Meister [43]. Reaction mixtures (500 μl) contained 110 mM hydroxyethylpiperazine–ethane sulfonic acid (HEPES) buffer, pH 8.5; 10 mM $MgCl_2$; 5 mM γ-L-glutamyl-*p*-nitroanilide; 22 mM glycylglycine; and the appropriate dilution of tissue homogenates or commercial porcine or bovine GGT.

Half of the reaction mixture was immediately mixed with 5 vol of 1 M acetic acid (blank) and the rest was incubated at 37°C for 20–60 min. Five volumes of 1 M acetic acid were then added, followed by centrifugation for 5 min at 10,000 ***g*** at room temperature. The absorbance of the released *p*-nitroaniline was determined at 410 nm, and its amount was calculated from the increase of A_{410} (ϵ = 8800) and a standard curve of commercial *p*-nitroaniline. Significant release of *p*-nitroaniline was not detected in reactions incubated up to 90 min in the absence of GGT, was linear with time up to 60–70 min in its presence and was almost completely inhibited in the presence of a 5 mM serine and 10 mM borate [44]. Reaction rate in the absence of glycylglycine was decreased to 20–25% of that of complete reaction mixtures [45]. The above indicate that the activity can be attributed to GGT. Peritoneal fluid from normal and tumor-bearing mice and growth media from lymphoma and rat hepatocyte cultures contained low, constant GGT activities and did not contain GGT inhibitors as judged by the addition of various amounts of the above to reactions containing known GGT amounts.

The specific activity of GGT was calculated from those homogenate dilutions in which enzyme activity was linearly proportional to protein concentration. Protein was determined according to the

method of Lowry *et al.* [46].

A unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol *p*-nitroaniline/min. Specific activity was expressed in terms of mU/mg protein. GGT specific activity values cited from other works throughout this paper were re-calculated and expressed in mU/mg protein.

Fluorimetric assay of GGT

In order to detect the low activities of GGT in lymphomas and other mouse tissues, or in small numbers of cells derived from tissue culture, the sensitive fluorimetric GGT assay according to Smith *et al.* [47] was employed, using γ-L-glutamyl-2-amino-4-methyl coumarin as substrate. The assay procedure was essentially as described [47], with the appropriate dilution of tissue homogenates and commercial bovine GGT as standard. Reactions were stopped with 50 mM glycine buffer, pH 10.4. The fluorescence of the free aminomethylcoumarin was measured in a Perkin–Elmer MFP-44B spectrofluorimeter (λ_{ex} = 370 nm, λ_{em} = 440 nm).

Standard calibration curves were constructed daily by measuring the net relative fluorescence of a series of freshly prepared solutions of 7-amino-4-methylcoumarin in 50 mM glycine buffer, pH 10.4. The amount of aminomethylcoumarin released by the enzyme was calculated from the increase in fluorescence and the standard curve. Specific activity of GGT was calculated as described above.

The specific activities of GGT measured fluorimetrically were about 12% higher than those measured spectrophotometrically in the same samples. For example, three determinations of the same normal mouse kidney homogenate yielded 598 ± 151 and 658 ± 186 mU/mg protein in the spectrophotometric and the fluorimetric assays, respectively. Most of the presented values were obtained by the fluorimetric assay. In our hands, fluorimetry allows the detection of 50 nM changes in aminomethylcoumarin concentrations. Statistically significant differences of GGT activities between normal and tumor-bearing mice were determined by Student's *t* test at a 99% level of probability ($P < 0.01$).

Histochemistry of GGT

Mouse tissues and solid tumors were fixed in 10% formalin and were embedded in paraffin according to Dempo *et al.* [48]. Sections (6 μm) were stained for GGT according to Rutenberg *et al.* [49] and with hematoxylin–eosin for the detection of pathological changes or infiltrations of lymphoma cells into mouse organs.

Materials

Bovine and porcine GGT, glycylglycine, γ-glutamyl-*p*-nitroanilide, γ-glutamylamino-

Table 1. Distribution of GGT in normal tissues of BALB/c mice, rats and humans

	GGT activity					
	mU/mg protein		Relative activity (kidney = 100)			
	Mice*		Rats†	Mice‡	Rats§	Humans‖
Kidney	660 ± 212	(4)	352	100	100	100
Pancreas	63.3 ± 15.1	(5)	N.T.	9.6	N.T.	8.3
Brain	3.7	(1)	N.T.	0.56	N.T.	0.5
Testis	2.08 ± 0.95	(3)	N.T.	0.32	N.T.	N.T.
Lung	1.73 ± 1.1	(4)	0.46	0.26	0.13	0.31
Spleen	1.14 ± 1.21	(4)	0.36	0.17	0.10	1.5
Heart muscle	0.41 ± 0.31	(4)	N.T.	0.06	N.T.	0.045
Skeletal muscle	0.30	(1)	N.T.	0.05	N.T.	0.067
Liver	0.12 ± 0.08	(4)	0.29¶	0.02	0.08	3.9
Small intestine	N.T.		3.36**	N.T.	0.95	0.95

*GGT specific activities were calculated according to Materials and Methods. Values are means ± S.D. Numbers in parentheses are numbers of individual mice examined.

†Mean of GGT activities in Fischer, Kx and Buffalo rat strains, calculated from Table 6 in [23].

‡Relative specific activities of GGT calculated for mice; kidneys specific activity was arbitrarily designated as 100.

§Calculated from second column as in (c).

‖Taken from [43].

¶We have tested some Charles River pregnant females. GGT activity was 0.46–0.52 mU/mg protein.

**Fischer rats, taken from Table 1 in [23].

Table 2. GGT activity levels in tissues of tumor-bearing BALB/c mice

Mouse No.	Kidney	Heart	Lung	Testis	Liver	Spleen	Solid tumor	Ascitic tumor
1*	469	0.27	0.70	1.24	0.05	*4.54*	0.84	N.T.
2‡	285	*3.75*	0.31	0.80	*2.20*	0.40	0.85	1.03
3†‡	433	*1.99*	1.17	1.43	*0.36*	0.67	1.51	0.91
4	604	*1.71*	0.98	*11.20*	0.22	0.40	1.59	0.82
5	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	1.90	1.58
6	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	2.22	1.10
7*	739	0.74	*4.73*	N.T.	*0.44*	2.91	2.33	N.T.
8	542	N.T.	0.40	*7.84*	*0.38*	0.64	2.43	0.87
9	336	0.02	1.59	1.17	0.02	0.44	3.06	0.98
10	346	0.13	1.47	2.51	0.02	0.57	3.48	1.81
11	514	0.64	*14.19*	3.14	0.02	0.51	3.70	0.62
12‡	459	0.38	0.59	0.95	0.62	*9.13*	3.99	1.51
13	476	0.25	0.81	1.63	0.02	0.49	3.99	N.T.
14	815	0.42	0.64	1.81	0.10	0.32	4.27	N.T.
15	353	0.02	1.32	1.67	0.05	1.02	4.76	1.81
16	446	0.38	1.97	*4.90*	0.08	1.44	4.90	1.47
17	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	5.60	1.45
18‡	430	0.07	0.79	0.30	0.20	1.57	5.71	0.44
19	321	0.02	0.47	2.53	*0.44*	2.00	10.2	1.23
20‡	710	0.09	1.43	0.90	0.17	0.91	19.2	0.60
21‡	860	0.85	1.75	2.00	*0.97*	*7.78*	19.5	1.57
22	420	0.62	1.13	0.65	*0.60*	1.16	24.5	N.T.
23	480	0.58	1.47	0.87	*0.90*	0.91	32.9	N.T.
24	540	0.18	2.60	0.80	0.17	2.08	61.2	N.T.
25	355	N.T.	N.T.	N.T.	0.031	1.69	2.67	0.85
26	314	N.T.	N.T.	N.T.	*0.37*	1.26	2.43	1.70
27	304	N.T.	N.T.	N.T.	0.09	0.33	1.73	0.98
28	505	N.T.	N.T.	N.T.	*0.90*	0.86	2.18	1.24
29	585	N.T.	N.T.	N.T.	*0.38*	0.91	1.98	2.95
30	475	N.T.	N.T.	N.T.	*3.84*	1.16	21.0	2.00

Mice Nos 1–24 were injected with 10^7 T–25 lymphoma cells (if not indicated otherwise) and were killed after 14 days. Tissues were homogenized and assayed for GGT as described above. GGT activities are expressed in mU/mg protein. Numbers in italics are GGT levels significantly higher than those of normal mice (*t* test, $P < 0.01$; see Table 1). (*) Mice injected with 100 cells; (†) mouse injected with 1000 cells; (‡) ascitic and solid tumors from these mice were used for the preparation of tissue cultures. Mice Nos 25–30 were injected i.p. with 10^7 T–25 lymphoma cells and killed at 9 days (mouse No. 30), 13 days (nos 25–27) and 14 days (Nos 28–29) post-inoculation. Parts of the liver, spleen and solid tumors were used for the preparation of 6 μm sections, and were stained for GGT as described in Materials and Methods. The remaining of the tissues were assayed for GGT. T–25 cell infiltrations were observed in all of the livers and spleens (see figures).

methylcoumarin, and aminomethylcoumarin were from Sigma Chemical Co., St. Louis, MO. Media for cell culture were from Gibco, Grand Island, NY. Sera were from Sera Laboratories, Crowly Down, West Sussex, U.K.

RESULTS

Distribution of GGT in tissues of tumor-bearing mice

Table 1 demonstrates the distribution of GGT activity among various tissues and organs of control BALB/c mice. the distribution of GGT in mice is similar to that observed in rats and in humans (Table 1).

Table 2 shows the distribution of GGT activity among the various tissues of tumor-bearing mice. In most cases GGT activity values were statistically indistinguishable from those of control mice, and in some cases it was significantly elevated (italics in

Table 2). An increase in GGT activity which deviated from the mean specific activity at a confidence level of 99% ($P < 0.01$; t test) was considered statistically significant.

T-25 lymphoma cells were found to infiltrate the liver, testis and spleen, and thus were designated as 'involved' tissues. Other tissues were designated as 'uninvolved' ones [23]. Tumor-bearing mice were analyzed for GGT activity in 'involved' and 'uninvolved' tissues. Table 3 shows that approximately 45% of tumor-bearing mice had increased GGT activity in the liver, whereas the frequencies of organs with increased GGT activities in the spleen, testis, heart or lung tissue were low. Four out of six mice bearing tumors with infiltration(s) in the liver (as judged by microscopy, see below) showed increased GGT of the liver (mice 25–30, Table 2). Microscopical analysis of the spleens of these six mice revealed that all the spleens contained infiltrations of the lymphoma cells as well, but GGT levels in the spleens remained low (Table 2). Thus, in contrast to the situation in rats, where liver and other tissues do respond to the presence of tumors by elevated GGT levels, irrespective of the tumor type or the enzyme activity within the tumors, [23], there is no clear pattern of relationship between GGT levels in mouse tissues and the presence of lymphoma, except in the case of liver. GGT increase in the infiltrated liver may be due to local biliary statis caused by the infiltrating tumor rather than the expression of GGT by the tumor cells. This is supported by the histochemical results which give no indication of elevated GGT in lymphoma cells infiltrating the liver.

In addition, there was no correlation between elevated GGT levels and the number of lymphoma cells inoculated (determining the duration of host exposure to proliferating tumors) or the passage number of the inoculated cells.

Table 3. Distribution of elevated GGT levels in various organs among mice bearing T-25 lymphoma

	No of mice examined	No. of mice with increased GGT*	%†
Kidney	27	0	0
Lung	22	2	10
Heart	20	3	15
Testis	20	3	15
Spleen	27	3	11
Liver	27	12	44

*Levels of GGT were derived from Table 2. Differences between normal and tumor-bearing mice were statistically significant at $P < 0.01$ (t test).

†Frequency of mice with elevated GGT levels among tumor-bearing mice.

GGT activity in solid and ascitic tumors

The S49 lymphoma produces both solid and ascitic tumors in the abdominal cavity of i.p.-inoculated mice. It was thus possible to examine GGT levels of the tumor cells growing in the same animal in two forms.

Various mouse lymphoma lines grown *in vitro* have been shown to contain GGT activities lower than those of the solid tumors from which they had been derived [27]. Table 2 shows that all ascitic tumor components contained low GGT levels with a relatively small fluctuation between individuals, whereas those of solid tumors varied greatly.

In vitro suspension cultures were prepared from solid and ascitic tumors of six mice (indicated in Table 2) and GGT levels were determined during

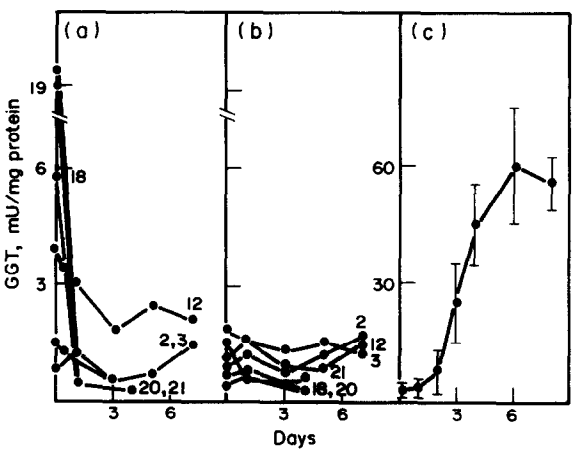

Fig. 1. GGT levels in T-25 cells and fetal rat hepatocytes growing in cultures. Panels A and B: GGT in cultures from solid and ascitic S49 tumors. Six mice bearing the T-25 subline were killed. Tissue cultures, were prepared from both ascitic and solid tumor cells derived from each mouse as described in Materials and Methods. Cells from duplicate plates were harvested at the indicated time points, and were assayed for GGT and for protein as described above. Numbers within panels pertain to mouse numbers in Table 2. A — cultures derived from solid tumors; B — cultures derived from ascitic tumors. Panel C: Induction of GGT in cultured fetal rat hepatocytes. Cultures of fetal rat hepatocytes were prepared as described in Materials and Methods. GGT induction was meausred as described above. Plotted are means of GGT levels ± S.D. (bars)

their growth. Figure 1 (A, B) shows that GGT levels in cultures derived from ascitic tumors were comparable to those of ascitic cells grown *in vivo* and did not vary greatly. Cultures which had been derived from solid tumors containing high initial GGT activity lost it rapidly, whereas those which had been derived from solid tumors with low initial GGT activity did not manifest this decrease (Fig.

1A, B). The enzyme levels in the latter were comparable to those of ascitic tumors grown *in vivo* or *in vitro*.

Low GGT activities in suspension cultures incubated for several days could result from (i) the presence of GGT inhibitors in the growth medium; (ii) the small number of cultured cells assayed; (iii) the true decrease in enzyme activity; or (iv) the composition of growth medium which could affect the induction of GGT [8, 50]. The growth medium (and the ascitic fluid) did not contain inhibitors of GGT (see Materials and Methods). In order to test the ability to detect significant changes of GGT activity in a small number of cells in culture we have used the known capacity of rat hepatocytes to express GGT *in vitro* [41]. Figure 1(C) shows that significant changes of GGT levels could be easily detected in rat-hepatocytes cultures.

Distribution of GGT in tissues of lymphoma-bearing mice as judged by histochemistry

The variability of GGT levels in solid tumors, and the facts that (i) normal pancreas contains very high levels of GGT (Table 1) and (ii) that it was difficult, and many times impossible to distinguish macroscopically between pancreas and solid tumors, led us to suggest that pancreas cells may be present within many of the solid tumors.

Six mice were inoculated i.p. with S49 and killed at 9, 13 and 14 days post-inoculation. Kidney, spleen, liver and solid tumor tissues were used to prepare histological sections. The remaining parts of the tissues were homogenized and assayed fluorimetrically for GGT. Figure 2 shows that pancreas tissue is indeed embedded in tumors examined 9 and 13 days (Fig. 2C, D) post-inoculation. Tumor cells were invariably GGT-negative, both in solid tumors (Fig. 2) and in infiltrations into the spleen and liver (Fig. 3). Table 2 shows the specific activity of GGT in those tumors. It is thus safe to assume that the apparent high GGT activity of the solid tumors can be attributed to pancreas cells.

The variability of GGT activity among solid tumors can be explained by the presence of varying numbers of pancreas cells in them. The rapid decrease of overall GGT activity in cultures of solid tumors containing high initial enzyme levels can also be explained: lymphoma cells with low GGT proliferate with time, whereas GGT-rich, normal pancreatic cells do not.

These highly malignant cells invade other organs as well: in addition to infiltration in and around the pancreas, all six tumor-bearing (examined histologically) mice showed infiltrations in the liver and spleen (Fig. 3). The infiltrating tumor cells (Fig. 3) as well as the surrounding tissue (Fig. 3, Table 2) contained low GGT levels.

GGT activity in other mouse lymphomas

Four additional mouse lymphoma lines were grown in culture and examined for their GGT content. Table 4 shows that GGT activities were

Table 4. GGT activity in various murine lymphomas

Line	Solid tumor	Ascitic tumor cells	Tumor cells in culture
T-25	7.8 ± 1.8 (17)	1.16 ± 0.43 (17)	1.01 ± 0.44 (29)†
			1.36 ± 1.0 (24)‡
L12	—	—	1.55 ± 0.21 (4)§
230-23-8(C57black)	—	—	2.34 ± 0.62 (4)§
2M3			1.82 ± 0.26 (4)§
RA3-2C2	—	—	2.10 ± 0.30 (4)§
MPC 104	0.65*	—	0.30*
3469	0.57*	—	0.17*
1748	0.75*	—	0.17*
MPC 11	—	—	1.25*
471	—	—	0.17*
HPC 76	—	—	0.17*
PU5R	—	—	0.17*
183	—	—	0.55*

GGT activity is expressed as mU/mg protein. Numbers in parentheses pertain to the number of mice examined.

*Calculated from [27].

†Cultures from acitic murine lymphomas, this work.

‡Cultures from solid murine lymphomas, this work. GGT activity in culture medium was 0.1 mU/mg protein.

§Cultures of murine lymphomas other than TAS49, this work.

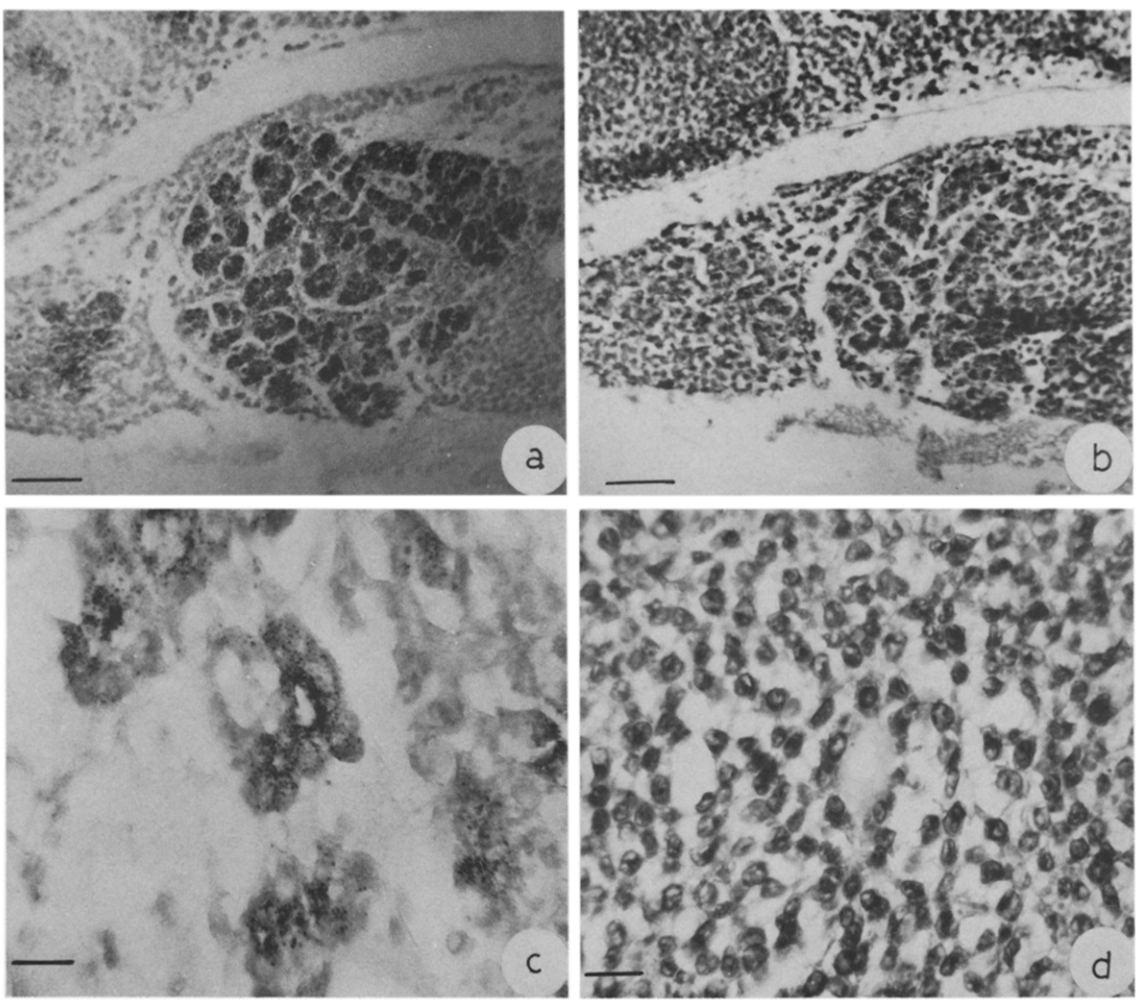

Fig. 2. Presence of pancreatic cells within tumor tissue. (a) Histological section of a solid tumor examined 9 days *post-inoculation. GGT staining with hematoxylin as counterstain. Acini of GGT-positive cells surrounded by GGT-negative tumor cells. (b) Adjacent section stained with hematoxylin–eosin. (c) Section of a solid tumor examined* 13 days *post-inoculation. Small acini of GGT-positive pancreas cells surrounded by GGT-negative tumor tissue. GGT staining. (d) Adjacent section, hematoxylin–eosin staining. Notice the acinus at the centre of the field. Original magnifications: a, b,* ×63; *c, d,* ×252.
Bars: a, b, 50 μm; *c, d,* 10 μm.

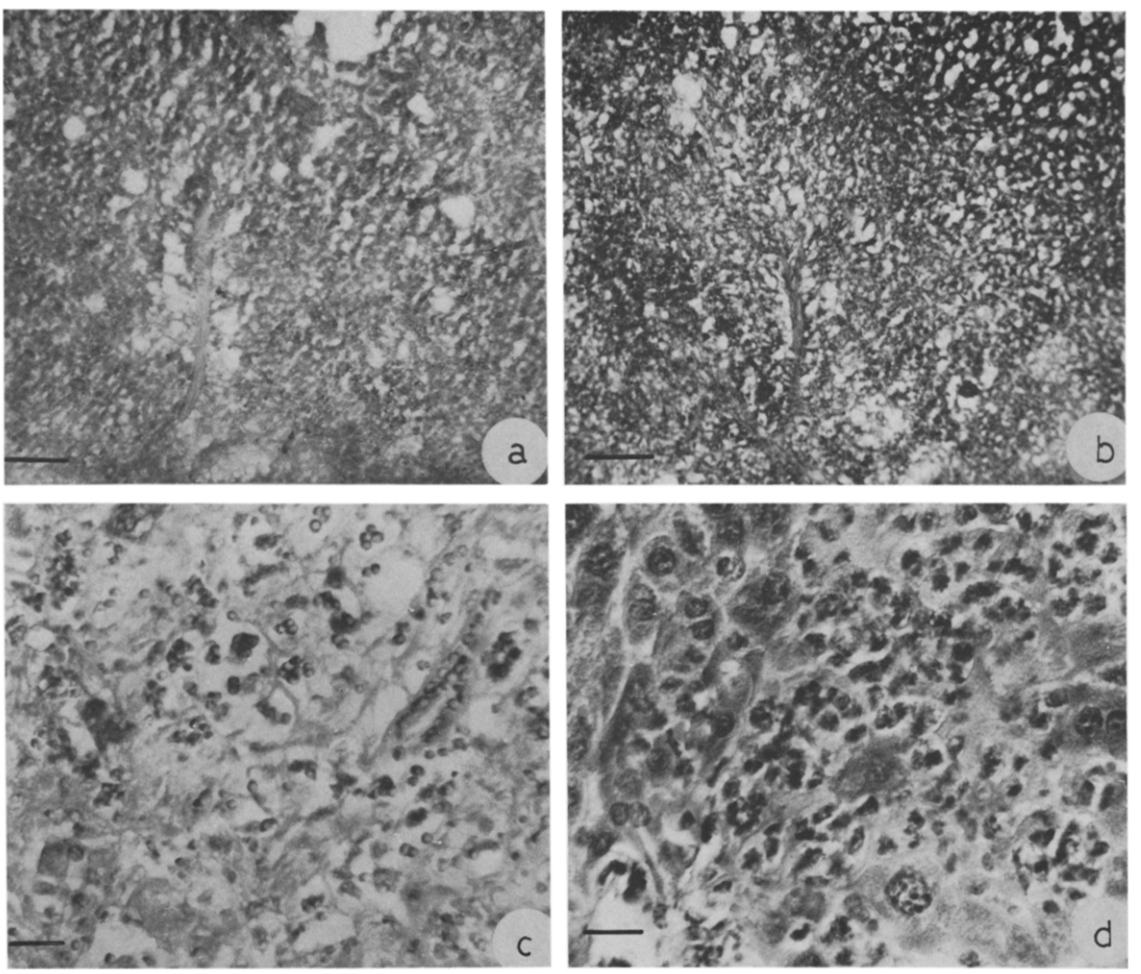

Fig. 3. Infiltration of T-25 cells into the spleen and the liver. (a) Histological section of spleen massively invaded by S49 cells. (b) Adjacent section, hematoxylin–eosin staining. Notice that the original anatomy is hardly preserved. (c) Section of liver infiltrated by S49 cells. GGT staining. The nuclei of the infiltrating cells are strongly stained with hematoxylin (counterstain) but are GGT-negative. The cytoplasm of these cells is GGT-negative (compared with 2a, 2c). (d) Adjacent section, hematoxylin–eosin staining. S49 cells surrounded by hepatocytes. Original magnifications: a, b, ×63; c, d, ×252.
Bars: a, b, 50 μm; c, d, 10 μm.

comparable to that of the T–25 tumor cells grown in suspension cultures or ascitic tumors grow *in vitro*.

DISCUSSION

Low GGT level seems to be a typical property of tumors originating in the immune system: the five murine lymphoma lines studied here, eight additional murine lymphoma lines [27] and several human cases including acute myelogenous and lymphatic leukemias, gastrointestinal lymphoma and heavy chain disease were all low in GGT as judged by direct examination of the enzyme in tumor cells [27] or indirect examination of plasma of patients suffering from various lymphomas [51]. By contrast, GGT was elevated in many tumors originating in epithelial cells or in epitheloid cells involved in transport or secretion processes: hepatocytes and biliary ductular cells in liver cancer [34, 52, 53], and epithelial cells in the mammary gland [19], colon [17] and thyroid [21]. Also, elevated GGT was found in plasma of humans suffering from melanoma, breast and lung carcinoma and hypernephroma [51].

It is possible that increased GGT in tumors reflects the type, physiological function(s) or developmental stage of differentiation of the cells from which those tumors arose, rather than play a crucial role in the tumorigenic process. This hypothesis is supported by (i) the lack of correlation between GGT activity and tumor cell proliferation rate, degree of malignancy or invasiveness; (ii) the phenotypic diversity of GGT activity among individual cells within a tumor [9, 22, 23, 33, 54]; and (iii) the fact that many tumors are not GGT-rich [21, 24–29]. However, many studies dealing with GGT in various cancers suffer from the fact that GGT levels were analyzed in advanced stages of tumor development after most (or all) relevant events had already occurred (e.g. this work and [27]). Therefore it is impossible to establish, on the basis of such studies, whether GGT is involved functionally in the early development stages of tumors originating in the immune system. If GGT is involved in the early stages of such tumors, then GGT-rich putative early precancerous cells are apparently selected against during carcinogenesis.

It has been proposed recently that low GGT activity can be linked to cell proliferation whereas high activity can be associated with terminal differentiation and low proliferative potential [54, 55]. This could be true also in the case of lymphoid tissues, where GGT increases in normal mitogen-exposed lymphoblasts from humans and rats but proliferating lymphoid tumor cells are GGT-poor. GGT increase was suggested to indicate the synthesis and secretion of immunoglobulins and lymphokines, as manifested by a GGT-rich human myeloma line which was also active in the synthesis and secretion of the light chain [27]. This argues for the increased expression of GGT as a marker for cell differentiation rather than for the functional association of the enzyme with tumor development.

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