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Differences in liver ganglioside patterns in various inbred strains of mice

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The ganglioside patterns in the liver of different inbred and hybrid strains of mice were investigated. The inbred strains were Balb/cAnNCr1BR, C57BL/6NCr1BR, DBA/2NCr1BR, C3H/HeNCr1BR; the hybrid strain was the Swiss albino. The following major gangliosides were found to be present in mouse liver: $G_{M3\text{-}NeuAc}$; $G_{M3\text{-}NeuGl}$, G_{M2} [a mixture of one species carrying *N*-acetylneuraminic acid (NeuAc) and one carrying *N*-glycollylneuraminic acid (NeuGl)], G_{M1} and $G_{D1a\text{-}(NeuAc,NeuGl)}$. The qualitative and quantitative patterns of liver gangliosides were markedly different in the various inbred strains of mice: in Balb/cAnNCrlBR strain, ganglioside G_{M2} was preponderant (99.2% of total ganglioside content); in C57BL/6NCr1BR, the major ganglioside was G_{M2} (90.4%), followed by $G_{M3\text{-}NeuAc}$ (5.6%) and $G_{M3\text{-}NeuGl}$ (4.0%); in DBA/2NCr1BR, G_{M2} accounted for 77.1%, $G_{D1a\text{-}(NeuAc,NeuGl)}$ 18.9% and G_{M1} 3.1% of gangliosides; in C3H/HeNCr1BR, G_{M2} constituted 50.6%, G_{M1} 22.8% and $G_{D1a\text{-}(NeuAc,NeuGl)}$ 22.1%. In the hybrid Swiss albino mice, liver ganglioside composition markedly varied from one animal to another, $G_{M3\text{-}NeuGl}$, G_{M2} and $G_{D1a\text{-}(NeuAc,NeuGl)}$ being the predominant gangliosides in the various cases.

Glycosphingolipids are normal components of the plasma membranes of mammalian cells. A number of reports have illustrated the species, sex and organ specificity of the different glycosphingolipids (Coles *et al.*, 1970; Kriterovich *et al.*, 1970; Wiegandt, 1973; Seyfried *et al.*, 1978; Taki *et al.*, 1978). It has been also demonstrated that the glycosphingolipid composition of the same organ or tissue may vary in different inbred strains of the same animal species (Adams & Gray, 1968). With special reference to gangliosides it is known that in red cells the most abundant ganglioside, G_{M3}, contains *N*-acetylneuraminic acid in some dog and cat breeds and *N*-glycollylneuraminic acid in others (Yasue *et al.*, 1978; Hamanaka *et al.*, 1979). Significant differences in the brain ganglioside patterns of various strains of mice have been recently noted (Dreyfus *et al.*, 1982; Seyfried *et al.*, 1979). Finally the chemical differences between genetically obese (*ob/ob*) mice and normal strain (C57 BL/6J) include changes in the ganglioside content and composition in both liver and brain (Sena *et al.*, 1982).

We have initiated a systematic study on the genetic correlates of ganglioside composition in mammalian tissues. The present paper defines the liver ganglioside patterns in different inbred strains of mice.

Abbreviations used: $G_{M3\text{-}NeuAc}$, II^{3}NeuAc-LacCer; $G_{M3\text{-}NeuGl}$, II^{3}NeuGl-LacCer; G_{M2}, II^{3}NeuAc-GgOse$_3$-Cer; G_{M1}, II^{3}NeuAc-GgOse$_4$Cer; $GD_{1a\text{-}(NeuAc,NeuGl)}$, II^{3}NeuAc,IV^{3}NeuGl-GgOse$_4$Cer; NeuAc, *N*-acetylneuraminic acid; NeuGl, *N*-glycollylneuraminic acid; GalNAc, *N*-acetylgalactosamine. Abbreviations for gangliosides are those recommended by Svennerholm (1963) and IUPAC–IUB [*Lipids* (1977) **12**, 455–468; *J. Biol. Chem.* (1982) **257**, 3347–3351].

Experimental

Chemicals

N-Acetylneuraminic acid and *N*-glycollylneuraminic acid were purchased from Sigma (St. Louis, MO, U.S.A.); *Vibrio cholerae* sialidase (EC 3.2.1.8) was from Behringwerke (Marburg, Germany).

Animals

Four inbred strains of mice, Balb/cAnNCr1BR; C57BL/6NCr1BR, C3H/HeNCr1BR and DBA/2NCr1BR and hybrid Swiss albino mice [Crl:CD^R-1(CR)BR], provided by Charles River (Milan, Italy), were employed. All mice of the same strain

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originated from at least ten different litters, from which they were randomly drawn directly by the dealer. This was valid for all strains, the Swiss albino hybrid included. As guaranteed by the dealer, all animals were screened for commoner mouse viruses. Male mice, aged 45–50 days, were killed by decapitation and the livers were removed, washed in ice-cold 0.9% NaCl solution, dried and immersed immediately in liquid N_2. Organs were kept at −30°C and thawed immediately before analysis.

Isolation of gangliosides

Gangliosides were extracted and purified from each individual liver by the procedure of Tettamanti *et al.* (1973) already applied to extraneuronal tissues (Brunngraber *et al.*, 1976). The ganglioside content in the final extract was determined by the resorcinol/HCl method (Svennerholm, 1957; Miettinen & Takki-Luukkainen, 1959).

T.l.c. separation and quantification of gangliosides

Separation of gangliosides into individual species was attained by t.l.c. under the following experimental conditions: HPTLC plates (from Merck, Darmstadt, Germany) were used; solvent was chloroform/methanol/aq. 0.2% $CaCl_2$ (5:4:1, by vol.); temperature was 18–20°C; detection of the spots was by treatment with an Ehrlich spray reagent and heating at 120°C for 15 min. Densitometric quantification was done with a Camag scanner.

Isolation and partial characterization of individual gangliosides

Each of the five major gangliosides occurring in mice liver (termed A, B, C, D and E) was isolated from the strain (25–100 animals) in which it was abundant (Fig. 1). Thus ganglioside A from C57BL/6NCr1BR strain, B from Swiss albino hybrid, C from Balb/cAnNCr1BR, D from C3H/HeNCr1BR and E from DBA/2BCr1BR were isolated. Chemical analyses on isolated gangliosides A, B, C D and E included determination of carbohydrate content and composition, determination of sphingosine content and composition, cleavage, isolation and recognition of acylneuraminic acid, partial acid hydrolysis followed by isolation and characterization of the neutral glycosphingolipids formed and exhaustive treatment with *Vibrio cholerae* sialidase. The details for the procedures used for isolation and characterization of gangliosides have been described previously (Ghidoni *et al.*, 1980).

Results and discussion

Available information on the ganglioside composition of mouse liver is poor (Shaposhnikova *et al.*, 1981; Sena *et al.*, 1982). Fig. 1 shows that five

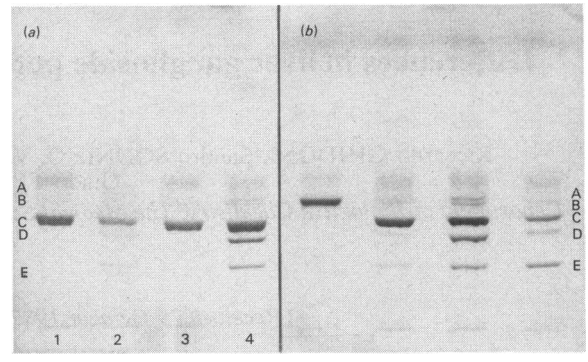

Fig. 1. *T.l.c. of mouse liver gangliosides*
(*a*) Different inbred strains: 1, C57BL/6NCr1BR; 2, DBA/2NCr1BR; 3, Balb/cAnNCr1BR; 4, C3H/HeNCr1BR. (*b*) Different individual animals of the hybrid Swiss albino.

different gangliosides, A, B, C, D and E are present in the liver of the four inbred mouse strains used and in the hybrid Swiss albino mouse. Control experiments showed that each of these gangliosides had identical t.l.c. behaviour in all strains. As shown in Table 1, ganglioside A contained sphingosine, glucose, galactose and NeuAc in the theoretical molar proportions of 1:1:1:1, ganglioside B contained sphingosine, glucose, galactose and NeuGl in the proportions of 1:1:1:1, ganglioside C contained sphingosine, glucose, galactose, GalNAc and sialic acid in the proportions of 1:1:1:1:1, the sialic acid being a mixture of NeuAc (40%) and NeuGl (60%), ganglioside D contained sphingosine, glucose, galactose, GalNAc and NeuAc in the proportions of 1:1:2:1:1 and ganglioside E contained sphingosine, glucose, galactose, GalNAc, NeuAc and NeuGl in the proportions of 1:1:2:1:1:1. The presence of both NeuAc and NeuGl in the liver of a hybrid mouse strain (CBA × C57BL/6F) was already reported by Shaposhnikova *et al.* (1981). The proportion between NeuAc and NeuGl in ganglioside C was found to vary in the different mouse strains, indicating the presence of two ganglioside species, one carrying NeuAc and the other NeuGl, which are unresolved under our t.l.c. experimental conditions.

Gangliosides C and D were resistant to the action of *Vibrio cholerae* sialidase; gangliosides A, B and E were affected by sialidase, which released NeuAc from A and NeuGl from both B and E. Ganglioside E, prepared from the liver of each mouse strain, always liberated, upon sialidase treatment, NeuGl, indicating that this ganglioside is a single species, carrying a sialidase-labile NeuGl residue, and identical in all strains. The following neutral glyco-

Table 1. *Chemical composition of the different gangliosides isolated from mouse liver*

Ganglioside	Sphingosine and carbohydrate composition (molar proportion)* Sphingosine	Glucose	Galactose	GalNAc	Sialic acid	Type of sialic acid
A	1	1.05	1.08	—	1.10	NeuAc
B	1	1.06	1.02	—	1.05	NeuGl
C	1	0.97	1.03	0.94	1.08	NeuAc/NeuGl (2:3, by mol)
D	1	1.02	1.93	0.97	1.05	NeuAc
E	1	1.01	2.06	1.03	2.10	NeuAc/NeuGl (1:1, by mol)

* Sphingosine = 1.

Table 2. *Ganglioside content and pattern in the liver of four different inbred strains of mice*

All data are means ± S.D. of determinations accomplished on 11 adult animals of each strain. Animal age was 50 days (45 days in the case of C3H/HeNCr1BR strain). Total ganglioside content is expressed as μg of lipid-bound sialic acid/g of fresh tissue. Individual gangliosides are expressed as percentages of total lipid-bound sialic acid.

Parameter Mouse strain ...	Balb/CAnNCr1BR	C57BL/6NCr1BR	DBA/2NCr1BR	C3H/HeNCr1BR
Animal weight (g)	21.5 ± 1.3	22.0 ± 1.3	22.1 ± 1.4	24.0 ± 1.6
Liver weight (g)	1.07 ± 0.11	1.16 ± 0.11	1.02 ± 0.09	1.37 ± 0.09
Total ganglioside content	113.4 ± 10.0	110.5 ± 13.1	159.4 ± 23.3	125.3 ± 10.8
Ganglioside pattern				
(A) $G_{M3\text{-}NeuAc}$	0.44 ± 0.14	5.6 ± 0.7	0.52 ± 0.17	2.4 ± 0.4
(B) $G_{M3\text{-}NeuGl}$	0.36 ± 0.10	4.0 ± 0.4	0.38 ± 0.09	2.1 ± 0.4
(C) G_{M2}	99.2 ± 0.2	90.4 ± 0.8	77.1 ± 1.1	50.6 ± 2.5
(D) G_{M1}	Traces	Traces	3.1 ± 0.7	22.8 ± 2.1
(E) $G_{D1a\text{-}(NeuAc,NeuGl)}$	Traces	Traces	18.9 ± 1.1	22.1 ± 2.0

sphingolipids were obtained from the corresponding gangliosides by gradual acid hydrolysis: two compounds, one containing glucose, the other glucose and galactose (molar ratio 1.00:1.08), from gangliosides A and B; three compounds containing respectively (*a*) glucose, (*b*) glucose and galactose (molar ratio 1.00:1.05), (*c*) glucose, galactose and GalNAc (molar proportions 1.00:0.96:0.92) from ganglioside C; four compounds containing respectively (*a*) glucose, (*b*) glucose and galactose (molar ratio 1.00:1.09), (*c*) glucose, galactose and GalNAc (molar proportions 1.00:1.04:0.94) and (*d*) glucose, galactose and GalNAc (molar proportions 1.00:2.05:0.96) from gangliosides D and E. Thus the neutral glycosphingolipid core of the analysed gangliosides is Gal-Glc-ceramide for A and B, GalNAc-Gal-Glc-ceramide for C and Gal-GalNAc-Gal-Glc-ceramide for D and E. On the basis of these data it is possible to provisionally identify gangliosides A, B, C, D and E respectively as $G_{M3\text{-}NeuAc}$, $G_{M3\text{-}NeuGl}$, G_{M2} (a mixture of one species carrying NeuAc and one carrying NeuGl), G_{M1} and $G_{D1a\text{-}(NeuAc,NeuGl)}$. These conclusions concur with those obtained by Shaposhnikova *et al.* (1981) on a CBA × C57BL/6F hybrid strain.

The total liver content of gangliosides ranged from a minimum of 110.5 μg (as bound sialic acid)/g of fresh tissue in the C57BL/6NCr1BR strain to a maximum of 159.4 μg in the DBA/2NCr1BR strain (Table 2). These values are comparable with those reported by other authors in mouse (Shaposhnikova *et al.*, 1981; Sena *et al.*, 1982) and markedly higher than those reported in human (Kriterovich *et al.*, 1970; Seyfried *et al.*, 1978; Nilsson & Svennerholm, 1982) and rat (Taki *et al.*, 1978; Ueno *et al.*, 1982) liver. The pattern of liver gangliosides differed greatly in the various inbred strains of mice (Table 2). G_{M2} covered up to 99.2% of total ganglioside content in Balb/cAnNCr1BR strain. In C57BL/6NCr1BR strain the major ganglioside was G_{M2} (90.4%), followed by $G_{M3\text{-}NeuAc}$ (5.6%) and $G_{M3\text{-}NeuGl}$ (4.0%); this pattern is substantially close to that reported by Sena *et al.* (1982) for a C57BL inbred strain and by Shaposhnikova *et al.* (1981) for the hybrid CBA × C57BL/6F strain. In the DBA/2NCr1BR strain, G_{M2} accounted for 77.1%, followed by $G_{D1a\text{-}(NeuAc,NeuGl)}$ (18.9%) and G_{M1} (3.1%); in the C3H/HeNCr1BR strain G_{M2} constituted only 50.6%, G_{M1} 22.8% and $G_{D1a\text{-}(NeuAc,NeuGl)}$ 22.1%. As shown in Table 3, the liver ganglioside composition of hybrid Swiss albino mice markedly varied from one animal to another. The predominant ganglioside was $G_{M3\text{-}NeuGl}$ in some animals, and G_{M2} or $G_{D1a\text{-}(NeuAc,NeuGl)}$ in others. The preponderance of

Table 3. *Liver ganglioside patterns of 18 different mice of the Swiss albino line randomly collected*
All data are means of triplicate determinations. Animals age was 45 days. Mean (±S.D.) body weight was 23 ± 1.5 g. Mean (±S.D.) liver weight was 0.97 ± 0.12 g. Mean (±S.D.) ganglioside content, as μg of bound sialic acid/g of fresh tissue, was 153.4 ± 24.1.

	Ganglioside pattern (% of total lipid-bound sialic acid)				
Animal	$G_{M3\text{-}NeuAc}$ (A)	$G_{M3\text{-}NeuGl}$ (B)	G_{M2} (C)	G_{M1} (D)	$G_{D1a\text{-}(NeuAc,NeuGl)}$ (E)
1	4.1	89.7	0.7	1.2	4.3
2	3.7	88.6	0.6	2.6	4.5
3	1.3	86.2	0.8	3.8	7.9
4	2.2	1.0	75.3	8.4	13.1
5	4.0	5.1	61.8	20.3	8.8
6	2.1	3.0	61.5	17.0	16.4
7	5.6	7.9	60.8	11.6	14.1
8	2.9	5.2	60.5	19.1	12.3
9	2.8	6.2	59.0	15.0	17.0
10	2.9	11.0	55.0	13.1	18.0
11	3.3	4.0	54.8	19.3	18.6
12	3.7	9.0	51.7	15.4	20.2
13	3.2	1.9	46.1	18.6	30.2
14	6.0	3.5	40.1	15.3	35.1
15	2.1	1.3	37.6	19.0	40.0
16	3.9	3.0	34.6	18.3	40.2
17	1.5	4.7	23.0	29.8	41.0
18	4.0	3.8	16.0	32.0	44.2

$G_{M3\text{-}NeuGl}$ in the liver of some Swiss albino mice (a feature that is typical of human and rat liver) is consistent with the hypothesis that an original strain, from which the Swiss albino line was obtained, should have a liver ganglioside pattern in which $G_{M3\text{-}NeuGl}$ is the predominant species.

Recently Ueno *et al.* (1982) showed that the ganglioside distribution pattern of rat liver parenchymal cell differed from that of non-parenchymal cells. The different liver ganglioside pattern observed in the various mouse strains may reflect substantial differences in proportions between parenchymal and non-parenchymal cells, or, more likely, a different capability to biosynthesize gangliosides by the same type of cells in the various strains.

In conclusion, this work emphasizes the marked changes of liver ganglioside composition in different mouse strains and provides indications for studies aimed at establishing the genetic correlates between cell-surface recognition markers and cell social behaviour at the liver level.

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