GLYCOSPHINGOLIPIDS OF WILD-TYPE AND MUTANT LECTIN-RESISTANT CHINESE HAMSTER OVARIAN CELLS

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SUMMARY

The GSLs of wild-type CHO cells were examined. The principal GSLs detected were GM_3, GM_2 and monoglucosylceramide species. Mutant CHO cells, resistant to the cytotoxic effects of the lectins Con A and PHA-P, revealed no alterations of GSL profile as compared with the wild-type cells. The combination of these results suggests that the major GSLs of CHO cells are unlikely to be primary sites of interaction of CHO cells with these two lectins.

INTRODUCTION

GSLs are complex membrane-bound molecules present in most if not all eukaryotic cells (1). A central problem in the area of GSL biochemistry is the elucidation of the biological functions of these complex lipids. A variety of studies have implicated GSLs as cell receptors for various agents (2,3,4), in the abnormal biologic behaviour exhibited by tumor cells (cf. 5, 6) and as blood group substances in the red blood cells of certain species (7). However, in general, our knowledge of the functions of the various GSLs present in mammalian cells is still obscure.

Increasing use of tissue culture systems has been made in recent years to study aspects of the biochemistry of GSLs (8,9). Procedures have been developed for the isolation of mutant cultured cells that might permit a genetic approach to the investigation of the functions of membrane components (10); hopefully, this approach may prove applicable to GSLs. CHO cells are suitable for such studies because of their relative karyotypic stability (cf. 11). Knowledge of the GSL composition of CHO cells is a prerequisite

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Abbreviations: GSL, glycosphingolipid; CHO, Chinese hamster ovarian; Con A, concanavalin A; PHA-P, phytohemagglutinin-P; BHK, baby hamster kidney; WGA, wheat germ agglutinin; LBSA, lipid-bound sialic acid; PBSA, protein-bound sialic acid; GLC, gas-liquid chromatography; R, resistant to.

for studies of the above nature; as yet no studies of the GSLs of these cells have been reported. CHO cell lines resistant to the cytotoxic effects of the lectins Con A and PHA-P have been isolated (10,12). It has been suggested that GSLs on the surface of polyoma-transformed BHK cells may be involved in the agglutination of these cells by the lectin WGA (13). It therefore seemed possible that lectin-resistant mutant cells might exhibit changes of GSL profile relative to control cells. For these reasons we examined the GSLs of wild-type CHO cells and of the lectin-resistant mutants referred to above.

MATERIALS AND METHODS

The isolation, culture and properties of the wild-type and mutant CHO cells have been previously detailed (12).

Extraction and analyses of GSLs were also performed as previously described (14). The method of Miettinen and Takki-Luukkainen (15) was used to measure LBSA and the method of Aminoff (16) to measure PBSA on aliquots of non-lipid residues. The carbohydrate composition of the principal GSLs of CHO cells was determined by GLC of the trimethylsilyl ethers of their O-methyl glycosides (17,18). The nomenclature for the gangliosides is that of Svennerholm (19).

RESULTS

The results of quantitative determinations of the LBSA and PBSA contents of suspension and monolayer cultures of the wild-type and mutant CHO cells used in the present study are presented in Table I. These analyses revealed that the LBSA contents of the independently isolated mutant clones and wild-type CHO cells differed appreciably, both in suspension and monolayer cultures. For example, whereas the LBSA contents of the mutant clones Con A $\frac{R}{}$ 4, Con A $\frac{R}{}$ 1, Con A $\frac{R}{}$ 2 and PHA $\frac{R}{}$ 1 in suspension resembled each other (range of 3.20-3.90 nmoles LBSA per mg protein, and some 12-40% more than that of the wild-type cells), that of PHA $\frac{R}{}$ 2 was appreciably lower and that of Con A $\frac{R}{}$ 3 appreciably higher than that of the wild-type value. The various isolates in suspension culture contained 3-11 fold more sialic

TABLE I. Sialic Acid Distribution in Wild-Type and Mutant Chinese Hamster Ovary Cells in Suspension and Monolayer Culture.

Cells Analyzed	nmoles LBSA per mg Protein[a]		nmoles PBSA per mg Protein[a]	
	Suspension	Monolayer	Suspension	Monolayer
Wild-Type	2.85	5.95	18.20	35.90
Con A $\frac{R}{}$ 3	4.65	6.95	23.05	35.75
Con A $\frac{R}{}$ 4	3.65	5.00	12.60	27.20
Con A $\frac{R}{}$ 1	3.90	8.25	27.40	30.25
Con A $\frac{R}{}$ 2	3.20	5.05	19.25	31.00
PHA $\frac{R}{}$ 2	1.90	1.80	21.10	26.20
PHA $\frac{R}{}$ 1	3.90	10.40	30.25	24.65

[a] Results are expressed as the means of duplicate analyses on each purified ganglioside fraction and non-lipid residue from two batches of cells.

$\frac{R}{}$, signifies resistant to.

acid bound to protein than to lipid. In general, the LBSA and PBSA contents of the various cells increased from 1.5 to 2 fold when the cells were grown in monolayer as compared with suspension culture, although PHA $\frac{R}{}$ 2 and PHA $\frac{R}{}$ 1 cells provided exceptions to this statement. Qualitative data on the ganglioside patterns of the wild-type and mutant CHO cells in suspension and monolayer cultures are shown in Figs. 1 and 2 respectively. A relatively simple pattern of gangliosides was seen in the wild-type CHO cells (channel 1 of both figures), consisting of prominent resorcinol-positive bands corresponding in chromatographic migration to standards of GM_3 and GM_2; a band corresponding in migration to GD_{1a} was also visible. The ganglioside profiles of the wild-type and mutant cells grown in suspension and monolayer cultures were generally similar (comparison of Fig. 1 with Fig. 2). Quantitative studies on the distribution of sialic acid in the ganglioside zones of the wild-type cells revealed that approximately 90% of the sialic acid recovered from the chromatograms was present in the GM_3 zone, some 8% in the GM_2 zone and the remainder in the GD_{1a} zone.

Qualitative analysis by thin layer chromatography of the neutral GSL

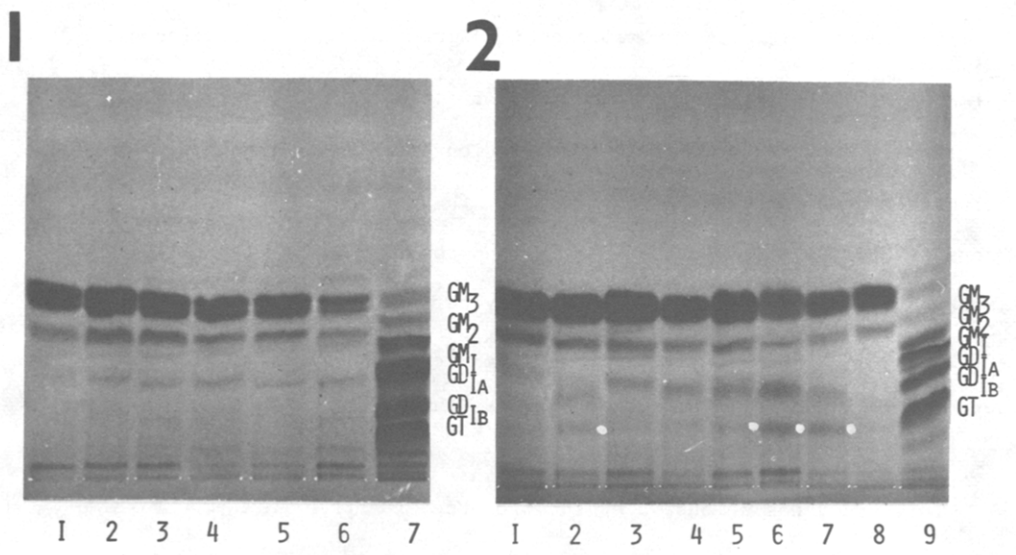

Fig. 1: Thin layer chromatogram of the gangliosides from wild-type and lectin-resistant mutants of CHO cells grown in suspension culture. Gangliosides of: 1, Wild-type cells; 2, Con A ^{R} 3 cells; 3, Con A ^{R} 4 cells; 4, Con A ^{R} 1 cells; 5, Con A ^{R} 2 cells; 6, PHA ^{R} 2 cells; 7, Human brain.

Fig. 2: Thin layer chromatogram of the gangliosides from wild-type and lectin-resistant mutants of CHO cells grown in monolayer culture. Samples 1 - 6 are as described in the legend to Fig. 1: 7, PHA ^{R} 1 cells; 8, PHA ^{R} 1 cells (in suspension); 9, Human brain.

In both chromatograms approximately 70 nmoles of lipid-bound sialic acid were spotted in each channel. The solvent system used for development was chloroform-methanol-ammonia-water (60:35:1:7, by volume) and the plates were sprayed with resorcinol reagent. The band indicated by the white dots in Fig. 2 was yellow and is a contaminant derived from dialysis tubing.

fractions of the wild-type and mutant CHO cells in suspension and monolayer culture was also performed. The principal neutral GSL exhibited by the wild-type cells corresponded in chromatographic migration to a standard of monoglycosylceramide; a lesser zone corresponding in migration to diglycosylceramide was also detected. The patterns of neutral GSLs of the wild-type CHO cells and of the different mutant clones, as evaluated by visual comparison, were identical, both in suspension and monolayer cultures.

The carbohydrate composition of the principal gangliosides of the wild-type CHO cells was determined by analysis by GLC of the trimethylsilyl ethers of the O-methyl glycosides. The sole carbohydrates detected in the gang-

lioside corresponding in chromatographic migration to GM_3 were glucose, galactose and sialic acid, in the ratio of 1.00:1.22:0.82 respectively. The ganglioside with a chromatographic migration of GM_2 contained glucose, galactose, galactosamine and sialic acid, in the ratio of 1.00:1.15:0.87:0.92 respectively. The sole carbohydrate found on analysis of the neutral GSL corresponding in migration to monoglycosylceramide was glucose, whereas analysis of the zone corresponding in migration to dihexosylceramide yielded approximately equimolar amounts of glucose and galactose.

DISCUSSION

This study has demonstrated the presence of appreciable amounts of gangliosides in CHO cells. The principal gangliosides corresponded in chromatographic migration to standards of GM_3 and GM_2; the analyses by GLC were compatible with them in fact being GM_3 and GM_2 species. A smaller amount of a ganglioside corresponding in migration to GD_{1a} was also detected, but partial characterization of this compound was not attempted. The neutral GSL content of the CHO cells was also found to be relatively simple, consisting principally of monoglucosylceramide and some lactosylceramide. The CHO cells did not possess significant amounts of the more complex neutral GSLs (such as tri-, tetra- and penta- glycosylceramides) sometimes seen in other cell lines (8). An increase of both LBSA and PBSA was found in monolayer cultures as compared with suspension cultures; this finding is compatible with the observation of Shen and Ginsburg (20) that the heteroglycan content of membrane fractions is higher when cells are grown in monolayer. Although there were appreciable differences in the total LBSA contents of the wild-type and mutant CHO cells (a finding consistent with previous observations on various 3T3 cell derivatives (14)), the ganglioside and neutral GSL profiles of the mutant cells did not differ significantly from these of the wild-type cells. In fact, the relative constancy of the ganglioside patterns of the different clonal isolates is in marked contrast to the wide variation in ganglioside patterns noted among clonal isolates of transformed cells (4,14,21,22).

The fact that the GSL patterns of the mutant cells did not differ from that of the wild-type cells suggests that the differences in sensitivity exhibited by the Con A-resistant mutant cell lines to Con A and other agents (pleiotropic effects) (12) are probably not due to marked alterations in their GSL composition. Con A is known to interact with compounds containing terminal α-D-mannopyranosyl or α-D-glucopyranosyl residues (23); none of the major GSLs detected in CHO cells in the present study would be anticipated to have these terminal α linkages (1). PHA-P reacts with a terminal N-acetyl-galactosaminyl residue (24); whether this lectin would react with this residue present in the GM_2 species of CHO cells has not been tested directly. On balance, it appears reasonable to conclude from the direct studies on chemical composition reported here and from the indirect evidence obtained from the mutant cells, that the GSLs of CHO cells are unlikely to be primary sites of interaction of CHO cells with the two lectins.

It is hoped that the baseline information obtained on the GSL composition of CHO cells may prove useful in designing selection procedures to permit a genetic approach to the study of the functions of the GSLs of mammalian cells.

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