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GANGLIOSIDE ACCUMULATION IN CULTURED SKIN FIBROBLASTS FROM GANGLIOSIDOSIS PATIENTS

Raju K. Pullarkat[1], Henry Reha[1], and Nicholas G. Beratis[2]

[1]Department of Neurochemistry and
[2]Department of Human Genetics, New York State Institute for Basic Research in Mental Retardation, Staten Island, N.Y. 10314

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SUMMARY

At late confluency (21 days after passage), cultured skin fibroblasts from G_{M1} gangliosidosis, type 1 patients showed approximately a 15-fold increase in G_{M1} ganglioside, and fibroblasts from Tay-Sachs and Sandhoff disease patients showed a 50- and 30-fold increase in G_{M2} ganglioside, respectively, when compared to normal fibroblasts. Since demonstration of storage material is important for accurate diagnosis of the lysosomal storage disorders, analysis of the accumulating lipids in late confluency fibroblasts can provide an additional tool for the diagnosis of the gangliosidoses and possibly other lysosomal disorders.

INTRODUCTION

Cultured skin fibroblasts have been widely used for the diagnosis and study of genetic disorders. Several inborn errors of metabolism are identifiable in these cells by either direct enzyme determinations or by analyzing the secondary metabolic consequences of the primary metabolic defect (1,2). Although the specific enzyme deficiency is easily detectable in cultured fibroblasts from patients with various gangliosidoses, no significant accumulation of sphingolipids has been previously found in these cells except for a 4-fold accumulation of G_{M2} in Sandhoff disease (3,4). In this report we demonstrate that, at late confluency, cultured skin fibroblasts derived from patients with three different gangliosidoses, Tay-Sachs disease, Sandhoff disease and G_{M1} gangliosidosis, type 1, accumulated significant amounts of specific gangliosides.

Ganglioside abbreviations used: G_{M3}, II^3NeuAc-LacCer; G_{M2}, II^3NeuAc-$GgOse_3$Cer; G_{M1}, II^3NeuAc-$GgOse_4$Cer; G_{D3}, $II^3(NeuAc)_2$-LacCer; G_{D1a}, IV^3NeuAc, II^3NeuAc-$GgOse_4$Cer.

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MATERIALS AND METHODS

Fibroblasts derived from three patients with Tay-Sachs disease, from two patients with Sandhoff disease, from two patients with G_{M1} gangliosidosis, type 1, and from six normal controls were studied. Fibroblast strains GM-221 and GM-502 (Tay-Sachs disease), GM-203 and GM-317 (Sandhoff disease) and GM-806 (G_{M1} Gangliosidosis, type 1) were obtained from the Institute for Medical Research, Camden, N.J. One Tay-Sachs fibroblast culture and one G_{M1} gangliosisosis, type 1 culture were derived from patients diagnosed by us. The normal strains were derived from subjects that were tested for heterogocity of various genetic disorders and found to be normal. All biopsies from children were obtained with the informed consent of their parents.

Skin fibroblasts were cultivated in RPMI 1640 medium supplemented with 16% fetal calf serum, 1% 200 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Grand Island Biological Co.). Cultures were replenished with fresh medium twice a week. Cells were passed in a split ratio of 1:4. Cultures were observed under an inverted microscope and harvested when the fibroblasts reached early confluency 4 to 6 days later. Late confluency fibroblasts were harvested 21 days after passage. Fibroblasts used in the study were between the fourth and eighth passage.

After harvesting, cells were washed and lysed by sonication. Protein was measured (5) and lipids were obtained by stepwise extraction with 1:1 chloroform-methanol followed by 2:1 chloroform-methanol. Gangliosides were isolated by the procedure of Fishman et al. (6) except that extensive dialysis was carried out after evaporating the methanol, instead of Sephadex column chromatography. In our hands the Sephadex chromatography step gave incomplete recovery and non-reproductive results. Gangliosides were separated by high-performance thin-layer chromatography (Silica gel-60, E. Merk), using a solvent system of chloroform:methanol:0.25% calcium chloride (60:40:8, v/v/v), and quantitated by densitometry (7).

RESULTS

The total gangliosides in the normal cells remained the same at early (4.2 ± 0.68 nmoles/mg fibroblast protein) and late (4.3 ± 0.74) confluency. Also, except for a reduction in the level of G_{M2}, which was present in all cases studied, the ganglioside pattern in the late confluency normal fibroblasts did not show any significant differences from that of the early confluency fibroblasts. The level of G_{D3}, however, was found to be increased at late confluency of some fibroblast strains.

Figure 1 illustrates the ganglioside pattern from early and late confluency fibroblasts derived from a normal subject and from a patient with Sandhoff disease. The amount of G_{M2} in the early and late confluency normal fibroblasts was 0.4 ± 0.10 and 0.10 ± 0.04 nmoles/mg protein, respectively. However, the amount of G_{M2} stored in the fibroblasts from two patients with

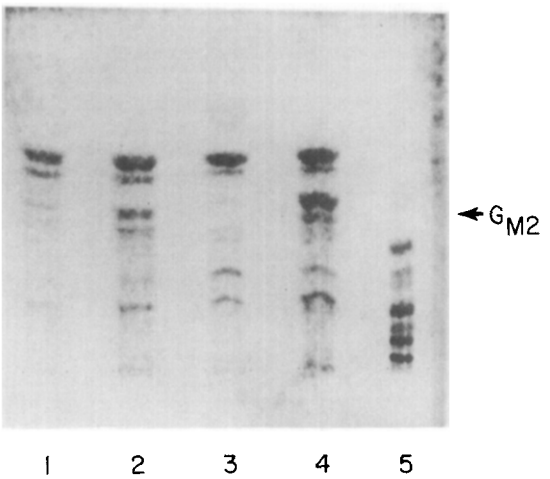

Fig. 1. High performance thin-layer chromatography of fibroblast gangliosides on silica gel-60 using a solvent system of chloroform:methanol:0.25% $CaCl_2$ (60:40:9, v/v/v). Visualization: resorcinol. Each sample contained gangliosides derived from 1 mg fibroblast protein. Channel 1, normal fibroblasts at early confluency; channel 2, Sandhoff fibroblasts at early confluency; channel 3, normal fibroblasts at late confluency; channel 4, Sandhoff fibroblasts at late confluency; channel 5, human brain gangliosides.

Sandhoff disease was 2.07 and 1.20 at early confluency, and 3.47 and 2.60 at late confluency. The level of the other gangliosides did not differ significantly between early and late confluency fibroblasts (Fig. 2).

Accumulation of G_{M2} also was observed in late confluency fibroblasts derived from patients with Tay-Sachs disease (Fig. 2). In the three fibroblast strains studied the amount of G_{M2} accumulated was 1.09 (range 0.6 to 1.4) and 3.86 (range 2.2 to 5.7) at early and late confluency, respectively. No G_{M2} could be identified in the cell-free medium of normal or Tay-Sachs fibroblasts cultivated for 24 hours in medium without fetal calf serum.

Storage of G_{M1} was observed in late confluency fibroblasts from patients with G_{M1} gangliosidosis, type 1 (Fig. 2). In early and late confluency normal fibroblasts the amount of G_{M1} was 0.07 ± 0.03 and 0.12 ± 0.4 nmoles/mg protein, respectively. In the two G_{M1} gangliosidosis fibroblast strains the mean amount of G_{M1} accumulated at early and late confluency was 0.1 (range 0.09 to 0.11) and 1.81 (range 1.04 to 2.58), respectively.

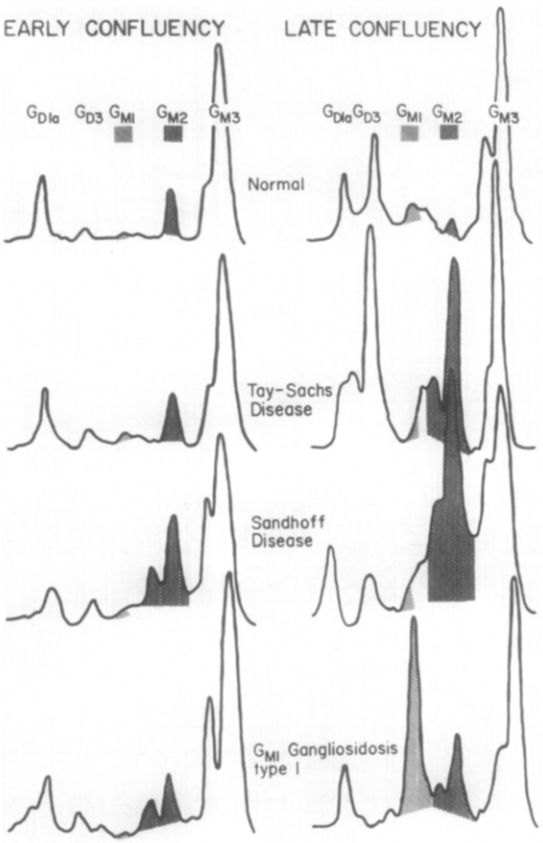

Fig. 2. Densitometry of gangliosides separated by high performance thin-layer chromatography on silica gel-60 as described by Ando et al. (7).

DISCUSSION

The major gangliosides detected in the present study are in agreement with previous reports (4,6), which have demonstrated that gangliosides G_{M3}, G_{M2}, G_{M1}, G_{D3}, and G_{D1a} are present in normal cultured skin fibroblasts. The most abundant ganglioside was G_{M3}, which constituted approximately 80 mol % of the total gangliosides. However, the reported amounts of gangliosides accumulated by fibroblasts from patients with G_{M1} gangliosidosis, type I and G_{M2} gangliosidosis, have been variable. Storage of G_{M1} could not be detected in two fibroblast strains from patients with G_{M1} gangliosidosis, type I (3,4), but it was found elevated in another case (8). Previously no abnormal accumulation of G_{M2} had been observed in fibroblasts from Tay-Sachs disease (3,9),

whereas a 3- to 5-fold increase in G_{M2} had been found in fibroblasts from Sandhoff disease (4). The data reported here indicate that cultured skin fibroblasts at late confluency from patients with three different gangliosidoses accumulated significant amounts of the ganglioside stored in each disorder. However, the level of this ganglioside at early confluency was within the normal range or it was only slightly increased. The degree of ganglioside accumulation in fibroblasts from gangliosidosis patients appears to be related to the rate of cell growth. This is supported from the fact that the Tay-Sachs fibroblast strain, which did not demonstrate G_{M2} accumulation at early confluency, was the fastest growing culture, whereas the other two Tay-Sachs fibroblast strains, which showed some G_{M2} accumulation at early confluency, were slowly growing. It is not certain, however, why fibroblasts from these patients only accumulate the macromolecule(s) which are not catabolized because of the enzymatic block at late confluency. It can be speculated that during the phase of active cell growth, the catabolic pathway of gangliosides is not very active. However, during the stationary phase the utilization of the gangliosides for the formation of new membranes is limited and, therefore, they are, to a large extent, catabolized. This, in gangliosidosis fibroblasts, would cause a significant accumulation of the macromolecule(s) catabolized by the deficient enzyme. Evidence for a more active catabolic pathway in late confluency fibroblasts is provided by the observation that the activities of a number of lysosomal enzymes are increased 3- to 10-fold at late confluency (10,11).

The demonstration that late confluency fibroblasts from gangliosidosis patients store significant amounts of gangliosides provides an additional tool for the diagnosis of these disorders. Identification of the stored material is important for accurate diagnosis, especially when new diseases or variants are described, since deficiency of an enzyme is not necessarily indicative of the primary defect (12). Measurement of the enzyme activity only, may lead to erroneous interpretations (13). Use of biopsy tissue, although

useful, has certain limitations because: organ and cell interactions in the patient's body may affect the degree of storage; and, more importantly, biopsy tissues consist of several cell types, the ratio of which may be altered in patients due to death of the most severely involved cells, such as the neuronal cells in the brain. Finally, under the conditions described, cultured fibroblasts provide the means for the dynamic study in vitro of the metabolic events in the gangliosidoses and possibly in other lysosomal disorders. This could help to distinguish between primary and secondary events and determine the pathogenesis of these disorders.

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