Cloning of a Mouse β1,3 N-Acetylgalcosaminyltransferase GlcNAc(β1,3)Gal(β1,4)Glc-ceramide Synthase Gene Encoding the Key Regulator of Lacto-series Glycolipid Biosynthesis*

Timothy R. Henion‡§, Dapeng Zhou¶, David P. Wolfer‡, Firoze B. Jungalwala‡, and Thierry Hennet**

From the ‡Eunice Kennedy Shriver Center, University of Massachusetts Medical School, Waltham, Massachusetts 02452 and the Institutes of ¶Physiology and §Anatomy, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Received for publication, April 11, 2001, and in revised form, May 30, 2001
Published, JBC Papers in Press, May 30, 2001, DOI 10.1074/jbc.M102979200

The Journal of Biological Chemistry
Vol. 276, No. 32, Issue of August 10, pp. 30261–30269, 2001
Printed in U.S.A.

The distinction between the different classes of glycolipids is conditioned by the action of specific core transferases. The entry point for lacto-series glycolipids is catalyzed by the β1,3 N-acetylgalcosaminyltransferase GlcNAc(β1,3)Gal(β1,4)Glc-ceramide (Lc3) synthase enzyme. The Lc3 synthase activity has been shown to be regulated during development, especially during brain morphogenesis. Here, we report the molecular cloning of a mouse gene encoding an Lc3 synthase enzyme. The mouse cDNA included an open reading frame of 1131 base pairs encoding a protein of 376 amino acids. The Lc3 synthase enzyme efficiently utilized the lactosyl ceramide glycolipid acceptor. The identity of the reaction products of Lc3 synthase-transfected CHOP2/1 cells was confirmed by thin-layer chromatography immunostaining using antibodies TE-8 and 1B2 that recognize Lc3 and Gal(β1,4)GlcNAc(β1,3)Gal(β1,4)Glc-ceramide (nLc4) structures, respectively. In addition to the initiating activity for lacto-chain synthesis, the Lc3 synthase could extend the terminal N-acetyllactosamine unit of nLc4 and also had a broad specificity for ganglio-series glycolipids. The mouse Lc3 synthase gene was mainly expressed during embryonic development. In situ hybridization analysis revealed that the Lc3 synthase was expressed in most tissues at embryonic day 11 with elevated expression in the developing central nervous system. Postnatally, the expression was restricted to splenic B-cells, the placenta, and cerebellar Purkinje cells where it colocalized with HNK-1 reactivity. These data support a key role for the Lc3 synthase in regulating neolacto-series glycolipid synthesis during embryonic development.

Glycolipids are ubiquitous constituents of cellular membranes. The pattern of glycolipid expression varies according to cell type and is developmentally regulated. Functionally, glycolipids have been implicated in various biological processes related to cell activation (1, 2), differentiation (3, 4), and adhesion (5, 6). Glycolipids also play important roles in infectious diseases, because they function as receptors for some viruses (7, 8) and bacterial toxins (9, 10). Moreover, cellular transformation is often accompanied by changes in the patterns of glycolipid expression (11, 12), which may be of prognostic and diagnostic values.

Glycolipids can be classified according to their respective core oligosaccharide structures. In mammals, four major classes can be distinguished (13). Isoglobulo-, globo-, lacto- and ganglio-series glycolipids are differentiated by the third saccuhrate unit added after the common lactosyl core (Fig. 1). This selective step is achieved by specific glycosyltransferase enzymes, which by their presence or absence in cells control the nature of the glycolipid repertoire. The molecular cloning of key glycosyltransferase genes allows a precise analysis of the dynamics of glycolipid expression and the possible correlation of changes in glycolipids with specific biological processes.

The β1,3 N-acetylgalcosaminyltransferase Lc3 synthase is the key enzyme controlling the expression of lacto-series glycolipids. This class comprises both neutral and charged compounds and are characteristic glycolipids of human colonic carcinomas and leukemia cells (12, 14). A number of sialosyl-, disialosyl-, fucosyl- and sulfoglucuronosyl-lactoglycolipids have been characterized in the nervous system (15, 16). The pattern of expression of the HNK-1-reactive sulfoglucuronosylglycolipids (SGGL) and other neolacto-glycolipids have been shown to be conditioned by the key Lc3 synthase activity (17). The Lc3 synthase enzyme is highly regulated during development, being most active by embryonic day 15 (E15) in the rat cerebral cortex and then decreasing to undetectable levels by postnatal day 10. In the adult cerebellum, the Lc3 synthase activity is restricted to Purkinje cells (18). Restoration of SGGL synthesis in primary granule neuron culture promotes neurite outgrowth and cell aggregation (19) suggesting that the control of SGGL expression by Lc3 synthase may have significance during development of the nervous system. In the present report, we describe the cloning of a Lc3 synthase gene and the character-
Cloning and Expression of the Lc3 Synthase Gene—The human expressed sequence tag fragment AI589087 was retrieved from the EST division of GenBank™ as it showed significant similarity to the β1,3 galactosyltransferase-I, -II, -III, and -IV (21, 22) and β1,3 N-acetylgalactosaminyltransferase-I (23) proteins. The mouse and human cDNAs were recently reported (20).

Northern Blotting—The Lc3 synthase mRNA levels were detected using multiple-tissue membranes of mouse adult tissue and embryonic stage RNA from Sigma. A 1.8-kbp SalI-XhoI fragment from the mouse Lc3 synthase cDNA was labeled with [α-32P]CTP (Hartmann Analytics, Braunschweig, Germany) by random priming and hybridized to the poly(A)+ RNA blots. Blots were washed in 0.1× SSC and 0.1% SDS heated up to 55 °C and exposed for 4 days between intensifying screens at −70 °C.

In Situ Hybridization—Digoxigenin (DIG)-labeled sense and antisense riboprobes were synthesized using a DIG RNA synthesis kit (Roche Molecular Biochemicals) from a 0.7-kbp HindIII-BglII fragment of the mouse Lc3 synthase cDNA subcloned into the plasmid pGEM3zf(+) (Promega) following the instructions of the manufacturer. For in situ analysis of spleen Lc3 synthase expression, spleens from C57BL/6 mice aged 8–12 weeks were frozen in Tissue-Tek (Miles, Elkhart, IN). Cryostat sections of 10 μm were cut at −20 °C and thaw-mounted on Superfrost plus microscope slides (Menzel-Gläser, Freiburg, Germany). Sections were dried and fixed for 1 h by immersion in 30% sucrose.

Glycosyltransferase Activity Assays—Donor and acceptor substrates were from Sigma, except for the O(CH2)nCO2Me,desacetylated acceptors, which were kindly provided by Dr. Markus Streiff (Novartis, Basel, Switzerland). Sf9 cells (5 × 10^8) infected with wild-type or with recombinant baculoviruses were lysed at 72 h post injection in 1 ml of 2% Triton X-100 in phosphate-buffered saline (PBS) on ice for 15 min. After removal of the nuclei by centrifugation, glycosyltransferase activity was assayed using 10 μl of cell lysate in 50 μl of 50 mM cacodylate buffer, pH 7.0, 20 mM MnCl2, 5% Me2SO, 0.75 mM ATP, 0.5 mM UDP-sugar donor substrate including 5 × 10^4 rpm of the corresponding UDP-[3H]sugar (Amersham Pharmacia Biotech) and various acceptors. Quantification of the reaction products was performed as described (21).

Thin-layer Chromatography Immunostaining of Lc3 Synthase-transfected Cells—The full-length coding region of the mouse and human Lc3 synthase gene were excised from pFastbac1 by BamHI and XhoI digestion and subcloned into pcDNA3 for transient transfection of CHOP2/1 cells. This Chinese hamster ovary glycosylation mutant is defective in Golgi sialic acid transport and in the Mgam1 gene required for initiation of N-linked glycan chain synthesis (24). As a result of this modifications these cells have greatly reduced glycoconjugate sialylation and N-linked glycosylation. After 70 h, cells were harvested by scraping, hand-homogenized in isopropanol:hexane:H2O (55:25:20), and dried under nitrogen. The residue was partitioned in chloroform:methanol:0.9% saline (20:10:6), and the lower phase was re-extracted with theoretical upper phase-saline (methanol:0.9% saline:chloroform, 48:47:3). The lower phase was extracted twice more with theoretical upper phase saline and dried under nitrogen. To reduce contaminating phospholipids, the residue was subjected to alkaline hydrolysis overnight with 0.2 M NaOH, neutralized with HCl, and purified by reverse phase C18-column chromatography. Glycolipid products were separated on aluminum high performance thin-layer chromatography plates (Merck, Darmstadt, Germany) using a solvent system of chloroform:methanol:0.25% CaCl2 (50:40:10) and immunostained using monoclonal antibodies TE-8 or IB2 (each at 1:5 dilution), essentially as described (25).

Fig. 1. Biosynthesis of the major classes of glycolipids. The common lactosyl ceramide (Gal[β1,4]Glc-Cer) structure is elongated by different glycosyltransferases, thereby defining the classes of glycolipids with their respective tri- or tetrasaccharide cores.

EXPERIMENTAL PROCEDURES

Cloning and Expression of the Lc3 Synthase Gene—The human expressed sequence tag fragment AI589087 was retrieved from the EST division of GenBank™ as it showed significant similarity to the β1,3 galactosyltransferase-I, -II, -III, and -IV (21, 22) and β1,3 N-acetylgalactosaminyltransferase-I (23) proteins. The mouse and human cDNAs were isolated from λ-phage brain cDNA libraries (CLONTECH) using a probing fragment derived from AI589087 generated by polymerase chain reaction with 50 ng of human genomic DNA as template with the primers 5′-GGTGATGTAGCTGCCAAAGTC-3′ and 5′-ATCTGCAGTATATTTGAC-3′. The mouse cDNA was subcloned as a SalI-XhoI 1.8-kbp fragment into the pFastbac1 vector (Life Technologies, Inc.) opened with the same enzymes, whereas the human cDNA was subcloned as a 1.2-kbp DraI-DraI fragment into the pFastbac1 vector linearized with SstI. The recombinant enzymes were produced with the baculovirus SF9 insect cell system as described previously (21).

Glycosyltransferase Activity Assays—Donor and acceptor substrates were from Sigma, except for the O(CH2)nCO2Me,desacetylated acceptors, which were kindly provided by Dr. Markus Streiff (Novartis, Basel, Switzerland). Sf9 cells (5 × 10^8) infected with wild-type or with recombinant baculoviruses were lysed at 72 h post infection in 1 ml of 2% Triton X-100 in phosphate-buffered saline (PBS) on ice for 15 min. After removal of the nuclei by centrifugation, glycosyltransferase activity was assayed using 10 μl of cell lysate in 50 μl of 50 mM cacodylate buffer, pH 7.0, 20 mM MnCl2, 5% Me2SO, 0.75 mM ATP, 0.5 mM UDP-sugar donor substrate including 5 × 10^4 rpm of the corresponding UDP-[3H]sugar (Amersham Pharmacia Biotech) and various acceptors. Quantification of the reaction products was performed as described (21).

Thin-layer Chromatography Immunostaining of Lc3 Synthase-transfected Cells—The full-length coding region of the mouse and human Lc3 synthase gene were excised from pFastbac1 by BamHI and XhoI digestion and subcloned into pcDNA3 for transient transfection of CHOP2/1 cells. This Chinese hamster ovary glycosylation mutant is defective in Golgi sialic acid transport and in the Mgam1 gene required for initiation of N-linked glycan chain synthesis (24). As a result of these modifications these cells have greatly reduced glycoconjugate sialylation and N-linked glycosylation. After 70 h, cells were harvested by scraping, hand-homogenized in isopropanol:hexane:H2O (55:25:20), and dried under nitrogen. The residue was partitioned in chloroform:methanol:0.9% saline (20:10:6), and the lower phase was re-extracted with theoretical upper phase-saline (methanol:0.9% saline:chloroform, 48:47:3). The lower phase was extracted twice more with theoretical upper phase saline and dried under nitrogen. To reduce contaminating phospholipids, the residue was subjected to alkaline hydrolysis overnight with 0.2 M NaOH, neutralized with HCl, and purified by reverse phase C18-column chromatography. Glycolipid products were separated on aluminum high performance thin-layer chromatography plates (Merck, Darmstadt, Germany) using a solvent system of chloroform:methanol:0.25% CaCl2 (50:40:10) and immunostained using monoclonal antibodies TE-8 or IB2 (each at 1:5 dilution), essentially as described (25).
Cloning of a β3GnT Lc3 Synthase

RESULTS

Several structural motifs are shared by the superfamily of β3 glycosyltransferases including β3,1,3-acetylglucosaminyltransferases. To confirm that this protein may represent an Lc3 synthase, the key enzyme in lactosylceramide synthesis, which were assayed at 2 mM, and Gal(1→4)Glc; Lac-N-tetraosetase, Gal(1→4)GlcNac(1→3)Gal(1→4)Glc (Table I). This strong activity toward lactosylceramide (1.23 nmol/min/mg protein) suggested that this protein of 378 amino acids displaying 77% identity with its human orthologous gene (GenBankTM accession number AF368169), which we isolated from a brain cDNA library, coded for a protein of 378 amino acids at the N-terminal region (Fig. 2). The predicted transmembrane region is marked in boldface. The ATG translation initiation codon is underlined and the motifs conserved in β3,1,3 glycosyltransferase proteins are shaded. The deduced amino acid sequence is shown in the single-letter code.

Table I

| Acceptor substrate specificity of the Lc3 synthase enzyme and other β3,1,3-N-acetylglucosaminyltransferases |
|-------------------------------------------------|
| Sph | Mock | β3GnT-I | β3GnT-III |
|------|------|--------|----------|
| pmol/mg prot added | Gal(1→4)GlcνAc | 2 | 96 | 26 | 4 |
| Gal(1→4)Glc | 3 | 230 | 89 | 5 |
| GalNaC(1→4)GlcνAc | 3 | 18 | 4 | 2 |
| Gal(1→4)GlcNac(1→4)GlcνAc | 3 | 11 | 3 | 5 |
| Gal(1→4)Glc-Cer | 1 | 1230 | 10 | 1 |
| Gal(1→4)GlcνCer(1→6)Gal | 3 | 924 | 1264 | 5 |
| Gal(1→3)GlcνNac(1→4)GlcνAc | 5 | 65 | 11 | 5 |
| Gal(1→3)GlcνNac(1→4)GlcνAc(1→6)GalνAc | 2 | 70 | 10 | 3 |
| Lac(-N)-tetraosetase | 2 | 293 | 46 | 3 |
| Lac(-N)-tetraosetase | 3 | 500 | 617 | 4 |
| Gal(1→4)Glc | 3 | 512 | 483 | 4 |
| Gal(1→4)GlcNac | 5 | 505 | 575 | 4 |
| asialo α1-glycoprotein | 8 | 243 | 346 | 13 |
| asialo-fetuin | 7 | 104 | 367 | 7 |

All acceptors were assayed at 5 mM except the (octyl)-derived acceptors, which were assayed at 2 mM, and Gal(1→4)Glc-Cer assayed at 0.25 mM.

Carbohydrate donor:
a) (octyl), O(CHO)_2CMe,
b) Lac-N-tetraosetase, Gal(1→3)GlcνNac(1→3)Gal(1→4)Glc,
c) Lac(-N)-tetraosetase, Gal(1→4)GlcNacβ3(1→3)Galβ3(1→4)Glc,
d) Gal(1→4)GlcNac(1→3)Gal(1→4)Glc-Cer (Table I). This strong activity toward lactosyl ceramide (1.23 nmol/min/mg protein) suggested that this protein may represent an Lc3 synthase, the key enzyme in lactosylceramide synthesis.

To confirm that this enzyme could function as an Lc3 synthase in vivo, the novel mouse cDNA, as well as its human ortholog, was overexpressed in CHO-P2/1 cells, which are devoid of the Lc3 synthase activity and sialylation (24). Extracted glycolipids from cells trans-
fected with a mock vector or vectors expressing the mouse and human cDNAs of interest were separated by high performance thin-layer chromatography. The Lc3 product was readily detected in cells transfected with mouse and human cDNAs by immunostaining using the specific antibody TE-8 (Fig. 3). As expected, a significant portion of the Lc3 product was further modified by endogenous $1,4$ galactosyltransferase activity to form Gal$[1,4]GlcNAc[1,3]Gal$,$[1,4]Glc-Cer, which was localized by the 1B2 monoclonal antibody (Fig. 3). In contrast, significant levels of Lc3 and nLc4 were not detected in mock transfected cells. Thus, this experiment clearly shows that the cDNA clones encode Lc3 synthase enzymes.

The Lc3 synthase displayed a uniquely broad level of activity with a variety of galactose-terminated glycolipid acceptors. The Lc3 synthase utilized Gal$[1,4]GlcNAc[1,3]Gal$,$[1,4]Glc-Cer with similar efficiency to lactosyl ceramide, demonstrating the ability to both initiate and extend lacto- and neolacto-chains. Unexpectedly, the enzyme also strongly utilized several gangliosides with exposed Gal$[1,3]GalNAc-R$ structures, including GA1, GM1, and GD1b (Table II). Thin-layer chromatography analysis confirmed the addition of GlcNAc to each acceptor to form a single species (Fig. 4), which was strongly immuno-reactive with mAb TE-8, specific for terminal GlcNAc$[1,3]Gal$ (data not shown).

To delineate the pattern of Lc3 synthase gene expression in vivo we have analyzed mRNA levels in mouse tissues by Northern blotting. Strong hybridization signals were detected in adult spleen and placenta, whereas faint signals were found in adult brain, lung, thymus, and muscle tissues (Fig. 5). During development, the Lc3 synthase gene was maximally expressed by E11. Then, the mRNA levels progressively decreased but remained elevated up to the day of birth (Fig. 5).

The expression pattern of the Lc3 synthase was also localized by in situ hybridization with whole E11 embryonic mice (Fig. 6, A and B). Most tissues demonstrated significant expression of Lc3 synthase mRNA, consistent with the intense signal detected at E11 by Northern analysis. Expression was relatively high in several regions of proliferative neuroepithelium in the developing central nervous system, including cerebral ventricular zones, which give rise to neurons of the adult cortex and the neuroepithelium of the neural tube. Additionally, elevated expression was found in derivatives of the somitic mesoderm, including dorsal root ganglia and the sclerotome compartment, which give rise to the axial skeleton. The mandibular compo-
Cloning of a β3GnT Lc3 Synthase

FIG. 5. Expression of the mouse Lc3 synthase in adult tissues and embryos as determined by Northern blot analysis. Each lane represents about 2 µg of poly(A)⁺ RNA. At the left, the size of the RNA markers is indicated in kilobases (kb).

FIG. 6. Expression of the Lc3 synthase gene during mouse development. Sagittal sections of a mouse embryo at E11 and of the developing eye at the same stage hybridized with a Lc3 synthase antisense probe (A and C) and a sense probe (B and D), respectively, are shown. The numbers on A mark the following organs: 1, telencephalic vesicle; 2, midbrain tectum; 3, fourth ventricle; 4, olfactory pit; 5, medial nasal process; 6, mandibular component of first brachial arch; 7, superior ganglion of vagus nerve; 8, pharynx; 9, heart; 10, hepatic primordium; 11, intestine; 12, sclerotome compartment; 13, floor plate; 14, spinal cord; 15, dorsal (posterior) root ganglion. Abbreviations for C and D are as follows: L, lens; NR, neural retina; RPE, retinal pigment epithelium. Note the staining in the sense control for the latter is caused by pigment granules and does not represent localized transcript.

To investigate Lc3 synthase gene expression in the developing central nervous system in more detail, brain sections from later embryonic time points were examined. By E14 widespread expression was detected throughout the rostral caudal extent of the telencephalon (Fig. 7, A and B). Intense expression was maintained in the proliferative ventricular and sub-
ventricular zone, with the exception of the ventromedial wall of the caudal telencephalon (Fig. 7A, arrows). This discontinuous ventricular expression pattern is very similar to that reported previously with monoclonal antibodies 7A in the mouse (29) and FORSE-1 in the rat (30). Both antibodies recognize fucosylated neolacto-glycolipids bearing Lewis-X structures (3-fucosyllactosamine), which are the predominant carriers of the Lewis-X determinant in the embryonic rodent cortex. At E17 additional staining in the expanding cortical plate became evident (Fig. 7C and D). At this time point, neurons migrate from the ventricular zone through the intermediate zone to form the characteristic laminar organization of the cortical plate in the adult forebrain. The accumulation of migrated cells from the strongly labeled ventricular zone may account for this signal. Substantial expression was also found in thalamic nuclei, the precerebellar neuroepithelium, and postmitotic neurons of the deep cerebellar nuclei (Fig. 7E and F). Several nuclei of the brainstem, including the inferior olive and pontine nuclei, also labeled strongly (not shown).

In the adult cerebellum, strong Lc3 synthase expression was found exclusively in Purkinje cell bodies in the Purkinje cell layer. The cell-specific expression pattern was confirmed by colocalization with the Purkinje cell-specific marker calbindin (31) (Fig. 8A). HNK-1 reactivity was confined to Purkinje cell bodies and their dendrites in the molecular layer, which precisely correlated with the Purkinje cell-specific expression of the Lc3 synthase gene (Fig. 8B).

The Northern analysis of adult mouse tissues also suggested the spleen to be a potential site of lacto-series glycolipid synthesis. In situ hybridization analysis of adult spleen revealed that Lc3 synthase-positive cells were localized in the follicle region. The hybridization signal matched with B-lymphocyte populations as shown by CD19 immunostaining (Fig. 9). By contrast, splenic regions enriched in T-lymphocytes did not yield a hybridization signal with the Lc3 synthase antisense probe.

DISCUSSION

We have reported here the molecular cloning of a β1,3 N-acetylgalcosaminyltransferase gene coding for an Lc3 synthase enzyme involved in lacto-series glycolipid biosynthesis. The Lc3 synthase protein included several of the structural motifs previously identified in members of the β1,3-galactosyltransferase superfamily. This conservation suggests a common origin, indicating that a basic glycosyltransferase has likely evolved by gene duplication events, thereby allowing a qualitative expansion of oligosaccharide structures in higher organisms. In fact, structurally related β1,3 glycosyltransferases are involved in nearly all types of glycoconjugate synthesis, including proteoglycan glycosaminoglycans, N-linked and O-linked glycans, and glycolipids.

Understanding the roles of specific classes of glycolipids in biological processes requires a clear view of the localization of these molecules in animal tissues. However, the in vivo detection of glycolipids themselves is hampered by the scarcity of reagents available that distinctly recognize specific structures. Furthermore, a selective detection of glycolipids can be difficult when the oligosaccharide moiety resembles those found on other classes of glycoconjugates. This last point is especially true for lacto-series glycolipids, which share several oligosac-
charide structures with glycoproteins. Therefore, the availability of specific genetic probes, as represented by core-specific glycosyltransferase genes, constitutes an essential tool in the study of glycolipids \textit{in vivo}. Along this line, the molecular cloning of the Lc3 synthase gene now enables a detailed investigation of the processes in which lacto-series glycolipids have been implicated. Noteworthy, the monoclonal antibodies recognizing lacto-series glycolipids such as TE-8 and 1B2 cannot be applied to detect the presence of the corresponding glycolipids \textit{in situ} as they also recognize carbohydrate epitopes on glycoproteins.

Previous studies have documented the expression of Lc3 synthase enzyme activity as a key regulatory step in the synthesis of lacto-series glycolipids in different tissues (12, 32) and cell lines (12, 33, 34). This is also true for the developing brain where the expression of Lc3 synthase enzyme activity specifically regulates the expression of SGGLs (18). \textit{In situ} colocalization of this new gene with HNK-1-reactive Purkinje cells supported its identity as the Lc3 synthase involved in SGGL synthesis. Additionally, the overlapping expression pattern of the Lc3 synthase with neolacto-glycolipids in the embryonic telencephalon (29), and the lens of the developing rodent eye (35), indicates that this Lc3 synthase may be regulatory for expression of other brain neolacto-series glycolipids, as suggested previously from enzyme data (16).

The regulation of SGGL synthesis in neural tissue has been related to changes in morphological and adhesion properties of neurons (19). Experiments where Lc3 synthase gene expression can be controlled in cell culture models will enable the role of SGGL in these biological processes to be assessed. Likewise, the high level expression of Lc3 synthase in zones of the proliferating neuroepithelium in the developing neural tube, cerebral cortex, and cerebellar anlage suggests potential roles for this class of glycolipids in early neuronal development that require further investigation.

Earlier reports based on the study of cell lines showed that the Lc3 synthase activity was not expressed in lymphoid cells

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{Restricted expression of the Lc3 synthase gene in adult mouse cerebellum. \textbf{Panels A} and \textbf{B} show regions of mouse cerebellum at different magnifications. Sagittal sections through the medial aspects of mouse cerebellum probed with antisense (\textit{upper left}) and sense RNA (\textit{upper right}) to mouse Lc3 synthase. \textbf{Lower panels} show double label immunofluorescence of sections adjacent to the \textit{in situ} sections stained with antibody to HNK-1 (\textit{lower left}) and the Purkinje cell marker calbindin, localized with Cy3 and Cy2-conjugated secondary antibodies, respectively. \textit{ML}, molecular layer; \textit{PCL}, Purkinje cell layer; \textit{IGCL}, intermediate granule cell layer.}
\end{figure}
The expression of LC3 synthase in splenic B-cells is interesting in light of the high level of activity with gangliosides GA1, GM1, and GD1b. A rare species of hybrid ganglioside structures containing extended lactosamine structures on a GM1 core was identified previously in glycolipid extracts of splenic B-cells but not T-cells (37). These were structurally identified as having the core structure GlcNAc(β1-3)Gal(β1-3)GalNAc-R. It seems likely that the LC3 synthase is involved in the synthesis of this GlcNAcβ1,3 linkage. Although the structural confirmation and significance of these awaits further analysis, these results may reveal a previously undescribed role for the LC3 synthase in regulating synthesis of these unique glycolipids. The exact functions of lecto-series glycolipids have remained elusive with the exception of few specific processes. The disruption of the LC3 synthase gene in mice should provide valuable insight into the functional involvement of this glycolipid class in developmental and physiological pathways.

Acknowledgments—We thank Rosmarie Lang and Dr. Laurent Pays for assistance in the in situ hybridization procedure, Dr. Eric Holmes for providing the TE-8 antibody, and Dr. James Dennis for providing the CHOP2/1 cells. We are also grateful to Dr. Denise Chou for advice on glycolipid isolation and immunostaining procedures.

REFERENCES

1. Felding-Habermann, B., Igarashi, Y., Fenderson, B. A., Park, L. S., Radin, N. S., Inokuchi, J., Strassmann, G., Handa, K., and Hakomori, S. (1990) Biochemistry 29, 6314–6322.
2. Norihisa, Y., McVicar, D. W., Ghosh, P., Houghton, A. N., Longo, D. L., Creekmore, S. P., Blake, T., Ortoldo, J. R., and Young, H. A. (1994) J. Immunol. 152, 485–495.
3. Nojiri, H., Takaku, F., Terui, Y., Miura, Y., and Saito, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 782–786.
4. Kojima, N., Kurosawa, N., Nishi, T., Hanai, N., and Tsuji, S. (1994) J. Biol. Chem. 269, 30451–30456.
5. Sheik, K. A., Sun, J., Liu, Y., Kawai, H., Crawford, T. O., Proia, R. L., Griffin, J. W., and Schnaar, R. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7532–7537.
6. Miura, R., Aspberg, A., Ethell, I. M., Hagihara, K., Schnaar, R. L., Russolhti, E., and Yamaguchi, Y. (1999) J. Biol. Chem. 274, 11431–11438.
7. Bhat, S., Spitalnik, S. L., Gonzalez-Searano, F., and Silberberg, D. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7131–7134.
8. Epand, R. M., Nir, S., Parolin, M., and Flanagan, T. D. (1995) Biochemistry 34, 1084–1089.
9. Lindberg, A. A., Brown, J. E., Stromberg, N., Westling-Ryd, M., Schultz, J. E., and Karlsson, K. A. (1987) J. Biol. Chem. 262, 1779–1785.
10. Ångström, J., Teneberg, S., and Karlsson, K. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11859–11863.
11. Singhai, A., and Hakomori, S.-I. (1990) Biochimica 12, 223–230.
12. Holmes, E. H., Hakomori, S., and Ostrander, G. K. (1987) J. Biol. Chem. 262, 15649–15658.
13. Fenderson, B. A., Eddy, E. M., and Hakomori, S.-I. (1990) Biochimica 12, 173–179.
14. Hu, J., Stuits, C. L., Holmes, E. H., and Macher, B. A. (1994) Glycobiology 4, 251–257.
15. Chou, D. K., Iiyas, A. A., Evans, J. E., Costello, C., Quarles, R. H., and Jungalwala, F. B. (1986) J. Biol. Chem. 261, 11717–11725.
16. Chou, D. K., Suzuki, Y., and Jungalwala, F. B. (1996) Glycoconj. J. 13, 295–305.
17. Chou, D. K., and Jungalwala, F. B. (1993) J. Biol. Chem. 268, 21727–21733.
18. Chou, D. K., and Jungalwala, F. B. (1996) J. Biol. Chem. 271, 28868–28874.
19. Chou, D. K., Tobet, S. A., and Jungalwala, F. B. (1998) J. Biol. Chem. 273, 6580–6587.
20. Togayashi, A., Akasaka, T., Okubo, R., Kudo, T., Nishihara, S., Iwasaki, H., Natsume, A., Mori, M., Inokuchi, J., Imamura, T., Sasaki, K., and Narimatsu, H. (2001) J. Biol. Chem. 276, 22002–22040.
21. Hennet, T., Dinter, A., Kohsert, P., Mattu, T. S., Rudd, P. M., and Berger, E. G. (1998) J. Biol. Chem. 273, 58–65.
22. Zhou, D., Berger, E. G., and Hennet, T. (1999) Eur. J. Biochem. 263, 571–576.
23. Zhou, D., Dinter, A., Gutierrez Gallego, R., Kamerling, J. P., Vliegenthart, J. F. G., Berger, E. G., and Hennet, T. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 406–411; Correction (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11673–11675.
24. Cummings, L., Warren, C. E., Granovsky, M., and Dennis, J. W. (1993) Biochem. Biophys. Res. Commun. 193, 814–822.
25. Zhou, D., Hennet, T., Jungalwala, F. B., Berger, E. G., and Hennet, T. (2000) J. Biol. Chem. 275, 22651–22656.
26. Miyazaki, H., Fukumoto, Y., Okada, M., Hasegawa, T., and Furukawa, K. (1997) J. Biol. Chem. 272, 24794–24799.
27. Shiraiishi, N., Natsume, T., Togayashi, A., Endo, T., Akasaka, T., Yamada, Y., Imai, N., Nakagawa, S., Koizumi, S., Sekine, S., Narimatsu, H., and Sasaki, K. (2000) J. Biol. Chem. 275, 4389–4397.
28. Okajima, T., Nakamura, Y., Uchikawa, M., Haslam, D. B., Numata, S. I., Furukawa, K., Urano, T., and Furukawa, K. (2000) J. Biol. Chem. 275, 40189–40193.
29. Yamamoto, M., Boyer, A. M., and Schwarting, G. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3045–3049
30. Tole, S., Kaprielian, Z., Ou, S. K., and Patterson, P. H. (1995) J. Neurosci. 15, 957–969
31. Nordquist, D. T., Kozak, C. A., and Orr, H. T. (1988) J. Neurosci. 8, 4780–4789
32. Hendricks, S. P., He, P., Stults, C. L., and Macher, B. A. (1990) J. Biol. Chem. 265, 17621–17626
33. Nakamura, M., Tsunoda, A., Sakoe, K., Gu, J., Nishikawa, A., Taniguchi, N., and Saito, M. (1992) J. Biol. Chem. 267, 23507–23514
34. Stults, C. L., and Macher, B. A. (1993) Arch. Biochem. Biophys. 303, 125–133
35. Ogiso, M., Saito, N., Hoshi, M., and Komoto, M. (1997) Glycoconjugate J. 7, 605–615
36. Macher, B. A., Lee, W. M., and Westrick, M. A. (1982) Mol. Cell Biochem. 47, 81–95
37. Nohara, K., Nakauchi, H., and Spiegel, S. (1994) Biochemistry 33, 4661–4666
