Glycolipid stage-specific embryonic antigens (SSEA-1) in kidneys of male and female C57BL/6J and beige adult mice

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Abstract SSEA-1 glycolipids from the kidneys of normal male and female as well as beige mutant mice were isolated and their structures were examined by component analysis, mass spectrometry, immunoblotting, and permethylation studies. These antigens were shown to be extended globoside derivatives as reported by Sekine et al. (1987. *J. Biochem.* **101:** 553–562). Quantitative high performance liquid chromatography analyses revealed that the concentration of SSEA-1 glycolipids were four- to fivefold greater in male than female mice. Essentially no SSEA-1 glycolipids were excreted in the urine of normal male mice and thus are not components of the multilamellar lysosomes normally excreted. Testosterone is known to induce the hypertrophy of proximal tubule cells that involves the formation of multilamellar lysosomes and results in the accumulation and excretion of these bodies and associated lysosomal enzymes and specific glycolipids. The present results indicate that in male mice there is also an increase in subcellular structures that contain SSEA-1 glycolipids. The amount of SSEA-1 glycolipids in male beige mice were greater than in normal mice on a per kidney basis. Thus, the increase is in proportion to the kidney hypertrophy seen in beige mouse kidneys. Beige mutant mice appear to have a primary defect in the excretion of multilamellar lysosomes which produces a secondary hypertrophy with an accompanying increase in SSEA-1 glycolipids.—**Williams, M. A., S. K. Gross, J. E. Evans, and R. H. McCluer.** Glycolipid stage-specific embryonic antigens (SSEA-1) in kidneys of male and female C57BL/6J and beige adult mice. *J. Lipid Res.* 1988. **29:** 1613–1619.

Supplementary key words kidney • galabiosylceramide • globotriaosylceramide • globoside • 3-fucolactosaminyl β1–6(galactosyl β1–3) globotetraosylceramide • testosterone • HPLC

Testosterone produces hypertrophy of the mouse kidney which primarily affects the proximal tubule cells. The concomitant androgen induction of β-glucuronidase was first shown by Fishman and Farmeland in 1953 (1). Other lysosomal enzymes are now known to be thus induced. Kidney cells actively excrete lysosomal enzymes into the urine and the rate of enzyme excretion increases markedly during androgen induction (2, 3). Electron micrographs have shown that near the brush border of induced cells there is a concentration of enlarged lysosomes filled with myelin-like figures. The same membranous material, present in the lumen of the proximal tubule, indicates an active excretion of these multilamellar lysosomes (4). We have demonstrated a concomitant increase in neutral glycosphingolipids (GSLs) both in the kidney and in the urine of males and testosterone-treated females (5). This phenomenon has also been documented in several inbred strains of mice (6). These studies indicate that testosterone induces the formation of multilamellar kidney lysosomes which contain specific GSLs ($GaOse_2Cers$ and $GbOse_3Cers$) and that a large percent of these bodies is normally excreted into the urine each day. There is also an induction of GalCer and $GbOse_4Cer$, which are not found in the urine of these animals and thus are not components of the excreted lysosomes. These data suggest that within proximal tubule cells, different glycolipids reside in specific subcellular compartments.

Novak et al. (7) and Novak and Swank (8) have described mouse pigmentation mutants that have lysosomal enzyme excretion defects. These mutants have increased lysosomal enzyme levels in the kidney and decreased excretion of these enzymes. We have previously shown that there is also a decrease in GSL excretion by these mutant mice and an accumulation in their kidneys of the GSLs

Abbreviations: NFA, nonhydroxy fatty acid; HFA, hydroxy fatty acid; HPLC, high performance liquid chromatography; AUFS, absorbance units full scale; GSLs, glycosphingolipids; SSEA-1, stage-specific embryonic antigen 1; HPTLC, high performance thin-layer chromatography; GLC, gas–liquid chromatography; MS, mass spectrometry; EI, electron impact ionization; CI, chemical ionization; FAB, fast atom bombardment; DCI, direct chemical ionization. Nomenclature used for glycosphingolipids is that recommended by IUAC (1976. *Lipids.* **12:** 455–468): GlcCer, glucosylceramide; GalCer, galactosylceramide; LacCer, lactosylceramide; $GaOse_2Cer$, galabiosylceramide, Gal(α1–4)Gal(β1–1)Cer; $GbOse_3Cer$, globotriaosylceramide, Gal(α1–4)Gal(β1–4)Glc(β1–1)Cer; $GbOse_4Cer$, globotetraosylceramide, GalNAc(β1–3)Gal(α1–4)Gal(β1–4)Glc(β1–1)Cer.

that are normally excreted (9). These data are consistent with the concept that specific GSLs are associated with the organelles whose normal exocytosis is blocked as a result of the genetic defects.

Fox et al. (10, 11) reported the presence of stage-specific embryonic antigen-1 (SSEA-1) in the mouse kidney. The SSEA-1 epitope was found to be the X-determinant trisaccharide Galβ1-4(Fucα1-3)GlcNAc (12) and the antibody has been shown to react with both glycoproteins and glycolipids (11).

Recently, Sekine et al. (13) reported the isolation from mouse kidney of two neutral glycolipids carrying SSEA-1 and SSEA-3 epitopes. The SSEA-3 antigen was shown to be Galβ1-3globotetraosylceramide and the structure of the SSEA-1 glycolipid was proposed to be Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3)-globotetraosylceramide. Sekine, Yamakawa, and Suzuki (14) also demonstrated the occurrence of polymorphic variation of these two glycolipids in inbred strains of mice.

In the present study, to further investigate the possible association of individual glycolipids with specific cellular structures, we have examined the SSEA-1 glycolipid in the kidneys and urine of beige mutant mice as well as normal male and female mice. The SSEA-1 glycolipids were isolated from C57BL/6J mouse kidneys and their structures were examined by component analysis, mass spectrometry, immunoblotting, and permethylation studies. Their concentration in the kidneys of normal and beige male and female mice was measured by HPLC.

MATERIALS AND METHODS

Materials

HPLC grade methanol and other reagent grade solvents and chemicals were obtained from Fisher Chemical Scientific (Fairlawn, NJ); Iatrobeads 6RS-8060 and 6RS-8010 were from Iatron Industries (Tokyo, Japan); DEAE-Sephadex (A-25) was from Pharmacia Fine Chemicals (Piscataway, NJ); Unisil was from Clarkson Chemical Company (Williamsport, PA); and HPTLC plates were obtained from E. Merck (Darmstadt, Germany). Bond Elut C18 reversed phase and Bond Elut NH_2 (aminopropyl) cartridges were obtained from Analytichem International (Harbor City, CA). A DB-1 GLC capillary column was purchased from J & W Scientific Inc. (Rancho Cardova, CA). Poly(isobutyl methacrylate) was obtained from Polyscience (Warrington, PA). Glycolipid standards were prepared as previously described (15). A monoclonal antibody with SSEA-1 specificity was made available by Drs. Yamamoto and Schwarting (16).

HPTLC and immunoblots with SSEA-1 antibody

Fractions to be tested were dissolved in 200 μl of chloroform–methanol 2:1 (v/v) and aliquots representing 1–2 mg of protein were spotted on aluminum-backed HPTLC plates and developed with chloroform–methanol–water 60:35:8 (v/v/v). The GSLs were visualized with orcinol spray reagent (17). Immunoblots were run according to modifications (16) of the procedure of Brockhaus et al. (18).

Isolation and analysis of SSEA-1 glycolipids from kidney

SSEA-1 positive glycolipids were isolated from pooled kidneys (37 g) of normal and pigmentation mutant male and female C57BL/6J mice. The kidneys were cleaned of all adhering tissue and the capsules were removed. Seven volumes of methanol was added and the tissue was homogenized with a Polytron. The homogenate was then heated at 55°C for 15 min, 14 volumes of chloroform was added, and the sample was mixed and filtered. The residue was re-extracted with 10 volumes of chloroform–methanol 1:1 (v/v). The sample was again filtered and the filtrates were combined and dried under nitrogen. The residue was suspended in 0.6 M methanolic NaOH, maintained at room temp for 1 hr and neutralized with aqueous 0.4 M HCl. Chloroform was added to form a partition according to Folch, Lees, and Sloane Stanley (19) and the upper phase lipid was recovered by C18 reversed-phase chromatography (20). These polar lipids (dissolved in methanol) were applied to a DEAE Sephadex A-25 (acetate) column and the neutral glycolipids were eluted with methanol (21). This neutral glycolipid fraction was examined by HPTLC and immunoblotting and was shown to contain a major and a minor band which were SSEA-1 positive. One to 2 mg of material at a time was further fractionated on a 10 μM Iatrobead column (0.45 cm × 50 cm) with a linear 2 hr gradient of isopropanol–hexane–water from 55:44:1 to 55:35:10 (v/v/v) at a flow rate of 1 ml/min (22). Three-ml fractions were collected and examined by HPTLC. Fractions containing single bands with the same R_f values were pooled. It was necessary to make several HPLC runs to obtain quantities of the major and the lower minor SSEA-1 positive bands sufficient for structural analysis.

Isolation and analysis of urinary glycolipids

Urine was collected from four C57BL/6J male mice for a 20-hr period. A total of 4.5 ml was collected. To the urine was added 36 ml of chloroform and 23 ml of methanol. The mixture was shaken for 1 hr at room temperature and then centrifuged. The residue was then re-extracted with chloroform–methanol 2:1 (v/v). The combined supernatants were taken to dryness. The sample was treated with 2 ml of 0.6 M methanolic NaOH for 60 min at room temperature and then neutralized with 3 ml of 0.4 M HCl. The lipid was desalted with a C18 reversed-phase Bond Elut cartridge, and eluted with chloroform–

methanol (1:1, v/v). The sample was subjected to Unisil and DEAE chromatography as described for kidney.

Sugar and fatty acid analysis

For sugar and fatty acid analysis, the glycolipids were subjected to methanolysis in anhydrous 0.75 M methanolic HCl as described (23). The fatty acid methyl esters were analyzed by GLC on a DB-1 0.25 mm × 30 m fused silica capillary column (J & W Scientific, Inc.) with helium as the carrier gas at 20 psi. A Hewlett-Packard model 5890 chromatograph equipped with a flame ionization detector was used and the column oven was programmed from 140–275°C at 10°C/min. The methyl glycosides were analyzed as their trimethylsilyl derivatives on the same DB-1 column as described (23) but the temperature was programmed from 140–250°C at 10°C/min.

Permethylation analysis

Approximately 100 μg of the major band was permethylated according to Larson et al. (24). One-tenth of the permethylated antigen was then taken for hydrolysis and subsequent mass spectometric analysis of the partially O-methylated alditol acetates (23). The remainder of the sample was used for the mass spectrometric analysis of the intact glycolipid.

Mass spectrometry

Gas–liquid chromatography–mass spectrometry for analysis of fatty acid methyl esters and partially methylated alditol acetates was performed with a Finnigan model 4500 mass spectrometer interfaced to a Hewlett-Packard model 5890 GLC and controlled by Teknivent Vector/One GLC–MS data system. A DB-1 0.25 mm × 30 m fused silica capillary column (J & W Scientific, Inc.) was used with helium as the carrier gas at 20 psi. Electron impact ionization (EI) was performed at 70 eV with the ion source at 150°C. Chemical ionization (CI) was performed with ammonia as the reagent gas at 0.5 torr and the same electron energy and source temperature. Fatty acid methyl esters were analyzed with a column temperature program from 170–300°C at 10°C per min under EI conditions scanning from m/z 70 to m/z 470 at 2 sec/scan. Partially methylated alditol acetates were analyzed under both EI and CI conditions by holding the column temperature at 170°C for 2 min, programming to 192°C at 2°C/min and to 232 at 10°C/min where the temperature was held for 1 min. The CI conditions used selected ion monitoring for the $[M + H]^+$ ions of amino sugars and the $[M + NH_4]^+$ ions of neutral hexoses to specifically identify the type of sugar and its degree of methylation. EI, used for linkage analysis, used selected ion monitoring for 14 ions of interest.

Fast atom barbardment (FAB) and direct ionization (DCI) MS were performed on the intact permethylated GSLs with a VG model 70-250 SE high resolution mass spectrometer. DCI was performed in the positive ion mode under ammonia CI conditions. One to 5 μg of permethylated GSL was dried on the DCI filament and desorbed at 1.5 A. Scans were acquired from 100–2500 amu at 1 sec/decade at 3,000 resolution and 7 kV acceleration. FAB spectra were acquired with 3-nitrobenzyl alcohol, and xenon fast atom source (Ion Tech, Ltd.) operated at 1.2 mA and 8 kV. Sodium acetate was added to the matrix to enhance formation of sodium adducts. Spectra were acquired from 100–2500 amu with 3,000 resolution at 10 sec/decade and 7 kV acceleration. Because the FAB spectra persisted for 10 to 20 scans, they were averaged to improve the signal to noise ratio.

Quantitative HPLC analysis

Each kidney was homogenized with a Polytron (Brinkmann Instruments, Westbury, NY) in the presence of 1 ml of water. An aliquot was taken for protein determination (25) and each sample received 9×10^4 cpm of tritium-labeled SSEA-1 as an internal standard to assess recovery. The tritiated SSEA-1 glycolipid was prepared by the galactose oxidase–sodium borohydride method (26) in Dr. M. D. Ullman's laboratory. The homogenate was extracted with chloroform–methanol 2:1, centrifuged, and the supernatant taken to dryness. The residue was hydrolyzed with 0.6 M methanolic NaOH for 1 hr at room temperature and then neutralized with aqueous HCl (15). Solvents were added to satisfy Folch conditions (19) and the phases were separated. The lower phase was washed three times with theoretical upper phase and the upper phases were combined. The upper and lower phase GSLs were isolated separately and then combined to obtain the total neutral GSLs (TNGSL).

The combined upper phases were applied to a C18 Bond Elut (0.2 g) cartridge. The eluate was re-applied and the cartridge was washed with 5 ml of H_2O. The lipid material was eluted with 5 ml chloroform–methanol 1:1 (v/v) (20). The sample was taken to dryness under N_2 and dissolved in methanol and the neutral and acidic upper phase lipids were separated on an aminopropyl cartridge. Aminopropyl cartridges (0.1 g) were converted to the acetate form with 5 ml 0.8 M ammonium acetate in methanol. Each cartridge was washed with 10 ml of methanol and the sample was applied with 3 × 1 ml of methanol. The cartridge was then washed with an additional 2 ml of methanol.

The Folch lower phase lipids were taken to dryness, dissolved in chloroform, and applied to a 125-mg Unisil column. After a chloroform wash the GSLs were eluted with 4 ml of acetone–methanol 9:1 as previously described (15). This eluate and the methanolic eluate from the aminopropyl cartridge were combined to form the TNGSLs. This combined fraction was benzoylated (27), cleaned up, and dissolved in 200 μl carbon tetrachloride. Ten percent aliquots were taken for counting to determine recovery

and 5-μl aliquots were injected onto a Zipax column as described previously (27). The GSLs were eluted with a linear gradient of 1 to 40% dioxane in hexane in 26 min at a flow rate of 2 ml/min.

RESULTS

HPTLC and immunoblot analysis

The total neutral glycolipid extracts from kidneys of male and female C57BL/6J and beige mice were obtained (see Methods) and equal aliquots of each sample were analyzed by HPTLC along with the large scale preparation from pooled kidneys as shown in **Fig. 1**. The dramatic differences between the quantities of glycolipids, including SSEA-1, from male (lanes B and C) and female (lanes D and E) kidneys, in both normal (B and D) and beige (C and E) mice are apparent. The kidney extracts were also spotted on an aluminum-backed HPTLC plate and after development blotted with SSEA-1 antibody as shown in **Fig. 2**. In each case two distinct bands were visualized; the upper band was heavy and somewhat spread and probably consists of more than one substance. In the normal and mutant female kidneys, lanes 2 and 4, the lower band was much lighter than the upper, whereas this difference in intensity in the male (lanes 1 and 3) was much less. The bands from the male beige mutant (lane 3) were much heavier than those from the normal male (lane 4) when quantities equivalent to equal portions of a kidney were spotted as shown in Fig. 2.

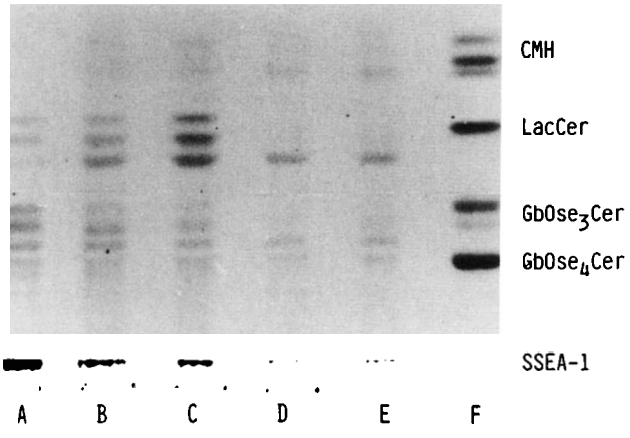


Fig. 1. HPTLC of mouse kidney total neutral glycosphingolipid (TNGSL) fractions. An equivalent of 2 mg of protein was spotted for lanes B to E. Lane A, upper phase neutral glycolipids from the pooled kidney preparation; B, C57 male TNGSL; C, beige male TNGSL; D, C57 female TNGSL; E, beige female TNGSL; F, glycolipid standards. Mouse kidney GSLs except for SSEA-1 have been previously characterized (5). The components running just below LacCer are $GaOse_2Cers$.

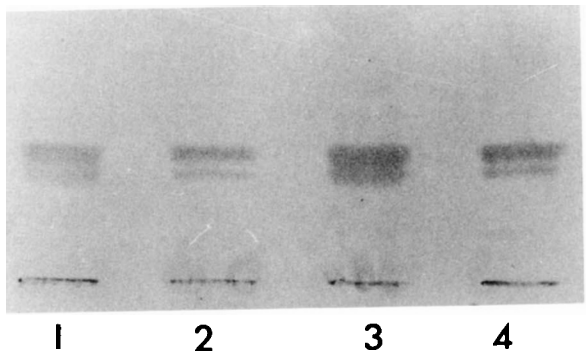


Fig. 2. SSEA-1 immunoblot of mouse kidney total neutral glycosphingolipids. GSLs, equivalent to 1 mg of protein, were spotted on each lane: 1, C57 male; 2, C57 female; 3, beige male; 4, beige female. See Methods for description of procedures. The origin appears as a line just above the lane numbers and does not give an antibody reaction.

Structural characterization

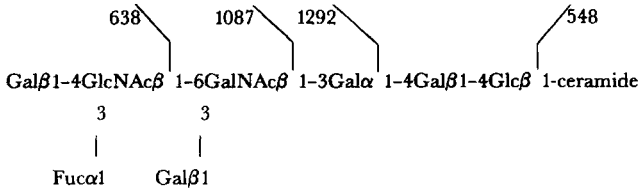
The two SSEA-1 positive glycolipid bands, as shown in Fig. 2, were isolated from pooled kidneys as described in Methods. Analysis of the fatty acid methyl esters and methyl glycosides of these two bands revealed that the major band contained 16:0 (35%), 18:0 (4%), 22:0 (20%), 24:0 (32%), and 24:1 (8%) fatty acids. The more polar band contained 16:0 (80%), 18:0 (11%), and 20:0 (9%) fatty acids. No hydroxy fatty acids were detected. Gas-liquid chromatographic analysis of the methyl glycosides of the SSEA-1 bands showed the following monosaccharide Glc:Gal:GalNAc:GlcNAc:Fuc ratios: 1.0:4.5:1.1:0.8:1.0 for the major band and 1.0:4.1:0.98:0.87:1.1 for the minor band.

The permethylated glycolipids were hydrolyzed and the partially methylated alditol acetates were examined by EI- and CI-GLC-MS with specific ion monitoring. Ion plots from CI-GLC-MS for $[M + NH_4]^+$ ions of trimethylfucose (m/z 210) and the tetramethylhexose (m/z 340) gave peaks that indicated the presence of fucose and galactose as terminal residues. EI-GLC-MS indicated the presence of a terminal galactose residue with a m/z 205 peak at the appropriate retention time; peaks seen with signals at m/z 117 and 233 indicated the presence of 2,3,6-O-methyl glucose and 2,3,6-O-methyl galactose derived from 4-O-substituted residues. A signal at m/z 161 with appropriate retention time indicated the presence of 2,4,6-O-methyl galactose derived from a 3-O-substituted residue. The presence of 6-O-methyl GlcNAc was indicated by the presence of an appropriate peak in the m/z 158 signal and 4-O-methyl GalNAc was indicated by peaks with the m/z 189 and 274 scans which are derived from 3,4-substituted GlcNAc and 3,6-substituted GalNAc.

The intact permethylated glycolipids were examined by mass spectrometry with DCI and FAB ionization. For the

major component, the FAB spectra provided molecular weight information with a sodium adduct ion at m/z 2396 consistent with the structure containing a 24:0-dl8:1 ceramide, two acetylhexosamines, five hexoses, and a deoxy hexose. A FAB signal at m/z 2284 for the minor permethylated glycolipid was obtained consistent with the presence of a 16:0-d18:1 ceramide component. Both the FAB and DCI spectra of the major component showed ions at m/z 638, 1087, and 1292 which indicated the presence of fragments of the terminal trisaccharide, pentasaccharide, and hexasaccharide of the proposed structure. Corresponding oligosaccharide fragments were also obtained for the minor component.

These data are entirely consistent with an extended galactosyl globo structure with a terminal SSEA-1 trisaccharide as shown below and as reported by Sekine et al. (13).



Quantitative determination of SSEA-1 in kidneys from male and female C57BL/6J and beige-J mice

Kidneys from male and female C57BL/6J mice and those from the beige-J pigmentation mutant on C57BL/6J background were extracted and total neutral glycosphingolipids were isolated as described in Methods. These samples were perbenzoylated and quantitated by HPLC. A representative chromatogram is shown in **Fig. 3**. The SSEA-1 peak was collected and the radioactivity was determined. The amount of SSEA-1 glycolipid present was calculated from the peak areas and the percent recovery of the internal standard. The results are summarized in **Table 1**. The kidneys of both normal and mutant male mice contain 4–6 times as much SSEA-1 per mg protein as the female kidneys. There appears to be no significant difference between normal and mutant mice in either males or females.

The mouse urine GSL fraction was examined for SSEA-1 glycolipids as shown in **Fig. 4**. Immunoblots and orcinol detection showed almost no glycolipid antigen in urine.

DISCUSSION

From the data obtained by antibody reactivity, component and methylation analysis, and mass spectrometry, the neutral glycolipids isolated from C57BL/6J kidney carrying the SSEA-1 epitope are galactosylgloboside derivatives consistent with the structure proposed by Sekine et al. (13). The SSEA-1-reactive glycolipids isolated from human erythrocytes and human teratocarcinoma (22) are known to be derivatives of neolactotetraosylceramide, thus the structures of SSEA-1 glycolipids detected

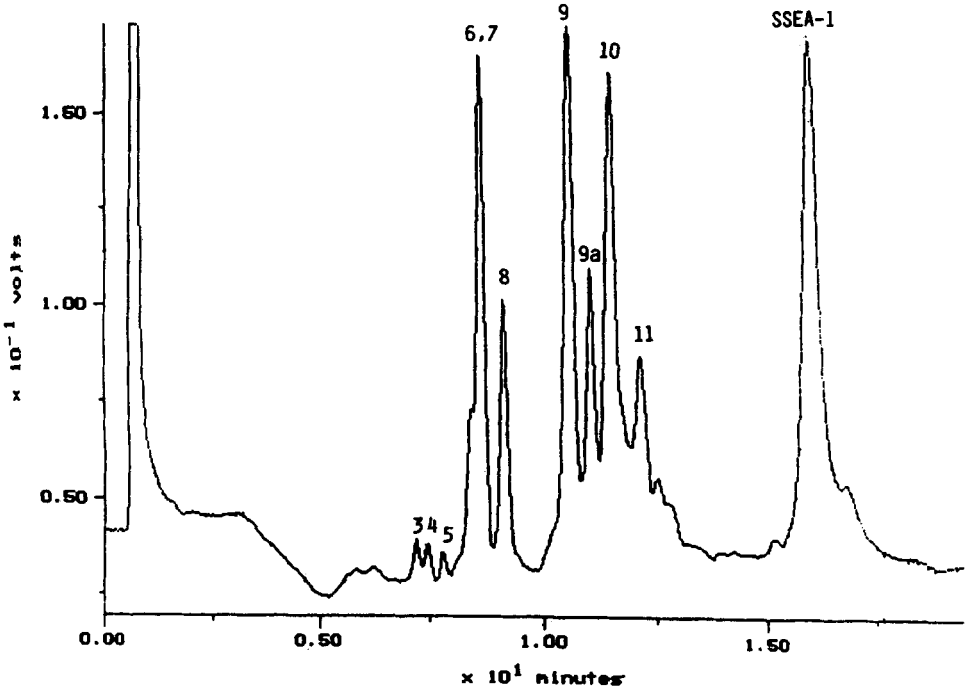


Fig. 3. HPLC chromatogram of a representative male C57BL/6J mouse kidney perbenzoylated total neutral glycosphingolipids. See Methods for the isolation and HPLC procedures. The compositions of peaks 3 to 11 are given in references 5 and 29.

TABLE 1. Content of SSEA-1 glycolipid in the kidneys of male and female C57BL/6J and beige-J mice[a]

Sex	C57BL/6J Mice		Beige-J Mice	
	nmol/mg protein ± SD, n = 3			
Male	0.75	0.15	0.69	0.15
Female	0.19	0.07	0.11	0.02
	nmol/kidney ± SD, n = 3			
Male	11.42	3.48	14.44	3.72
Female	2.21	0.34	1.65	0.42

[a]Kidney wet weights (g) were: C57BL/6J, male 0.145 ± 0.015, female, 0.119 ± 0.008; beige, male, 0.195 ± 0.019, female, 0.126 ± 0.019.

in other sources need to be established. We have recently found that the SSEA-1 glycolipids accumulating in the brain of a fucosidosis patient are of the lacto series (28).

Quantitative HPLC analysis of the kidney SSEA-1 glycolipids demonstrated a four- to fivefold elevation in the concentration of these glycolipids in male as compared to female mouse kidneys. This suggests that these lipids are components of the structures that hypertrophy in response to testosterone, primarily proximal tubule cells, and that their synthesis is probably induced by testosterone as has been shown for other kidney glycolipids (29). However, the SSEA-1 glycolipids were not detected in the urine of normal male mice, nor were they elevated in beige mice kidneys above that of normal male mice. Multilamellar lysosomal bodies containing only galabiosyl and globotriaosylceramides are excreted into the urine of normal male mice and accumulate in the kidneys of beige mutant mice. Thus, the SSEA-1 glycolipids are not associated with these same lysosomal membranes but appear to be components of other subcellular structures in the proximal tubule cells that hypertrophy in response to testosterone.

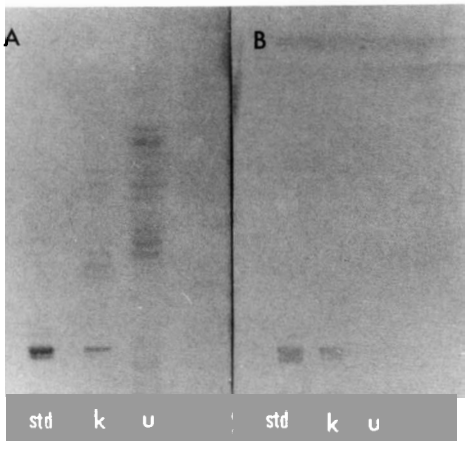


Fig. 4. HPTLC of male C57BL/6J mouse kidney (k) and urine (u). A: Orcinol spray for detection of GSLs; B: immunoblot with SSEA-1 antibody; STD is SSEA-1 antigen isolated from pooled mouse kidney.

These SSEA-1 glycolipids fall into a category with galactosylceramide and globotetraosylceramide which show male/female differences, but are not excreted into the urine and show minimal increases in the pigmentation mutants with excretion defects. The male beige mouse kidney is larger than the normal male mouse kidney, but the increased size and weight cannot be accounted for entirely by the accumulation of the multilamellar lysosomal bodies. The SSEA-1 glycolipids and globoside are maintained in the mutant kidney at levels roughly the same as in the normal tissue. It thus appears that there is a primary accumulation of multilamellar lysosomal bodies, which is accompanied by a general hypertrophy and a proportionate increase in other cellular structures and associated glycolipids such as globosides and SSEA-1 glycolipids. These phenomena indicate that individual glycolipids are associated with specific intracellular structures.

Fox et al. (11) suggested that the SSEA-1 antigen detected in SW kidney by immunohistochemistry was glycolipid and glycoprotein in nature. We have observed from polyacrylamide SDS electrophoresis and immunoblotting of kidney proteins that several protein bands serve as antigens for the SSEA-1 antibody. One of the several bands seen in normal and beige male mouse kidney proteins was absent from female protein blots (unpublished observations with Dr. M. Yamamoto). It has not been possible to distinguish glycolipid and glycoprotein antigens by immunocytochemistry and at the present time the morphological distribution of the SSEA-1 glycolipids cannot be assessed with certainty. We have not observed any obvious male–female differences by immunocytochemistry and, in view of the large sexual differences in kidney SSEA-1 glycolipid content reported here, it appears that the tissue-staining pattern observed (11) does not reflect just the glycolipid antigen.

In summary, the present studies show the SSEA-1 glycolipid antigens to be extended globoside derivatives as reported by Sekine et al. (14). Quantitative HPLC analyses revealed that the concentrations of SSEA-1 glycolipids were increased four- to fivefold in normal and beige male as compared to female kidneys. The total kidney SSEA-1 glycolipids were increased in male beige as compared to normal male mice proportional to the kidney hypertrophy. Essentially no SSEA-1 glycolipids were found to be excreted into the urine of normal male mice indicating that these glycolipids are not components of the excreted lysosomes. The androgen-dependent enlargement of the proximal tubule cells involves the formation of multilamellar lysosomes that contain galabiosyl and globotriaosylceramides. There is also a concomitant hypertrophy of other subcellular structures which contain SSEA-1 glycolipids. In beige mice there is a primary accumulation of multilamellar lysosomes that appears to produce a secondary cellular hypertrophy which involves a proportional increase in SSEA-1 glycolipids.

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