American Journal of Pathology, Vol. 149, No. 3, September 1996
Copyright © American Society for Investigative Pathology

Decrease of Glomerular Disialogangliosides in Puromycin Nephrosis of the Rat

Harry Holthöfer, Jukka Reivinen, Marja-Liisa Solin, Anni Haltia, and Aaro Miettinen

From the Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland

Puromycin aminonucleoside nephrosis (PAN) is a model for human minimal change nephropathy induced in rats by injection of puromycin. In PAN, defective sialylation of a major sialoprotein of podocytes, podocalyxin, has been demonstrated and the consequent decrease of anionic charge suggested as a causative factor for increased glomerular permeability and proteinuria. Whether defective sialylation is a general feature of PAN affecting also glomerular glycosphingolipids is not known. We have shown that rat glomeruli are rich in disialogangliosides GD_3 and O-acetyl GD_3, the functions of which are not known. Here, we made a sequential analysis of the glomerular gangliosides, especially of GD_3 and its O-acetyl derivative in acute PAN using immunohistochemical and biochemical techniques and compared the results with another rat model of glomerular disease, Heymann nephritis. The prominent immunohistochemical finding was the almost total disappearance of glomerular O-acetyl GD_3 and a substantial decrease of its precursor GD_3 peaking at 10 days after injection of puromycin. Segmental areas lacking these gangliosides remained in glomeruli still at 30 days after injection. The response was dose dependent. Semiquantitative analysis by thin layer chromatograms showed that O-acetyl GD_3 was decreased by 41% already at 3 days and by 60% at 10 days after injection of puromycin. Also GD_3, the immediate precursor of O-acetyl GD_3, was decreased by 20 and 19%, respectively, at 3 and 10 days after injection. At 3 days after injection, overt proteinuria had not started. At these times, no other changes were observed in the glomerular gangliosides. The decrease of glomerular GD_3 and O-acetyl GD_3 indicates a decrease of GD_3 synthase activity and perhaps of O-acetyltransferase activity in PAN nephrosis. As these changes preceded the overt proteinuria, they may have a causal relationship to it. In the glomeruli of Heymann nephritic rats, no similar changes were seen, suggesting that the sialylation defect is not due to proteinuria but is a consequence of targeted puromycin action on cells. (Am J Pathol 1996, 149:1009–1015)

Sialylated glycosphingolipids (gangliosides) are ubiquitous components of mammalian cell membranes.[1] These molecules are synthesized by a stepwise addition of sugar residues to the glycan side chain of a ceramide backbone by specific enzymes to yield developmentally regulated tissue and cell-type-specific structures.[1–3] Gangliosides may modulate cell adhesion molecules and are involved in cellular differentiation and malignant transformation.[4–6] Additionally, gangliosides can bind to cell surface receptors and may modulate receptor tyrosine kinase activity and activate membrane-bound protein kinase C.[7,8] Despite the variety of functions suggested for gangliosides, little is known of their roles in specific tissues and cell types except for neuronal cells.[9–11]

Gangliosides are particularly abundant in the kidney tissue.[12] We have recently reported that the disialogangliosides GD_3 and O-acetyl GD_3 are found in the visceral epithelial cells (podocytes) of rat kidney glomeruli.[13,14] GD_3 has a role in the mesenchymal-epithelial interaction during the development of mouse kidney,[15] but otherwise little is known of the functions of renal gangliosides. Due to their abun-

Supported by grants from the Sigrid Juselius Foundation, the Paulo Foundation, the Päivikki and Sakari Sohlberg Foundation, the Finnish Foundation for Cancer Research, the Finnish Foundation of Nephrology, and the Academy of Finland.

Accepted for publication May 8, 1996.

Address reprint requests to Dr. Harry Holthöfer, Department of Bacteriology and Immunology, PO Box 21 (Haartmaninkatu 3), FIN-00014 University of Helsinki, Helsinki, Finland.

dance and electronegative charge, gangliosides could be involved in the maintenance of the charge-selective filtration barrier of glomeruli.[16] A decrease in the sialylation, without changes in the total amount of podocalyxin, a major sialoprotein of podocytes and endothelial cells, has been reported in puromycin aminonucleoside nephrosis (PAN) of the rat,[17,18] suggesting that changes in the glycan chains may be important in the pathogenesis of proteinuria.

PAN is a widely used experimental model of glomerular disease in which the damage affects mainly podocytes.[19] However, the molecular mechanisms of proteinuria in PAN are still not known. Our results suggest that puromycin induces defective synthesis and modification of the disialogangliosides of the podocytes.

Materials and Methods

Animals and Induction of Nephritis

PAN was induced in two series of Sprague-Dawley rats. In experiments 1 and 2, 10 and 40 male rats (Department of Bacteriology and Immunology, University of Helsinki), respectively, weighing 175 to 250 g, with free access to tap water and standard rat chow throughout the study, were used.

To induce PAN, 30 mg/100 g of aminonucleoside of puromycin (or, in experiment 2, 15 mg/100 g, see below; Sigma Chemical Co., St Louis, Mo) in phosphate-buffered saline (PBS) was injected intraperitoneally, and 18-hour urinary samples were collected every second day in metabolic cages. All animals developed significant albuminuria by day 5 after injection, as measured by nephelometry (Behring nephelometer 100 analyzer, Behringwerke, Marburg, Germany[20]). The animals in experiment 1 (five PAN and five control rats) were sacrificed on day 10 at the peak of proteinuria. In experiment 2, 30 rats were injected, but with a lower dose of puromycin (15 mg/100 g rat weight), whereas 10 control rats received an equal volume of PBS. In experiment 2, groups of 10 rats were killed on days 3, 10, and 30 after injection after 18-hour urine collection, and a small cortical sample was taken from each kidney for immunofluorescence studies. In experiment 1, glomeruli were isolated from the kidneys of the injected and control rats and gangliosides were extracted from the isolated glomeruli as described earlier.[14] In experiment 2, one kidney was taken from each rat for the extraction of glomerular mRNA and the remaining kidney was taken for ganglioside extraction.

Autoimmune Heymann nephritis was induced as described earlier in detail.[21] Briefly, rats as above for PAN induction were immunized subcutaneously with 0.2 mg of renal brush border protein in 0.4 ml of Freund's complete adjuvant. The injections with incomplete adjustment were repeated 1 month after the first one. Urine samples were collected, and the rats were killed at 12 weeks after the first injection.

Immunofluorescence Microscopy

To study the presence and tissue distribution of GD_3 and O-acetyl GD_3 gangliosides, frozen sections of rat kidneys were cut at 3 to 4 μm, fixed in acetone at −20°C for 5 minutes, and washed in PBS. Thereafter, monoclonal antibodies against GD_3 ganglioside (clone R24; Biogenesis, Bournemouth, UK) or O-acetyl GD_3 (clone 27A; see Ref. 14) were flooded over sections for 30 minutes. After washing, the tissue sections were further incubated with rat anti-mouse IgG (Boehringer Mannheim, Mannheim, Germany) coupled with fluorescein isothiocyanate (FITC). The sections were also stained with FITC-conjugated anti-rat C3 (Cappel Laboratories, Cochranville, PA) or FITC-conjugated anti-rat IgG (Boehringer Mannheim) to detect immune deposits in the kidneys.

An Olympus OX50 microscope equipped with an epi-illuminator and a filter system for FITC fluorescence was used for microscopy.

Ganglioside Analysis

To analyze the glomerular gangliosides, the protocol was essentially as described before.[14,22] The pooled glomeruli isolated from PAN, Heymann, or control kidneys (1 vol) were homogenized in 3 vol of water at 0°C, 15 vol of chloroform/methanol (1:2 v/v) was added, and the mixture was agitated for 4 hours on a magnetic stirrer at 50°C. After repeated extractions with chloroform/methanol/0.1 mol/L aqueous KCl (5:10:4 v/v), the two supernatants were combined and partitioned according to Folch et al.[23] The upper phase containing the crude ganglioside fraction was collected and evaporated to dryness under nitrogen flow. After dissolving in a small amount of water, it was desalted using C18 Sep-Pac cartridges (Waters Associates, Milford, MA) and further purified with a DEAE Sephadex A-25 column and desalted with C18 Sep-Pac cartridges.

The total gangliosides thus obtained were separated on thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany) using chloroform/methanol/0.25% aqueous KCl (50:40:10) as solvent. Equal amounts of gangliosides as lipid-bound sialic acid were loaded on each lane. The hexoses were

then stained with orcinol/H_2SO_4 reagent.[14] Commercial gangliosides GM_3, GM_2, GM_1, GD_3, GD_{1a}, GD_2, and GT_{1b} were used as ganglioside standards.[14] The relative amount of gangliosides and their changes in 3-, 10-, and 30-day samples were analyzed from the thin layer chromatograms using direct densitometric scanning of the TLC plates with an Apple Color One scanner. Varying amounts of purified GM_3 ganglioside containing the same molar amounts of hexoses as the GD_3 or O-acetyl GD_3 gangliosides studied were run on the same plates with the glomerular samples. An 8-bit gray scale image was recorded from each lane, and the analysis was performed on a Power Macintosh computer using the NIH Imaging program v1.56 (written by W. Rasband, National Institutes of Health, Bethesda, MD). The detection of GD_3 or O-acetyl GD_3 was done by immune overlay, as described earlier.[14,22]

Urinary gangliosides were extracted from the lyophilized, pooled urine of the rats of experiment 1 and analyzed by TLC as described above.

Results

Proteinuria

In experiment 1 after administration of puromycin at 30 mg/100 g, rats developed increasing albuminuria reaching 210 ± 73 mg/24 hours (mean ± SD) at day 10, whereas the control rats had almost no albuminuria (0.2 ± 0.7 mg/24 hours). In experiment 2 after injection of puromycin at 15 mg/100 g, a minor elevation of urinary albumin could be detected on day 3 (4.7 ± 5.1 mg/24 hours) as compared with the controls (0.1 ± 0.7 mg/24 hours), whereas at day 10 or 30 the urinary albumin rates were 210 ± 102 mg/24 hours or 277 ± 114 mg/24 hours, respectively. The albuminuria with Heymann nephritis at the time of sacrifice (at 12 weeks from induction) was 30 ± 18 mg/24 hours.

Immunohistological Findings

No deposits of IgG or C3 could be seen in the glomeruli of PAN or control rats, but in the samples of the Heymann nephritic rats, diffuse granular deposits of IgG and C3 typical of membranous glomerulonephritis were found in all glomeruli. At day 3 after injection, glomerular staining of the injected rats for GD_3 or O-acetyl GD_3 was by visual evaluation comparable with that of the control rats (Figures 1 and 2). At day 10, a distinct decrease of GD_3 was observed in the glomeruli (Figure 3), whereas O-acetyl GD_3 was barely detectable (Figure 4). Notably, the higher dose of puromycin caused a more prominent loss of immunohistologically detectable O-acetyl GD_3 in the glomeruli. At day 30, both GD_3 and O-acetyl GD_3 immunoreactivities were back close to those seen in the control kidneys, although distinct loss of immunoreactivity within sectors of variable sizes was regularly detected within the glomeruli.

Heymann nephritic kidneys appeared closely similar to the controls; a prominent granular staining reminiscent of podocyte reactivity was detected in all glomeruli but not in other parts of the kidney (see Figure 1).

Biochemical Analysis of Glomerular Gangliosides

The major gangliosides of normal rat glomeruli are GM_3, GD_3, and O-acetyl GD_3 when analyzed by TLC and hexose staining, as we have earlier shown.[14,22] Normally, O-acetyl GD_3 is the major band between the GM_1 and GM_2 gangliosides (Figure 5, lane 2). This was was strongly reduced at day 10 in the glomeruli of the PAN rats of experiment 1 induced with the higher puromycin dose. In the samples of the rats with the lower puromycin dose (experiment 2), a similar but milder reduction in band intensity was detected already at day 3 (Figure 6, lane 3; see Table 1). The change was more prominent at day 10 (Figure 6, lane 4; Table 1) and remained at day 30 after injection. A decrease of GD_3 and concomitant increase of GM_3 was found already at day 3 (Figure 6, lane 3; see Table 1). These data show the time and dose dependency of the PAN effect for the gangliosides and was, notably, observed already before appreciable changes in the immunofluorescence microscopy. The Heymann nephritic kidney glomerular gangliosides closely resembled those of the control kidneys (results not shown).

To study whether the decrease of the glomerular O-acetyl GD_3 was due to its increased urinary secretion, we isolated the urinary gangliosides in experiment 1 and analyzed these on TLC immune overlays with 27A antibodies. In control and PAN rat urine, however, a comparable weak immunoreactivity was detected in both samples (results not shown), indicating that the loss of glomerular gangliosides in PAN nephrosis was not due to loss into urine.

Discussion

In this study, we demonstrate both immunohistochemically and biochemically that the normally

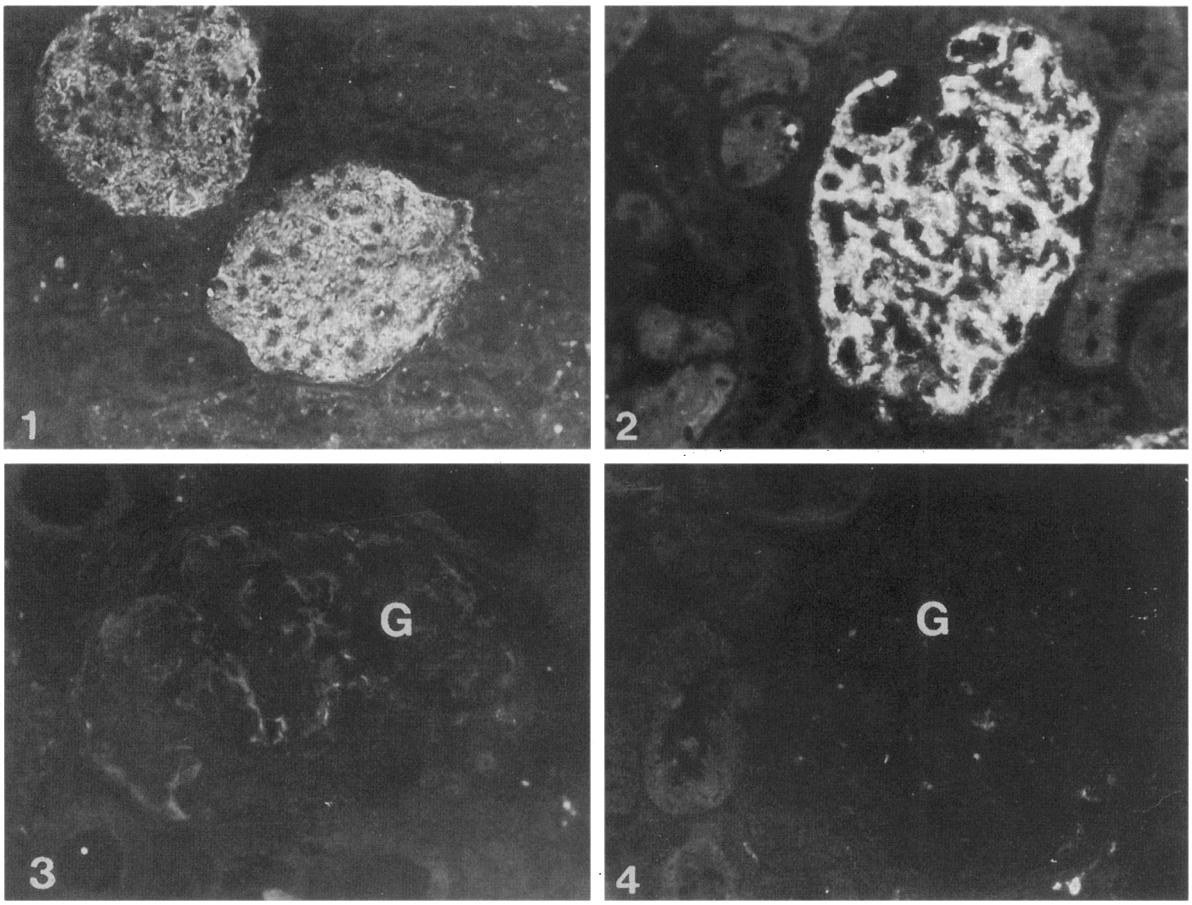

Figure 1. *Frozen section of normal rat kidney stained with R24 antibodies for GD_3 ganglioside. A granular staining pattern is seen within the glomeruli. Magnification, ×280.*
Figure 2. *Frozen section of normal rat kidney stained with 27A antibodies for O-acetyl GD_3. Granularity confined to the glomerulus in an epithelial pattern is evident. Magnification, ×360.*
Figure 3. *Kidney section of a rat with PAN nephrosis at day 10 stained for GD_3. Note the strong decrease of glomerular (G) staining as compared with the normal kidney in Figure 1. Magnification, ×360.*
Figure 4. *A complete loss of O-acetyl GD_3 reactivity within glomeruli (G) of PAN nephritic rat kidney at day 10. Magnification, ×360.*

abundant disialoganglioside O-acetyl GD_3 of glomerular podocytes becomes barely detectable and the relative amount of its immediate precursor GD_3 disialoganglioside markedly decreases in comparison with its monosialyl precursor GM_3 in the proteinuric phase of PAN nephrosis. The changes in glomerular gangliosides were dose dependent and could be observed already before the onset of prominent proteinuria on day 3 after injection. This indicates that the changes were not secondary to proteinuria and may have a causal relationship to it. In comparison, no changes were seen in the Heymann model of nephrotic syndrome caused by an immunological injury to glomerular podocytes. These results suggest that puromycin directly interferes with the terminal sialylation of gangliosides and that the target cell in PAN nephrosis is the podocyte.

The podocyte is morphologically a highly differentiated cell with its interdigitating foot processes con-

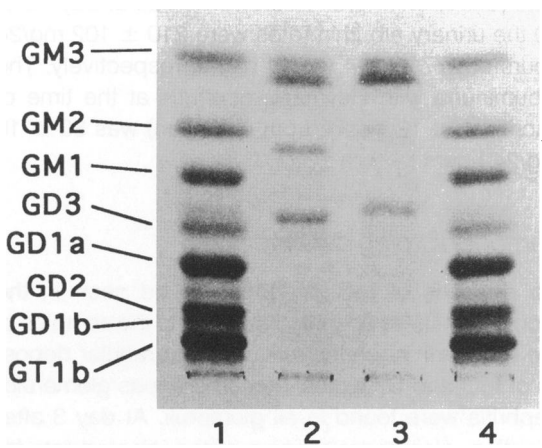

Figure 5. *Thin layer chromatogram (experiment 1) of the ganglioside extracts of glomeruli isolated from the kidneys of normal rats* (lane 2) *and rats with PAN nephrosis* (lane 3). *Note the almost total disappearance of the band migrating between standards* (lanes 1 *and* 4) *GM_1 and GM_2 (corresponds with O-acetyl GD_3) in* lane 3 *as demonstrated by hexose staining.*

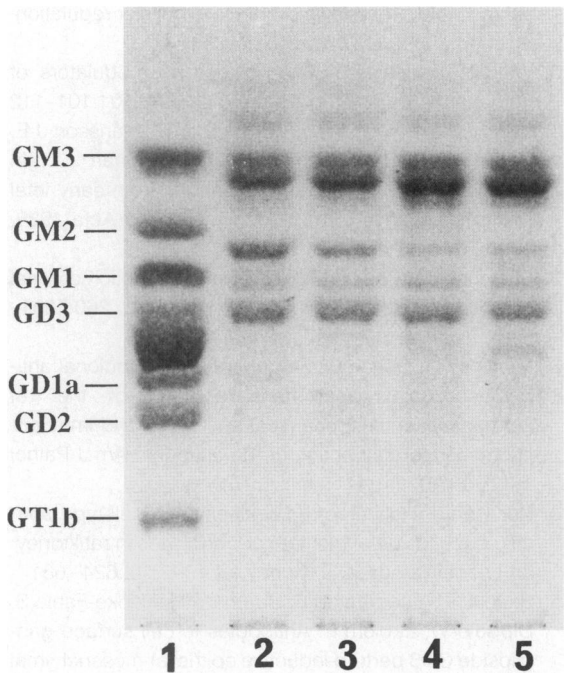

Figure 6. *Thin layer chromatogram (experiment 2) showing the time dependency of glomerular ganglioside changes in PAN. The amount of gangliosides loaded is the same in all lanes. Note the decreased intensity of the band migrating between standards* (lane 1) *GM_1 and GM_2 (corresponds with O-acetyl GD_3) and a simultaneous increase of the GM_3 already at post-injection day 3* (lane 3), *day 10* (lane 4), *and day 30* (lane 5). Lane 2 *shows the gangliosides of normal rat glomeruli.*

Table 1. *Proportional Changes of Glomerular GD_3 and O-Acetyl GD_3 Gangliosides Normalized to the Amount of GM_3 in PAN*

Ganglioside	Control	3 days	10 days	30 days
GM_3	100	100	100	100
GD_3	22.0	17.7 (80.5)	17.9 (81.4)	18.1 (82.3)
O-Acetyl GD_3	20.8	12.6 (60.6)	8.5 (40.9)	8.3 (40.0)

Results are from densitometric scanning of the TLC plate of Figure 6 (see Materials and Methods). Numbers in parentheses indicate the percentage from the respective ganglioside in the control samples.

nected by slit membranes (see Ref. 24). These cells form the outermost barrier for plasma proteins filtering through the fenestrated endothelium and the glomerular basement membrane. Puromycin aminonucleoside apparently has a specific effect on podocytes in glomeruli; after PAN injection, the foot processes retract and the intercellular slit membranes are replaced by tight junctions, closely associated with the onset of proteinuria.[25] The target molecules and mechanisms of PAN have, however, remained unknown. Earlier studies suggested that a decrease in the glomerular anionic charge, mainly composed of heparan sulfate proteoglycans and sialic acids, leads to increased permeability.[26] In support of this, neutralization of the glomerular anionic barrier by injection of protamine sulfate[27] or partial removal of sialic acids by neuraminidase digestion[28] leads to proteinuria and to similar morphological retraction of the foot processes. However, later studies using electron microscopic detection of polyethylene imine,[29] a marker for the fixed anionic charges, have not been able to confirm the reduction in charge density in PAN. Thus, a more specific explanation for the proteinuria in PAN must be sought. The known effects of the aminonucleoside of puromycin at the cellular level include inhibition of RNA and protein synthesis[30] by interfering with the peptide chain elongation at ribosomes.[31] However, Kerjaschki et al[17] have shown that the major sialoprotein of glomerular podocytes, podocalyxin, remains quantitatively unchanged in the peak proteinuric phase of PAN but that the total sialylation of this protein is decreased to less than 30% of controls. This suggests a causal relationship between the degree of sialylation and proteinuria at least for glomerular sialoproteins. The present results are well in agreement with and extend those of Kerjaschki et al[17] by showing that the sialylation of glomerular glycosphingolipids is also disturbed in PAN. The reason for this is not known, but it is possible that the activity in the synthesis of sialyl transferases are specifically disturbed by puromycin.

As the present results suggest that puromycin disturbs the metabolism of GD_3 and O-acetyl GD_3, we tried to estimate the amount of GD_3 synthase mRNA by Northern blotting, using probes for human GD_3 synthase obtained from Dr. Nishi (Kyowa Hakko Kogyo, Tokyo, Japan). However, the sensitivity of the technique using the probe specific for human GD_3 synthase was not enough as we could not demonstrate any GD_3 synthase mRNA in the samples of normal or PAN glomeruli. Because the rat GD_3 synthase has not been cloned, polymerase-chain-reaction-based techniques cannot be currently used to verify the changes in the mRNA levels of this key enzyme of ganglioside synthesis in PAN.

The main finding in this study was the dose- and time-dependent decrease of the O-acetyl derivative of GD_3 ganglioside in PAN. O-Acetylation is considered important in stabilizing the ganglioside structure in cell membranes by influencing the conformation and arrangement of glycoconjugates (see Ref. 32). O-Acetylation of sialic acids may also alter the ligand specificity of the sialic acids and sensitize cells to the alternate pathway of complement,[33] a well known mechanism associated with various types of glomerular diseases with proteinuria (see

Refs. 34 and 35). Complement activation products are not deposited in the kidneys, however, in PAN nephrosis. The precise relationship of O-acetylation of GD_3 and activation of complement in glomerular diseases with proteinuria remains to be studied in detail.

In summary, we demonstrate here that PAN of the rat is characterized by dose- and time-dependent changes in the terminal sialylation and O-acetylation of glomerular gangliosides of the disialoganglioside but apparently not of the monosialoganglioside series. In addition, these ganglioside changes seem to precede the onset of proteinuria. As no similar changes are seen in the Heymann nephritis model sharing proteinuria, we suggest that these findings represent the molecular effects of the aminonucleoside of puromycin in the glomerular podocytes.

Acknowledgments

The skillful technical assistance of Ms. Riitta Väisänen is gratefully acknowledged.

References

1. Hakomori S: Bifunctional role of glycolipids: modulators for transmembrane signaling and mediators for cellular interactions. J Biol Chem 1990, 265:18713–18716
2. Schwartzmann G, Sandhoff K: Metabolism and intracellular transport of glycosphingolipids. Biochemistry 1990, 29:10865–10871
3. van Echten G, Sandhoff K: Ganglioside metabolism: enzymology, topology, and regulation. J Biol Chem 1993, 268:5341–5344
4. Bremer EG, Hakomori S, Bowen-Pope DF, Ross R: Ganglioside mediated modulation of cell growth, growth factor binding, and receptor phosphorylation. J Biol Chem 1984, 259:6818–6825
5. Cheresh DA, Pytela R, Piersbacher MD, Klier FG, Ruoslahti E, Reisfield RA: An arg-gly-asp-directed receptor on the surface of human melanoma cells exists in a divalent cation-dependent functional complex with the disialoganglioside GD2. J Cell Biol 1987, 105:1163–1173
6. Hanai N, Nores GA, MacLeod C, Torres-Mendez RC, Hakomori S: Ganglioside-mediated modulation of cell growth. J Biol Chem 1984, 263:10915–10921
7. Kreutter D, Kim JY, Goldenring JR, Rasmussen H, Ukomadu C, Delorenzo RC, Yu RK: Regulation of protein kinase C activity by gangliosides. J Biol Chem 1987, 262:1633–1637
8. Nishizuka Y: The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 1984, 309:693–698
9. Hannun YA, Bell RM: Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. Science 1989, 243:500–507
10. Wu G, Ledeen RW: Gangliosides as modulators of neuronal calcium. Progr Brain Res 1994, 101:101–112
11. Svennerholm L, Boström K, Fredman P, Månsson J-E, Rosengren B, Rynmark BM: Human brain gangliosides: developmental changes from early fetal stage to advanced age. Biochim Biophys Acta 1989, 1005:109–117
12. Shayman JA, Radin NS: Structure and function of renal glycosphingolipids. Am J Physiol 1991, 260:F291–F302
13. Miettinen A, Dekan G, Farquhar MG: Monoclonal antibodies against membrane proteins of the rat glomerulus: immunochemical specificity and immunofluorescence distribution of the antigens. Am J Pathol 1990, 137:929–944
14. Reivinen J, Holthöfer H, Miettinen A: A cell-type specific ganglioside of glomerular podocytes in rat kidney: an O-acetylated GD3. Kidney Int 1992, 42:624–631
15. Sariola H, Aufderheide E, Bernhard H, Henke-Fahle S, Dippold W, Ekblom P: Antibodies to cell surface ganglioside GD3 perturb inductive epithelial-mesenchymal interactions. Cell 1988, 54:235–245
16. Mohos SC, Skoza L: Glomerular sialoprotein. Science 1969, 164:1519–1521
17. Kerjaschki D, Vernillo AT, Farquhar MG: Reduced sialylation of podocalyxin, the major sialoprotein of the rat kidney glomerulus, in aminonucleoside nephrosis. Am J Pathol 1985, 118:343–349
18. Kerjaschki D, Sharkey DJ, Farquhar MG: Identification and characterization of podocalyxin, the major sialoprotein of glomerular epithelial cells. J Cell Biol 1984, 98:1591–1596
19. Michael AF, Blau E, Vernier RL: Glomerular polyanion: alteration in aminonucleoside nephrosis. Lab Invest 1970, 23:649–657
20. Miettinen A, Westerlund B, Tarkkanen A-M, Törnroth T, Ljungberg P, Renkonen O-V, Korhonen TK: Binding of bacterial adhesins to rat glomerular mesangium *in vivo*. Kidney Int 1993, 43:592–600
21. Miettinen A, Törnroth T, Tikkanen I, Virtanen I, Linder E: Heymann nephritis induced by kidney brush border glycoproteins. Lab Invest 1980, 43:547–555
22. Holthöfer H, Reivinen J, Miettinen A: Nephron segment and cell-type specific expression of gangliosides in the developing and adult kidney. Kidney Int 1994, 45:123–130
23. Folch J, Lee M, Sloane-Stanley GH: A simple method for the isolation of total lipids from animal tissues. J Chromatogr 1957, 224:249–256
24. Tisher CC, Madsen KM: Anatomy of the kidney. The Kidney, Vol. 1, 4th ed. Edited by BM Brenner, FC Rector. Philadelphia, WB Saunders Co, 1991, pp 3–75
25. Ryan GB, Rodewald R, Karnovsky MJ: An ultrastructural study of the glomerular diaphragm in aminonucleoside nephrosis. Lab Invest 1975, 33:461–468

26. Andrews PM: Glomerular epithelial alterations resulting from sialic acid surface coat removal. Kidney Int 1979, 15:376–385
27. Seiler MW, Rennke HG, Venkatachalam MA, Cotran RS: Pathogenesis of polycation induced alterations (fusion) of glomerular epithelium. Lab Invest 1977, 36: 48–61
28. Kanwar YS, Farquhar MG: Detachment of endothelium and epithelium from the glomerular basement membrane produced by kidney perfusion with neuraminidase. Lab Invest 1980, 42:375–384
29. Groggel GC, Hovingh P, Border WA, Linker A: Changes in glomerular heparan sulfate in puromycin aminonucleoside nephrosis. Am J Pathol 1987, 128: 521–527
30. Gorski J, Aizawa Y, Mueller GF: Effect of puromycin *in vivo* on the synthesis of protein, RNA, and phospholipids in rat tissues. Arch Biochem Biophys 1961, 95:508–511
31. Hazelkorn R, Rothman-Denes B: Protein synthesis. Annu Rev Biochem 1973, 42:397–438
32. Schauer R: Sialic acids: metabolism of *O*-acetyl groups. Methods Enzymol 1987, 138:611–627
33. Varki A, Kornfeld S: An autosomal dominant gene regulates the extent of 9–O-acetylation of murine erythrocyte sialic acids. J Exp Med 1980, 152:532–544
34. Couser WG, Baker PJ, Adler S: Complement and the direct mediation of immune glomerular injury: a new perspective. Kidney Int 1985, 28:879–890
35. Morgan BP: Complement and renal disease. Complement: Clinical Aspects and Relevance to Disease. Edited by BP Morgan. London, Academic Press, 1990, pp 112–129