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Implication of glycolipids in lens fiber development*

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Mammalian lens contains Lewis^x, sialyl-Lewis^x and α-galactosyl epitopes in neolacto-series glycosphingolipids. The expression of these three epitopes is not observed in lens epithelial cells, but is immunohistochemically detected in the inner cortical fibers and the lens nucleus. In embryonic chick lens, sialyl-Lewis^x-containing gangliosides were also detected in the transitional zone and elongating lens fibers. Thus, the Lewis^x, sialyl-Lewis^x and α-galactosyl epitopes may be associated with the differentiation and maturation of lens epithelial cells to lens fibers.

Lens tissues in vertebrates are composed of multiple layers of fiber cells and a monolayer of epithelial cells. A cuboidal epithelial cell elongates to more than 100 times its original length to become a fiber cell (Bloemendal, 1977). Senile cataract is responsible for significant visual impairment in aged people worldwide. It is likely that oxidative stress implicated in cataract formation, and several risk factors are generally accepted as leading to the loss of lens transparency: age, corticosteroid use, ionizing radiation and diabetes (Chylack, 1984).

Our previous studies on lens glycosphingolipids (GSLs) revealed that humans and monkeys had Lewis^x (Le^x) and sialyl-Le^x epitopes on neolacto-series GSLs (Ogiso *et al.*, 1992; 1993; 1994a; 1995). Recently, using immunolocalization techniques, we revealed restricted expression of Le^x and sialyl-Le^x epitopes in the inner cortical and nuclear fibers of monkey lens, suggesting that the expression of neolacto-series GSLs is associated with the terminal differentiation and maturation of lens fibers (Ogiso *et al.*, 1998a).

Thus, differentiation of lens fiber cells may be accompanied by changes in cell-cell interaction, which is mediated through cell surface neolacto-series GSLs.

MATERIALS AND METHODS

Immunohistochemical study of GSLs in embryonic chick lenses. The localization of lens GSLs was immunohistochemically exam-

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; GSLs, glycosphingolipids; Le^x, Lewis^x.

ined using frozen sections as decribed elsewhere (Ogiso *et al.*, 1998a). Lenses from 15-, 17-, and 19-day-old embryos of White Leghorn chicken were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C overnight, and washed several times in cold phosphate-buffered saline. Cryosections of 6 μm were blocked with 10% rabbit serum and incubated with monoclonal anti-ganglioside antibodies to G_{M3} (M2590, 1:50, IgM, Meiji, Tokyo, Japan), G_{D3} (R_{24}, 1:20, IgM, Wako Pure Chemicals, Osaka, Japan) and sialyl-Le^x (CSLEX-1, 1:100, IgM, UCLA Tissue Typing Laboratory, LA, U.S.A.) at 4°C overnight. The immunoreaction was detected using the Nichirei alkaline phosphatase-conjugated SAB kit (Nichirei, Tokyo, Japan) and Vector substrate kit IV (Vector Lab., CA, U.S.A.). Experiments with control ascites fluid showed no immunoreaction.

Cultures of monkey lens epithelial cells. Non-cataractous lenses from 2- to 3-year-old rhesus monkeys (*Macaca mulatta*) were supplied under a co-operative program of the Primate Research Institute, Kyoto University, Inuyama, Japan. The anterior capsule with lens epithelial cells was peeled off from the lens cortex, and cut into pieces. The pieces were placed in 60-mm diameter collagen-coated culture dishes (25010-COL1, Corning, NY, U.S.A.) in Dulbecco's modified Eagle's medium (DMEM) (Gibco Lab., Grand Island, NY, U.S.A.) supplemented with 15% fetal calf serum (Boehringer Mannheim, Mannheim, Germany) in a humidified atmosphere of 95% air/5% CO_2, as described previously (Ogiso *et al.*, 1994b). The epithelial cells were dissociated with trypsin-EDTA after they had reached confluence, and the cells from third-generation subcultures were grown on 60-mm diameter dishes (3002, Falcon, Becton and Dickinson, CA, U.S.A.), which were coated with collagen type I or type IV (Cellmatrix-IP and -IV, Iwaki Glass, Tokyo, Japan), fibronectin from human plasma (Iwaki Glass), laminin from mouse EHS sarcoma (Iwaki Glass), vitronectin from human plasma (Iwaki Glass), or poly-L-lysine (Sigma, St. Louis, MO, U.S.A.). The cultures were maintained for 4 weeks with medium replacement every 3 days, and observed by Nikon TMS phase contrast microscopy.

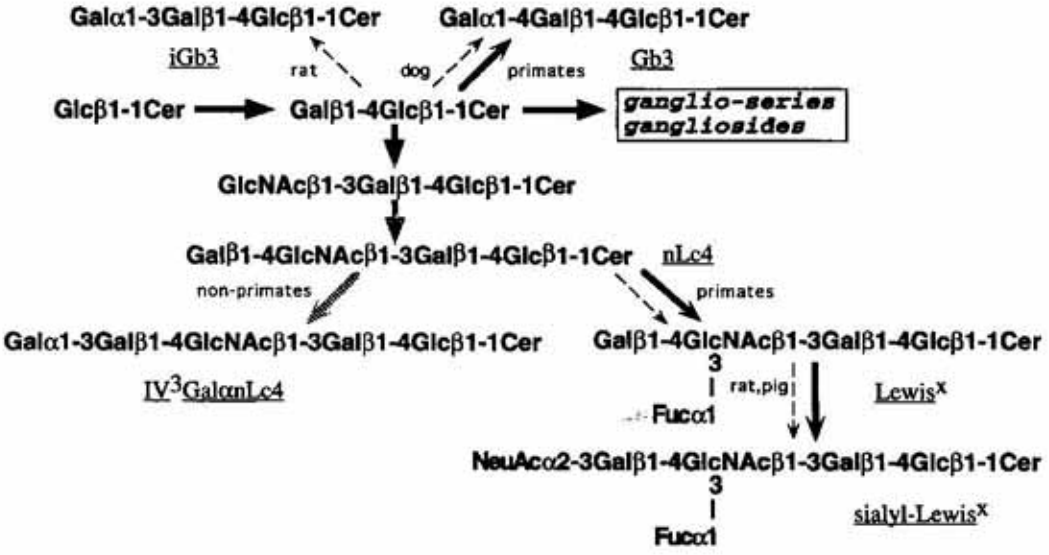

Figure 1. Composition of neutral GSLs in several mammalian lenses.

Sugar chain structures of neutral GSLs were identified in the mouse, rat, dog, pig, cow, rhesus monkey (*Macaca mulatta*), Japanese monkey (*M. fuscata*) and human lenses. The synthetic pathway of sialyl-Le^x gangliosides remains unclear because of the absence of sialyl-paragloboside (IV^3NeuAcnLc4). Neutral GSLs are abbreviated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (1977), but the suffix OseCer is omitted. Cer, ceramide.

RESULTS

Comparison of GSL compositions among several mammalian lenses

Mammalian lenses contain several GSLs with various sugar chain backbones, including ganglio-, neolacto-, globo-, and isoglobo-series sugar chains (Fig. 1). The α-galactosyl (Galα1-3Gal-R), Le^x(Galβ1-4(Fucα1-3)GlcNAc-R) and sialyl-Le^x(NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc-R) epitopes are formed on the non-reducing terminal of neolactotetraosylceramide Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (nLc4) (Ogiso *et al.*, 1992; 1993; 1994a). The α-galactosyl epitope is found in neutral GSLs from lens tissues of non-primate mammals (broken arrow), but not in those of humans and Old World monkeys. Instead, humans and Old World monkeys express Le^x epitopes in neutral GSLs (solid arrows). Sialyl-Le^xepitopes are found in rat and pig lenses as well as in human and Old World monkey lenses (thin, dashed arrows).

Ganglio-series gangliosides are principally composed of G_{M3} and G_{D3}, showing some species-specific differences (Fig. 2). Although mouse and rat lenses express several complex gangliosides, G_{M1}, G_{D1a} and G_{D1b}, primate lenses do not express complex b-series gangliosides (dashed arrows).

Composition and immunohistochemical localization of gangliosides in embryonic chick lens

In embryonic chick lens, G_{M3}, G_{D3} and two slow-moving gangliosides below G_{D1a} were observed in the ganglioside fraction. TLC-immunostaining using several anti-ganglioside antibodies immunologically identified the presence of G_{M3}, G_{D3} and sialyl-Le^x gangliosides from 15- through 19-day-old lenses (M. Ogiso *et al.*, unpublished).

Immunostaining for G_{M3} and G_{D3} revealed that their distribution profiles in embryonic chick lens were similar (Fig. 3). More intense staining in reaction to the antibodies was seen in the epithelia and transition zone. On the other hand, the immunoreaction to sialyl-Le^x gangliosides was more intense in the transition zone than in lens epithelia, which appears

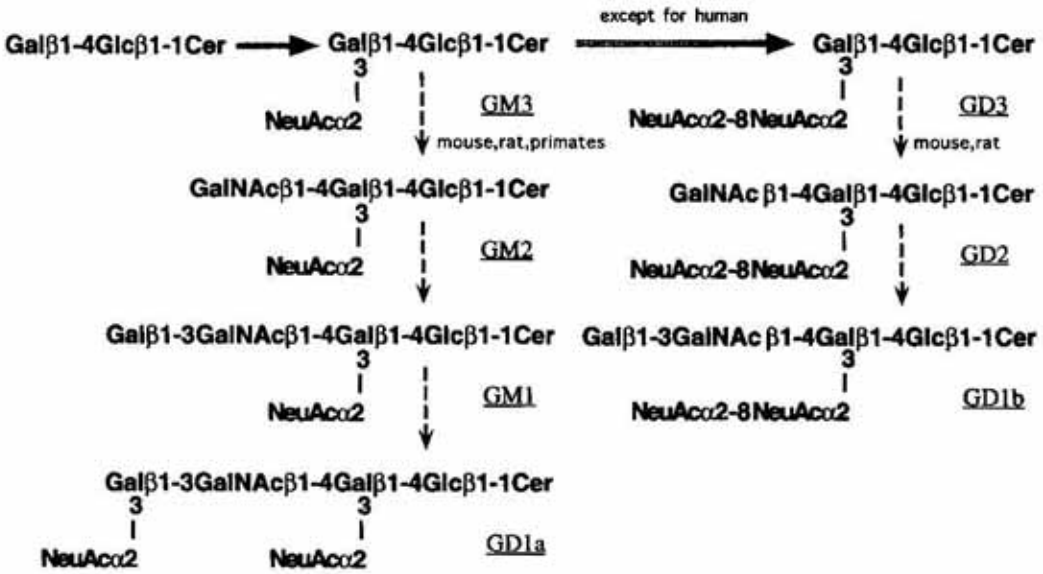

Figure 2. Composition of ganglio-series gangliosides in several mammalian lenses.

Sugar chain structures of ganglio-series gangliosides were identified from several mammalian lenses. Ganglio-series gangliosides are abbreviated according to Svennerholm (1964).

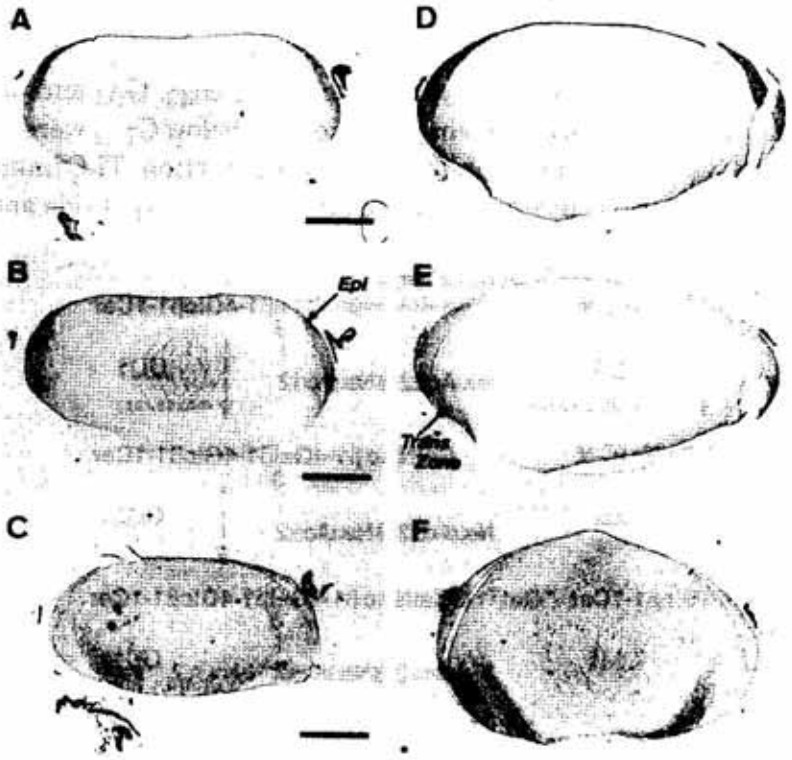

Figure 3. Immunohistochemical localization of G_{M3}, G_{D3} and sialyl-Le^x gangliosides in chick lens.

Cryosections of lenses from 15- (A–C) to 19-day-old (D–F) chicks were immunostained with anti-ganglioside antibodies. A,D, G_{M3}; B,E, G_{D3}; C,F, sialyl-Le^x epitope. Bars represent 0.5 mm.

to be related with the differentiation of lens fibers.

Cultures of lens epithelial cells from rhesus monkey

Monkey and human lenses contain essentially the same glycosphingolipids (Ogiso *et al.*, 1994a). In the monolayer culture of monkey lens epithelial cells, however, no Le^x or sialyl-Le^x epitopes were detected (Ogiso *et al.*, 1994b).

Prolonged cultures of lens cells showed morphological alterations, depending on cell-to-substratum adhesion (Fig. 4). On collagens (Col I and Col IV), fibronectin (FN) and laminin (LN), a filamentous sheath was observed in cells cultured for 4 weeks. The monolayer of cells cultured on vitronectin (VN) or polylysine (PL) assembled into aggregates after 4 weeks of culture. Cells cultured on vitronectin expressed a small amount of sialyl-Le^x gangliosides (Ogiso *et al.*, 1998b).

DISCUSSION

Vertebrate lens tissue is composed of a monolayer of anterior epithelial cells and multiple layers of fiber cells, which are derived from the epithelial cells and migrate to the lens nucleus throughout life (Bloemendal, 1977). It is believed that lens transparency is due to the characteristic architecture of lens tissue. A recent immunohistochemical study suggested that some neolacto-series GSLs

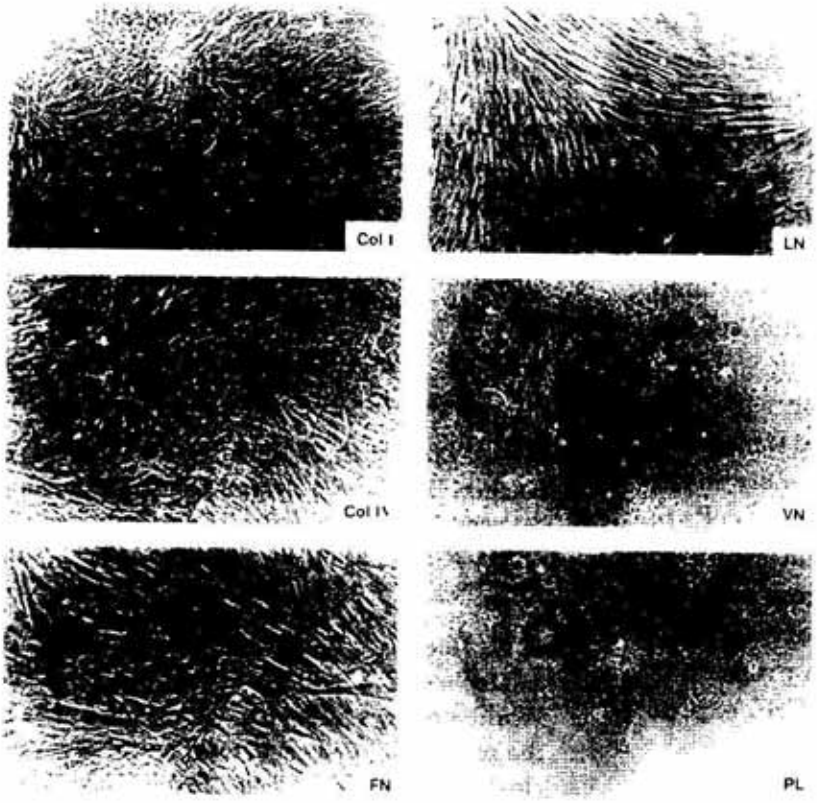

Figure 4. Phase contrast photographs of monkey lens epithelial cells at 4 weeks of culture on various cell substrata.

Monolayer cultures of epithelial cells were maintained in DMEM containing 15% fetal calf serum for 4 weeks. Col I, collagen type I; Col IV, collagen type IV; FN, fibronectin; LN, laminin; VN, vitronectin; PL, polylysine. Magnification 25×.

were involved in lens fiber development, in which the physiological roles of the α-galactosyl epitope were evolutionarily replaced by the Le^x and sialyl-Le^x epitopes in Old World monkeys and humans (Ogiso *et al.*, 1998a).

In birds, the epithelium and lens fibers are topographically separated by the transition zone (the annular pad), which consists of pseudostratified cells adjoining the epithelium, and of cells progressively elongating toward the fiber area (Maisel *et al.*, 1981). In chick lens, intense immunostaining of G_{M3} and G_{D3} was seen in the epithelium and transition zone. On the other hand, the distribution profile of sialyl-Le^x epitopes in the transition zone and elongating fibers suggested that they might contribute to the differentiation of lens epithelial cells to fiber cells.

Glycoconjugates carrying the α-galactosyl epitopes are known to be distributed in many types of cells in non-primate mammals and New World monkeys, but not in non-mammalian vertebrates, Old World monkeys, apes or humans (Galili *et al.*, 1987; 1988; Hendricks *et al.*, 1990). In addition, it is known that primates and birds have flat lenses able to accommodate by deformation of the lens and that accommodative activity in most mammals is lacking because the ciliary muscle, except in squirrels, is vestigial, if present. Thus our data on lens GSLs point to the possibility that evolution-related expression of α-galactosyl epitope serves as a differentiation-

associated antigen of lens fibers in non-primate mammals and New World monkeys; this epitope differs from the Le^x and sialyl-Le^x epitopes found in birds, Old World monkeys and humans.

Since lens tissue is completely separated from all other types of cells by the basement membrane (Bloemendal, 1977), lens epithelial cells can be cultivated without contamination by other cell types. In lens epithelial cells of rhesus monkey, the interaction between cells and extracellular matrices influenced the morphology and GSL composition. Changes in GSL composition, especially the expression of sialyl-Le^x epitope, were partly associated with cell aggregation of lens epithelial cells *in vitro*.

These findings may partly confirm that the differentiation of epithelial cells to lens fibres requires at least three consecutive steps in the cortical region: migration of epithelial cells to lens fibres; expression of Le^x and sialyl-Le^x epitopes; and final maturation accompanying denucleation and the breakdown of cell organelles. Further experiments are in progress to clarify the relationship between GSL expression and the extracellular matrix.

In various types of cataracts, soluble low molecular mass cytoplasmic proteins are known to become converted to soluble high molecular mass aggregates, transferred to insoluble phases, which leads to the formation of insoluble membrane-protein matrices (Chylack, 1984). However, it is plausible that cell adhesion between lens fibres is mediated through the Le^x and sialyl-Le^x epitopes, not the α-galactosyl epitope, in birds, Old World monkeys, apes and humans. In addition, human lens accumulates the Le^x epitope-containing GSL in an age-dependent, cataract-related manner (Ogiso *et al.*, 1992). The mechanisms of senile cataract formation seem to have changed within the order of primates after the divergence of Old World monkeys and humans.

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