Transformation of retinal glia cells into lens phenotype: Expression of MP26, a lens plasma membrane antigen

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ABSTRACT We describe experiments in which dissociated cells from differentiated, post-mitotic neural retina of late chicken embryos (13 and 16 days) rapidly and consistently transform (trans-differentiate) in vitro into lens-like phenotype and form spherical lentoids. Using immunohistochemical and other tests, we have established that the lentoids arise from the progeny of definitive retinal glia cells (Müller cells). An early event in their transformation is the appearance in the cell surface of MP26, a plasma membrane protein characteristic of lens but not found in the retina. The results support the hypothesis that the phenotype of definitive glia cells in the retina is stabilized by contact-mediated interactions with neurons; disruption of cell contacts and cell separation alter surface properties of the glia cells, decontrol their phenotype, and predispose them to phenotype transformation.

Contact-dependent cell interactions play a crucial role not only in embryonic cell differentiation and morphogenesis but also in maintaining the definitive phenotypic characteristics of differentiated cells (1). It has been suggested that disruption of normal cell contacts can lead to phenotype "destabilization" and can predispose cells to transformation (2). A particularly suitable experimental system for studying this important but little understood problem is the conversion of differentiated neural retina cells from late chicken embryos (13 and 16 days) into lens type cells (3). It is brought about by separating the cells and maintaining them for a few days in monolayer cultures.

The neural retina of chicken embryos contains several classes of neurons but only one kind of glia referred to as Müller cells. By the 13th day of embryonic development the retina has completed its overall histological organization and growth; both the neurons and the glia are post-mitotic and are phenotypically determined (4). However, if retina tissue from 13- or 16-day embryos is dissociated and the cells are plated monodispersed in monolayer cultures, the glia cells begin again to divide (5) and lose some of their characteristic traits (6). If, after 5–7 days, the cultured cells are aggregated by rotation, they assemble into numerous spherical clusters, each containing a compact core of lens-like cells (3). This core has been referred to as a "lentoid" because the cells accumulate cytoplasmic proteins characteristic of normal lens (7–10).

Earlier studies suggested that the lens-like cells arose from modified retinal glia cells and that their transformation was associated with changes in cell surface properties (3). The present work confirms these suggestions by identifying the progenitors of the lentoidal cells and by demonstrating that a lens-specific plasma membrane protein, MP26, becomes newly expressed on the surface of the transforming cells.

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FIG. 1. (A) NaDodSO₄ gel electrophoresis of MP26 purified from the lens of adult chicken (lane a), showing a single band in the region of M, 26,000. Lane b, molecular weight marker proteins (Bio-Rad). The gel was stained with Coomassie blue. (B) Double-immunodiffusion of anti-MP26 antiserum (center well) against lens cell membrane preparation (wells 1 and 2), purified MP26 (wells 3 and 4), and extract of neural retina tissue from 13-day chicken embryos (wells 5 and 6). The gel was stained with Coomassie blue. Monospecificity of the antiserum is indicated by a single precipitin line opposite wells 1–4. Absence of MP26 in retina tissue is indicated by the absence of a precipitation line opposite wells 5 and 6.

MATERIALS AND METHODS

Purification of MP26 from Chicken Lens. The plasma membrane of dissociated lens cells (lens fibers) is rich in an integral protein designated as MP26 because it has a M₉ of 26,000 (11, 12). This protein is known to be found only in the lens and has been studied most extensively in mammals (11, 13). We have isolated MP26 from the lens of adult chicken using the procedure of Kibbelaar and Bloemendal (14) for isolation of calf lens MP26. Final purification was by electrophoresis in 13% polyacrylamide/0.1% NaDodSO₄ slab gels. The gel region containing the M₉, 26,000 material was cut out and eluted with 20 mM NaPO₄ buffer/0.2% NaDodSO₄. The MP26-containing supernatant was Millipore-filtered, extensively dialyzed, lyophilized, and stored at -20°C.

Homogeneity of MP26 Preparation. Electrophoresis of the purified MP26 in polyacrylamide/NaDodSO₄ gel resulted in a single protein band in the M₉, 26,000 position (Fig. 1A).

Abbreviations: R¹³ and R¹⁶, neural retinas of 13- and 16-day chicken embryos, respectively, R¹³+7+2, cells dissociated from R¹³, cultured in monolayer for 7 days, and then aggregated for 2 days.

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Antiserum to MP26. BALB/c mice were injected intramuscularly at weekly intervals with the purified MP26 emulsified in Freund’s complete adjuvant. Serum was first collected 5 days after the third injection. Antibodies to MP26 were detected by enzyme-linked immunosorbent assay (ELISA) and by immunoreplica tests (15) with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin.

Immunoelectrophoresis of the MP26 antiserum against a lens cell membrane fraction and against purified MP26 resulted in a single precipitation line. In Ouchterlony immunodiffusion tests (Fig. 1B), the antiserum formed a single band against these two preparations and showed no reaction with homogenates of neural retina or other tissues, consistent with the lens specificity of MP26. Histological sections of chicken lens immunostained with the MP26 antiserum showed fluorescence in the plasma membrane of lens fibers, consistent with the previously reported localization of MP26 (12). Thus, by all of these criteria the antiserum was specific for MP26 and suitable for detection of this antigen.

Other Antiserum. Purification of carbonic anhydrase C and preparation of the antiserum (carbonic anhydrase C antiserum) have been described (16). Antiserum to lens cytoplasmic proteins (adult chicken lens) was raised in mice.

Culture Methods. Neural retina from 13-day chicken embryos (R13) was isolated aseptically and was dissociated by mild trypsinization into single cells. Procedures used for cell dissociation, preparation of monolayer cultures, and cell aggregation were as described (3). To obtain lentoids, R13 cells were maintained in monolayer culture for 5–7 days; the monolayers were then dispersed and the cells were aggregated by rotation for 48 hr in 25-ml Erlenmeyer flasks (5 x 10⁶ cells per 3 ml of culture medium) on a gyratory shaker (70 rpm) according to the standard procedure (3). The same protocol was used to obtain lentoids from cells of 16-day retina (R16).

Immunostaining with MP26 Antiserum. Samples were fixed in Carnoy’s fixative, embedded in paraffin, and sectioned at 5 μm. Rehydrated sections were exposed to MP26 antiserum (diluted 1:10) for 30 min at 37°C, washed in Tyrode’s solution, and

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**FIG. 2.** (a) Monolayer culture (7 days) of dissociated R13 cells, showing flattened gliocytes and clusters of neuronal cells. (×160.) (b) Forty-eight-hour aggregates obtained from R13 cells cultured in monolayer and then dispersed and aggregated by rotation. (c) Histological section of cell aggregates (R13-17), each showing a lentoid (★) and an outer zone (arrow; neuronal cells). (Hematoxylin/eosin stain.) (d) Section of cell aggregates (similar to e) immunostained with antiserum to adult lens proteins; lentoids (core cells) show immunofluorescence. (e) Section of cell aggregates (similar to c) immunostained with anticarbonic anhydrase C antiserum; immunofluorescence of the lentoids demonstrates their derivation from glial cells. (Bars = 0.5 mm.)
RESULTS

R^{13} tissue was dissociated into single cells and these were maintained in monolayer culture. After 7 days (R^{13+7}) the cultures contained, in addition to small clusters of nondividing neuronal cells, an abundant population of flattened epithelioid gliocytes (Fig. 2a) derived largely by multiplication from the original R^{13} Müller glia cells (5). The cultures were then dispersed and the suspended cells were aggregated by rotation in flasks for 2 days (R^{13+7+2}), as in previous work (3). In each flask, numerous spherical aggregates were formed, averaging 0.8 mm in diameter (Fig. 2b). Histological sections showed that virtually each aggregate contained an inner compact core of oblong cells with lentoidal morphology (Fig. 2c) and an outer zone of smaller, round, loosely adhering cells predominantly of neuronal origin (3).

The lentoidal identity of the core cells was confirmed by immunostaining the sections with antisera to cytoplasmic proteins (crystallins) of adult lens (Fig. 2d). As previously reported (3), the core cells reacted with the antisera, but the outer zone cells did not.

Glial Origin of the Lentoids. The possibility that the lentoids were formed by the epithelioid gliocytes——i.e., by modified progeny of Müller glia cells (3, 17)——was subjected to several tests. As previously described (16), Müller glia cells in post-mitotic differentiated chicken retina are characterized by the presence of carbonic anhydrase C. Because this enzyme continues to be expressed in gliocytes in monolayer cultures of R^{13} cells (18), it provided a marker for tracing the origin of the lentoids. Using carbonic anhydrase C antisera, we examined immunohistochemically lentoid-containing cell aggregates and found that the enzyme was localized in the lentoidal core cells (Fig. 2e)——i.e., in the same cells that also immunostained for lens crystallins proteins (Fig. 2d). The gliocytic origin of the core cells was further confirmed by a time-course study of cell aggregation: the carbonic anhydrase C-containing cells gradually segregated from the neurons and converged in the center of each aggregate to form the lentoidal core. Because carbonic anhydrase C is found also in normal lens (16), its retention by the modified gliocytes is consistent with a lentoidal phenotype.

Next, we determined that destruction of Müller cells in retina tissue depleted it of cells capable of forming lentoids. α-Amino acid is selectively cytotoxic to Müller glia cells in the retina of 13-day chicken embryos (19). Monolayer cell cultures were prepared from R^{13} tissue that had been pretreated in vitro for 2 days with this agent; the cultures consisted mostly of neurons and contained only sparse gliocytes. Cell aggregates obtained from such gliocyte-poor cultures showed only a few small lentoids. The number of residual gliocytes in these cultures directly correlated with the number and size of lentoids obtained from them.

On the other hand, depletion of neurons did not reduce the pool of lentoid-forming cells. This was demonstrated by using a ferric chelate of chinoform (5-chloro-7-iodo-8-quinolinol), which was found by Shinde and Egochi (20) to be selectively cytotoxic for neurons in long-term monolayer cultures of retina cells from early chicken embryos. We examined this agent in our experimental system and found that its addition (25 μM) to monolayer cultures of R^{13} cells on the 6th day resulted in rapid destruction of the neurons. When the remaining gliocytes were aggregated, they gave rise to numerous lentoids. Because of the absence of neurons, such lentoids were devoid of the outer zone; this further confirmed the neuronal identity of the outer zone cells and the gliocytic origin of the lentoidal cells (3, 17, 20).

Taken as a whole, the above results demonstrate that differentiated glia cells are the precursors of the lentoid-forming cells in this system. Accordingly, separation of glia cells from their histological contact relationships in R^{13} and their maintenance in monodispersed adherent cell cultures elicits changes resulting in their transformation into a lentoidal phenotype. Evidence described next shows that this transformation is reflected in corresponding modification of the cell surface.

Detection of MP26. Lentoid-containing aggregates, obtained as described above (R^{13+7+2}), were immunostained with MP26 antiserum. The lentoidal core cells consistently showed intense immunofluorescence of the surface membrane outlining their elongated shape (Fig. 3 a–c). Cells of the surrounding outer zone did not react with the antisera. Therefore, retinal

![Fig. 3](image_url) Sections of lentoids immunostained with anti-MP26 antiserum; (a–c) R^{13+7+2}. Immunofluorescence localized in the surface membrane of lentoidal cells. Outer zone cells do not show surface reaction with the antisera. Note the oblong shape of the lentoidal cells, resembling that of normal lens fibers, especially prominent in c. (d) Lentoids obtained from retina cells of 16-day embryo (R^{16+7+2}), showing immunostaining of cell membranes with anti-MP26 antiserum. (Bar = 0.5 mm.)
gliocytes transformed into lentoidal cells express MP26, a lens-specific membrane antigen. As previously reported (3), cells from 16-day retina also can give rise to lentoidal bodies under the same experimental conditions as R13 cells. Immunostaining of R16 cell aggregates (R16+7) with MP26 antiserum revealed membrane fluorescence in the lentoidal cells (Fig. 3d). Therefore, also the conversion of R16 gliocytes into lentoidal cells is marked by expression of this lens-specific membrane antigen.

Next, we investigated immunohistochemically if MP26 appeared only after the cells had aggregated into compact lentoids or earlier. MP26 could not be detected in retina tissue (Fig. 4a) (R15 or other ages), in freshly dissociated R13 cells, or in 1-day monolayer cultures of R13 cells. However, already in 3-day cultures of R13 cells, scattered gliocytes showed surface reaction with MP26 antiserum (Fig. 4b). The initial appearance of MP26 coincided with the presence of cells that immunostained for lens cytoplasmic proteins, but it is not yet clear if the cytoplasmic and the membrane lens antigens begin to be expressed simultaneously. After 5–7 days in monolayer culture, the majority of gliocytes showed membrane reaction with MP26 antiserum (Fig. 4c and d). Expression of MP26 in the gliocyte membrane was convincingly demonstrated in monolayer cultures in which neurons were destroyed by treatment with the chinoform compound (see above). Immunostaining with MP26 antiserum revealed the antigen in the surface membrane of virtually every remaining cell (Fig. 4 e–g).

Therefore, MP26 appears in the gliocyte surface membrane while the cells are still in monolayer culture. Accepting that expression of this lens-specific membrane molecule and of lens cytoplasmic proteins is diagnostic for gliocyte conversion into a lentoid phenotype, we conclude that this conversion is in progress already 2–3 days after separation of R13 cells and their plating in monolayer. Cell aggregation facilitates and expedites assembly of the converted cells into a lentoidal tissue configuration; however, some of the major changes indicative of this conversion occur while the cells are still dispersed. It is presently unknown whether continuous cell multiplication is obligatory for conversion of the separated R13 gliocytes into a lentoidal phenotype or whether persistent disruption of normal contact interactions with neurons would in itself elicit this conversion.

**DISCUSSION**

Lentoids were originally discovered in cultures of retina cells from very early chicken embryos (9, 21). In these cultures the starting cells were still undifferentiated, and a long period of
cultivation was required before lentoids began to appear. Because of this, it could not be determined which type of retinal cells gave rise to the lentoids. The more recent finding (3) that cells from differentiated retina of later embryonic ages can give rise to lentoids much faster, and with high frequency and consistency, made it now possible to establish the glial derivation of the lentoid-forming cells, as was previously suggested (3, 17).

The finding of MP26 expression in the membrane of the transformed gliocytes provides a new identity marker for their altered phenotype.

The fact that post-mitotic, definitive retinal glia cells ( Müller cells) are capable of conversion into a lens-like phenotype demonstrates that (i) their normal glialotypic differentiation does not irrevocably block a latent potential for expressing other programs and (ii) decontrol of mechanisms that stabilize the specialized traits of these glia cells can lead to phenotype modification. We have postulated that control of phenotype stability involves specific cell contacts (1, 3); in the present case, contact interactions of glia cells with neurons within retina tissue appear to have a key role in maintaining the phenotypic properties of definitive glia cells. Disruption of these cell contacts alters the surface properties of the glia cells, rapidly “switches off” some of their characteristic expressions such as inducibility of glutamine synthetase (1, 22), and triggers mitogenesis. Persistent separation predisposes these gliocytes to phenotype conversion. These considerations focus attention on changes in cell surface properties resulting from cell separation and on the possibility that such changes may reciprocally affect regulatory processes within cells (1).

Presently, two kinds of changes in surface properties of separated R13 gliocytes have been identified: (i) loss of their normal histological contact affinity for neurons, as evidenced by their segregation into internal cores in cell aggregates, and (ii) appearance of MP26, a lens cell membrane protein. With respect to cell affinities, it is known that histological cell associations in the retina are mediated by a retina-specific cell surface protein, R-cognin (23–25). Cell separation with trypsin degrades R-cognin; reassocation of the separated cells into a retinotopic tissue pattern requires functional restoration of R-cognin on the cell surface (23, 26). However, if dispersed gliocytes from differentiated retina are maintained in monolayer culture, they rapidly lose the capacity for R-cognin restoration or expression (18); thus, the absence of R-cognin could readily account for the loss of gliocyte affinity for neurons and acquisition of affinity for each other. As a hypothesis for future exploration, we suggest that such changes in the properties of the cell surface, initiated by disruption of normal cell contacts, are signaled into the regulatory centers of the gliocyte and trigger a cascade of processes that lead to phenotype modification.

The finding that MP26, a lens plasma membrane protein, becomes newly expressed in the surface of the modified gliocytes greatly expands this system’s usefulness for investigating mechanisms of phenotype stability and transformation. It also provides a promising experimental model for studying the still unresolved role of MP26 in the cell membrane (13, 27, 28). Because MP26 is not detectable in the neural retina, the possibility arises that gene(s) controlling the synthesis of this protein are “switched on” in retinal gliocytes due to changes elicited by disruption of contacts with neurons. Alternatively, normal glia cells may contain a conformational variant or a covert form of this protein that does not bind antibodies generated against MP26 from the lens. In any case, because MP26 is the most abundant and characteristic surface membrane protein in normal lens (27), its expression in modified retinal gliocytes validates the original identification of their phenotype as lentoidal (3, 9, 21). As to the role of MP26, it is unlikely that its expression on the modified gliocytes is directly responsible for the changes in their contact affinities because, according to recent studies on bovine lens (29), MP26 is not exposed on the outer surface of the cell membrane. Thus, the molecular basis for the affinity changes of the dispersed gliocytes as well as the specific function of MP26 remain to be elucidated.

Retina and lens have disparate origins in the embryo; therefore, it is puzzling why definitive retinal glia convert so readily into lens-like cells. Perhaps the experimental conditions presently used favor this particular redirection of the glial phenotype, and different conditions might result in other changes.

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