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Identification of a 185 kd *Maclura pomifera* Agglutinin Binding Glycoprotein as a Candidate for a Differentiation Marker for Alveolar Type II Cells in Adult Rat Lung

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In adult rat lung the lectin Maclura pomifera agglutinin (MPA) binds apically to alveolar type II (ATII) cells but not to alveolar type I (ATI) cells. This suggests that the presence of MPA binding glycoproteins might be a criterion by which to distinguish the differentiated state of these two adult alveolar epithelial cells. The authors therefore studied MPA binding glycoproteins of ATII cells, comparing, biochemically and cytochemically, MPA binding glycoproteins in freshly isolated ATII cells with those in cultures of ATII cells that are "dedifferentiating" or have "dedifferentiated" as a result of growth on tissue culture plasticware. A MPA binding glycoprotein (185 kd) that is present in freshly isolated "differentiated" ATII cells and then is subsequently lost as isolated ATII cells "dedifferentiate" in tissue culture has been identified. This 185 kd MPA binding glycoprotein alone, or expressed in conjunction with other proteins, is a candidate for a differentiation marker for ATII cells. Preliminary data suggests that this 185 kd MPA binding glycoprotein is not found in ATI cells. (Am J Pathol 1989, 134:277–285)

The pulmonary alveolus is lined by two kinds of differentiated epithelial cells, alveolar type I (ATI) and alveolar type II (ATII) cells. The squamous ATI cells are the site of gas exchange between the alveolar airspace and adjacent capillaries. The cuboidal ATII cells synthesize and secrete surfactant. In adult animals, ATII cells are also thought to be the progenitors for the cells that replace damaged ATI cells.[1–6] Such a repair process would require an ATII cell to lose its differentiated characteristics and acquire those of an ATI cell. Identification of biochemical markers for the differentiated state of both ATI and ATII cells would facilitate analysis of this transition because the disappearance and subsequent appearance of specific markers could be monitored. In adult rats, the lectin *Maclura pomifera* agglutinin (MPA) binds to ATII cells but not to ATI cells.[7,8] This suggests that the presence of MPA binding glycoproteins might be a criterion by which to distinguish the differentiated state of these two adult alveolar epithelial cells.

We have studied the suitability of using the presence of MPA binding glycoproteins in alveolar epithelial cells as a marker for differentiation. We used freshly isolated adult rat ATII cells as a source of potential differentiation markers and assumed that a differentiation specific marker would not be present in cultures of ATII cells that had been allowed to "dedifferentiate" in tissue culture. Our freshly isolated ATII cells retained the morphologic characteristics of differentiated ATII cells *in situ*.[9] ATII cells that have been cultured on tissue culture plasticware, however, lose these characteristics;[10,11] they are no longer cuboidal, and they lack apical microvilli and lamellar bodies. We compared "differentiated" freshly isolated ATII cells with "dedifferentiating" and "dedifferentiated" ATII cells that had been passaged on tissue culture plasticware for up to 25 months. Specifically, we identified MPA binding glycoproteins by lectin blot analysis of glycoproteins isolated from these cells and we observed MPA binding on the surface of these cells by electron microscopy. Both biochemically and cytochemically we found that the "undifferentiated" cells continue to express MPA binding glycoproteins for 25 months after loss of the ultrastructural characteristics associated with differentiated adult ATII cells. We conclude that scoring an alveolar epithelial cell plus or minus for MPA binding is inadequate for identifying its differentiated state. We found, however, a MPA binding glycopro-

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tein (185 kd) present in freshly isolated ATII cells that is lost in "dedifferentiating" ATII cells and suggest that this glycoprotein, alone or coordinately expressed with other proteins, is a candidate for a differentiation marker in ATII cells. In preliminary analysis, this prominent 185 kd MPA binding glycoprotein is not found in ATI cells.

Materials and Methods

Isolation of ATI and ATII Cells

ATI and ATII cells were isolated from adult rat lung as described previously.[8,9] ATII/ATI cell populations, mixtures of ATII and ATI cells, were obtained by following the isolation procedure for ATI cells and stopping before the final step, which would have removed ATII cells.[8]

Tissue Culture of ATII and ATII/ATI Cells

Freshly isolated ATII cells and ATII/ATI cells that were allowed to dedifferentiate by growth on tissue culture plasticware were cultured at 37 C in Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml Amphotericin B. Cells were plated at equal densities resulting in immediate confluency and observed for up to 10 days for mitotic figures. None were seen. These cells were harvested at days 2, 4, 6, 8, and 10, and processed as described below. Other freshly isolated cells used to establish long-term cultures were initially grown in a media mixture of 12.5% Dulbecco's, 12.5% Ham's F.12, 50% RPMI 1640 supplemented as described above except with 20% heat inactivated fetal calf serum, and the hormone supplements suggested by Taub and Sato[12] for epithelial cells. This was mixed 1:3 with the identical media conditioned by other ATII cells in culture. Freshly isolated cells were also grown on gelled Matrigel[13] (Collaborative Research, Lexington, MA) or on matrix secreted by "dedifferentiated" ATII cells.

Preparation of "Dedifferentiated" ATII Matrix

Cultures of "dedifferentiated" ATII cells were grown to confluency. The cells were then removed from their secreted substrates by washing with phosphate-buffered saline (PBS) and then with 1 mM EGTA/PBS, pH 7.4. For SDS-PAGE analysis, substrate was harvested by scraping with a rubber policeman and then solubilized by boiling in Laemmli sample buffer[14] and stored at −25 C.

Transmission Electron Microscopy

Isolated cells were fixed according to the procedure of Hirsch and Fedorko[15] and processed as described previously.[8,9] Cells grown in monolayer were either scraped from the substrate with a rubber policeman and processed as described for a pellet or embedded in monolayer and sectioned perpendicular to the substrate.

Binding of Maclura pomifera Agglutinin to Lung Tissue, Freshly Isolated Cells, and "Dedifferentiated" ATII Cells in Monolayer Culture

Minced adult rat lung tissue which had been perfused and lavaged, freshly isolated rat lung cells, and ATII cells grown in monolayer tissue culture were labelled at 4 C with MPA as described previously for isolated ATII cells.[8] Controls were as previously described.[8] In addition, some of the isolated rat lung cells and some of the ATII cells in monolayer culture were prefixed with 2% paraformaldehyde in PBS, pH 7.2, or with 2% paraformaldehyde, 0.19% glutaraldehyde in PBS, pH 7.2, before being labelled with MPA at 4 C as described.

Analysis of ATII Cells by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Freshly isolated ATII cells, and ATII cells grown on tissue culture plasticware and then harvested by scraping with a rubber policeman were solubilized and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[14] as follows. Cells were washed twice with PBS, pH 7.2, and then extracted in suspension at room temperature for 10 minutes in 3 and 2.5 times their volumes, respectively, of extraction buffer (0.1 M PIPES, 1 mM $MgSO_4$, 2 mM EGTA, 0.2% NP40 and 0.2 TIU/ml Aprotinin, pH 6.9). The unsolubilized cytoskeletons and nuclear components were removed from the samples by centrifugation at 1500*g* for 10 minutes at room temperature. The supernatant of "soluble" proteins was boiled in Laemmli sample buffer[14] and stored at −25 C. For analysis by SDS-PAGE, samples were separated on resolving gels with acrylamide content of 7.5%, 5–15% gradient or 5–7.5% gradient with stacking gels of 5, 3, or 3% respectively. Comparisons between samples were made on the basis of equal volumes of packed cells.

Preparation and Analysis of Control Samples for SDS-PAGE

Secondary rat lung fibroblasts were obtained by outgrowth from tissue explants of perfused, lavaged, minced lung tissue from rats identical to those used for ATII cell isolations. These cells were cultured and processed for SDS-PAGE as described above for "dedifferentiated" ATII cells. Baby hamster kidney (BHK-21) cells, an established line of cells, were cultured on tissue culture plastic, harvested, extracted and analyzed by SDS-PAGE using procedures identical to those for ATII cells grown in monolayer tissue culture on plastic. Rat lung alveolar macrophages were harvested by pulmonary lavage with PBS, pH 7.2, from rats identical to those used for ATII cell isolations. Macrophages were solubilized and analyzed using procedures identical to those used for freshly isolated ATII cells except that they were solubilized in 4 times their volume. The albumin content of fetal calf sera (Hyclone, Logan, UT), of normal goat sera (GIBCO, Grand Island, NY) and of rat sera obtained from rats identical to those used for ATII cell isolations, was significantly reduced using reactive blue-2-sepharose CL-6B (Sigma, St. Louis, MO) as recommended by Pharmacia Fine Chemicals, Piscataway, NJ.[16] These sera were then boiled in Laemmli sample buffer and stored at −25 C until analysis. Rat red blood cell (RBC) ghosts were prepared at 4 C from rats identical to those used above by washing RBC three times with 0.155 M NaCl, two times with ghost buffer (0.2 M NaH_2HPO_4, 0.2 M Na_2HPO_4, 0.0929 M NaCl and 0.00362 M KCl), and then lysing the RBC in 20 times volumes of a 1:20 dilution of ghost buffer. The resulting membranes were washed 4 times with 20 times volumes of 1:20 dilution of ghost buffer, boiled in Laemmli sample buffer and stored at −25 C.

Identification of Maclura pomifera Agglutinin Binding Glycoproteins

Proteins that had been separated by SDS-PAGE were passively transferred by diffusion[17] from the acrylamide gel to 0.2 μ nitrocellulose (Schleicher and Schuell) placed on each side of the gel thereby yielding two mirror image replicas of the distribution of the proteins within the gel. After incubating the nitrocellulose blots in 2% hemoglobin/PBS, pH 7.2, MPA binding glycoproteins were localized by exposing the nitrocellulose blots to peroxidase conjugated MPA (E. Y. Laboratories, San Mateo, CA; 80 μg/ml of 2% hemoglobin/PBS, pH 7.2) at 4 C for 10–12 hours in the dark. For control preparations, sugar hapten was included with the lectin as described for electron microscopy. The nitrocellulose blots were then washed with PBS and the bound peroxidase conjugate localized by incubating the nitrocellulose blot in 0.002% H_2O_2, 0.34 mM 4-chloro-1-naphthol (Bethesda Research Labs, Bethesda, MD) in 5:1 PBS:methanol until the desired color developed. Nitrocellulose blots which were used to show protein distribution were stained with India Ink (Pelikan Drawing Ink Z, 50 Black), according to the procedure of Hancock and Tsang.[18] Comparisons between samples were made between samples separated on the same gel and transferred simultaneously to one piece of nitrocellulose.

Results

Maclura pomifera Agglutinin Binds to ATII but not to ATI Cells

Rat lung tissue was perfused, lavaged, minced, cooled to 4 C and, then, exposed to peroxidase-conjugated MPA. As seen in Figure 1A, ATII cells in adult rat lung bound MPA at their apical surfaces whereas ATI cells did not. The specificity of the reaction was demonstrated by the absence of reaction product in the appropriate controls, such as mixing MPA with 1 mM 1-0-alpha-methyl-D-galactopyranoside, a specific sugar hapten for MPA.

Freshly isolated adult rat lung cells, kept at 4 C, were exposed to peroxidase-conjugated MPA. MPA bound to the surface of isolated ATII cells (Figure 1B) but not to isolated ATI cells. Where the polarity of the ATII cells could be inferred from the position of microvilli, it was apparent that the MPA was limited to the apical surface. The same results were seen with cells prefixed with 2% paraformaldehyde or with 2% paraformaldehyde, 0.19% glutaraldehyde. As seen with lung tissue, the lack of reaction product in the appropriate controls, such as inclusion of specific sugar hapten with the lectin (Figure 1C), demonstrated the specificity of the reaction.

Isolated ATII and ATII/ATI Cells Retain the Morphologic Characteristics of Differentiated ATII and ATI Cells in Short-Term Tissue Culture

When freshly isolated ATII cells were cultured on Matrigel, a basement membrane matrix,[13] the ATII cells continued to maintain their polarity as the majority of them reassociated and organized to form polarized alveolarlike structures (see Figure 2). The cells in these clusters had microvilli facing the lumen of the "alveolus" and adjacent cells were joined at their apical ends by junctional complexes. They retained their lamellar bodies. When freshly isolated ATII/ATI cells were cultured on extracellular matrix secreted by "dedifferentiated" ATII cells, some of the ATII

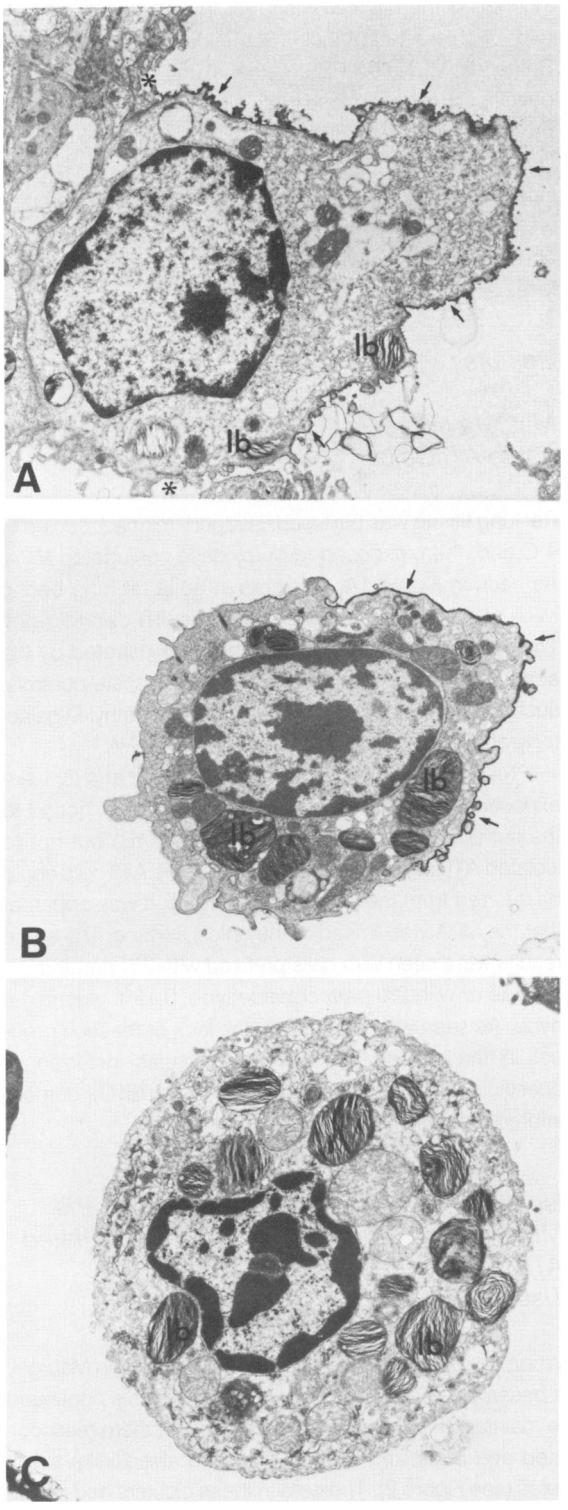

Figure 1. *Binding of Maclura pomifera agglutinin to ATII cells. Peroxidase-conjugated MPA bound to the apical surface of ATII cells (arrows) in situ in adult rat lung but not to ATI cells (asterisks)* (**A**, *×8000*). *Freshly isolated ATII cells, labelled in the same manner as lung tissue, retained the ability to bind peroxidase conjugated MPA in a specific and polar manner* (**B**, *arrows, ×6710*). *The specificity of the MPA binding was demonstrated by the absence of reaction product when the sugar hapten, 1-0-alpha-methyl-D-galactopyranoside was included with the lectin* (**C**, *×7320*). *lb, lamellar bodies.*

cells reassociated with each other to form alveolarlike structures as described above. Other ATII cells were associated with ATI cells in a manner similar to that seen *in situ* (see Figure 3). Both ATI and ATII cells reestablished polarity with cytoplasmic extensions from ATI cells extending partway up the side of adjacent ATII cells and forming junctional complexes with the ATII cells. Analysis of freshly isolated ATII/ATI cell preparations by transmission electron microscopy revealed that the freshly isolated cells were a single cell suspension rather than groups of alveolar epithelial cells. This observation supports the conclusion that the ATII and ATI cells reassociated *in vitro*. Observation of freshly plated cultures by phase contrast light microscopy also supports this conclusion. It can be concluded, then, that the isolation procedure did not damage the morphologic characteristics associated with the "differentiated" ATII cell. Furthermore, the ATII and ATI cells retained the ability to reassociate *in vitro* so that their *in situ* morphologic characteristics, including their polarity, were retained.

When freshly isolated ATII cells were maintained in culture on tissue culture plasticware for extended periods of time, however, even though the cells retained their epithelial character with desmosomes and junctional complexes (Figure 4A), the ATII cells lost their lamellar bodies, apical microvilli, and cuboidal shape (see Figure 4B, 4C). They were morphologically dedifferentiated. Analysis of our freshly isolated ATII cell preparations by electron microscopy[11] showed that contamination of our ATII cell preparation was consistently limited to a maximum of 1% ATI cells, 1% lymphocytes, 1% macrophages, and a possible 3% unidentified cells. We are confident that our cell cultures that were considered to be "dedifferentiated" ATII cells were derivatives of ATII cells rather than other epithelial cells because we did not see any localized clonal proliferation within our cultures that could represent proliferation of 3% of the cells. In addition, when samples of "dedifferentiating" cells were obtained by plating and maintaining cells at confluency, and then harvesting them at different time points up until 10 days after plating, we observed no mitotic figures during those 10 days.

MPA Binding to "Dedifferentiated" ATII Cells Cultured on Tissue Culture Plasticware

When ATII cell "derivatives," which had been grown on tissue culture plasticware, were exposed to peroxidase-

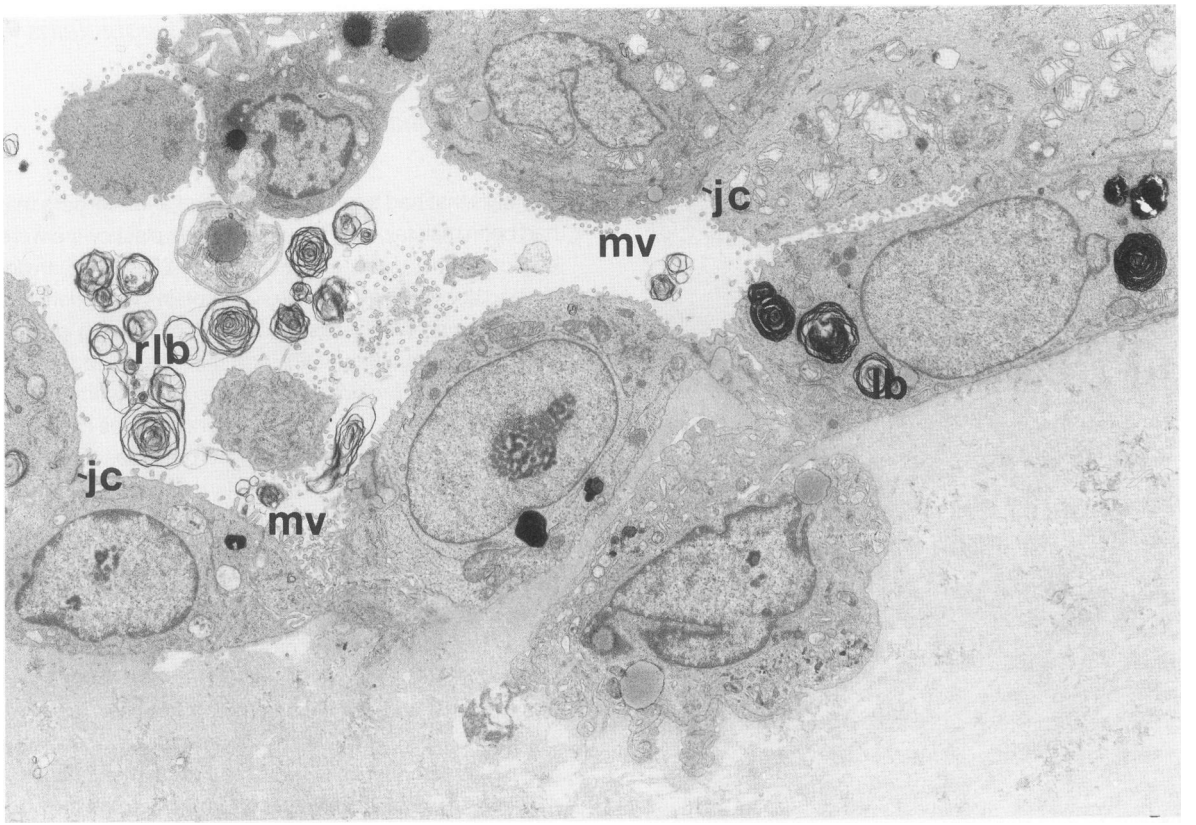

Figure 2. *ATII cells cultured on matrigel retain morphologic characteristics of "dedifferentiated" ATII cells. When ATII cells, isolated as described in Materials and Methods were plated on gelled Matrigel they reassociated: by 3 days the majority of the ATII cells rearranged to form polarized alveolarlike structures. In some instances lamellar body contents (rlb) appeared to be extruded into the "lumen". The cells in these clusters had microvilli (mv) projecting into the "lumen" and adjacent cells were joined at their apical ends by junctional complexes (jc). lb, lamellar body, ×3750.*

conjugated MPA as described above and then examined by transmission electron microscopy, it was apparent that all of the morphologically "dedifferentiated" cells that came in contact with MPA retained the ability to bind the lectin (Figure 4B). The specificity of this binding was demonstrated by the lack of reaction product on cells exposed to a mixture of 1-0-alpha-methyl-D-galactopyranoside and MPA (Figure 4C). ATII cell "derivatives" were examined at several time points: 10 days, 24 days, 3 months, 10 months, 16 months, and 25 months after isolation and culturing of the cells. In all instances, the cells retained the ability to bind peroxidase-conjugated MPA on their exposed apical surfaces. Extracellular staining, presumably of extracellular matrix, also was seen.

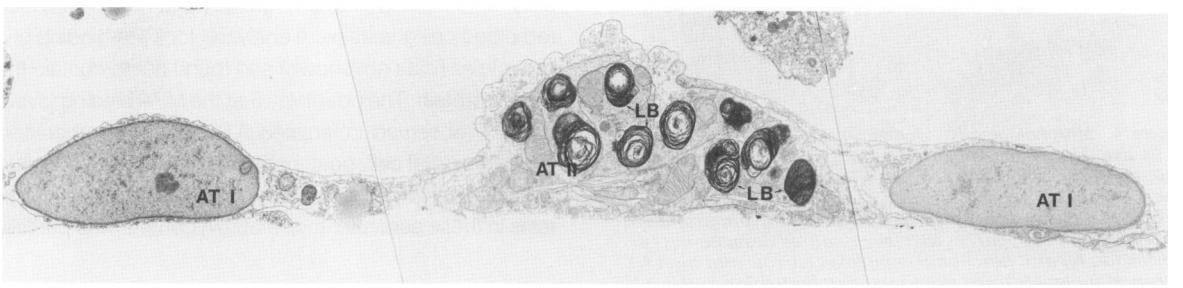

Figure 3. *ATII/ATI cells cultured on matrix secreted by ATII cells. When freshly isolated ATII/ATI cells were cultured together on matrix secreted by "dedifferentiated" ATII cells, some of the ATII and ATI cells re-associated in a manner similar to that seen in situ* with an established polarity. ATI cells were identified by their squamous morphology and lateral association with ATII cells; cytoplasmic extensions of the ATI cells extended partway up the sides of ATII cells and formed junctional complexes with the ATII cells. Cells were fixed after 3 days in culture. ATI, alveolar type I cell. ATII, alveolar type II cell. LB, lamellar body, ×3795.

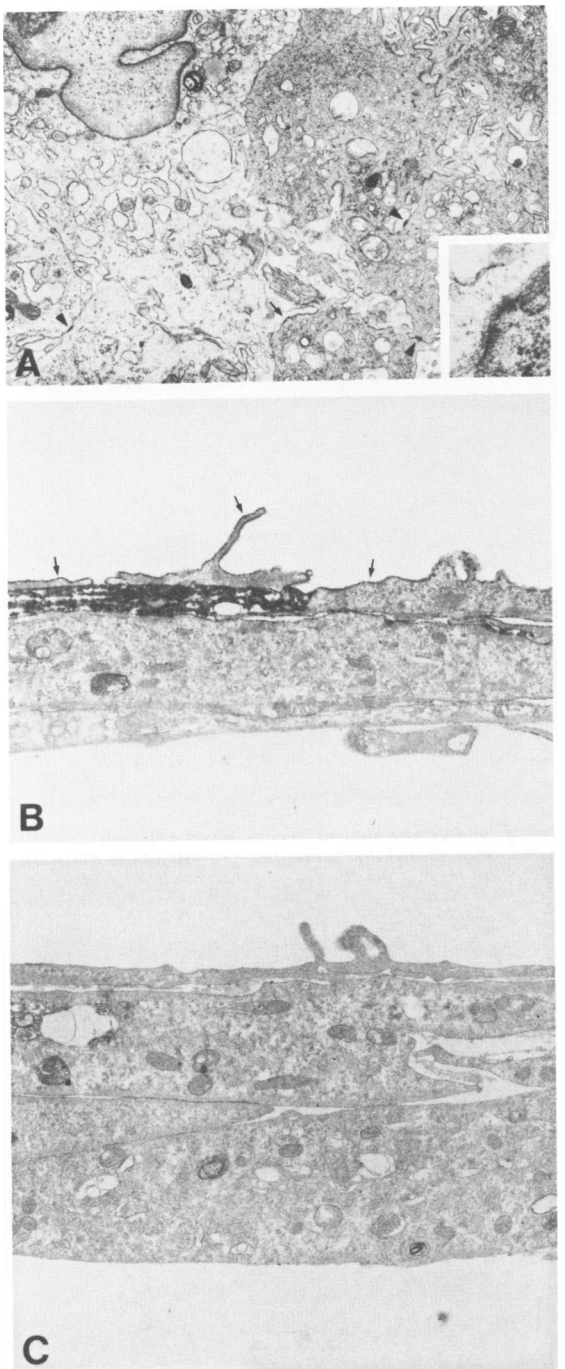

Figure 4. *Morphologically "dedifferentiated" ATII cells. ATII cells that had been grown on tissue culture plasticware for months retained some of their epithelial characteristics but lost the morphologic characteristics of differentiated ATII cells. When the cells were scraped from the substrate and examined as a pellet it was apparent that they retained desmosomes (arrowheads,* **A***) and junctional complexes (arrow and inset* **A***). When monolayers were examined perpendicular to the substrate it was apparent that the cells had lost their cuboidal shape, apical microvilli, and lamellar bodies (***B** *and* **C***). These "dedifferentiated" cells did, however, still bind peroxidase conjugated MPA on their surfaces (***B***, arrows). The extracellular matrix also appeared to bind MPA (***B***). MPA binding was inhibited in control preparations (***C***) when the sugar hapten 1-0-alpha-methyl-D-galactopyranoside was included with the lectin.*

Identification of MPA Binding Glycoproteins in Freshly Isolated ATII Cells and Morphologically "Dedifferentiating" and "Dedifferentiated" ATII Cells

ATII cells that had been freshly isolated and ATII cells that had been maintained in tissue culture on plasticware were collected, solubilized, and analyzed by SDS-PAGE as described above. MPA binding glycoproteins were identified by exposing nitrocellulose blots of the resulting gels to peroxidase-conjugated MPA and then localizing the peroxidase. MPA binding glycoproteins which were unique to freshly isolated ATII cells were identified (see Figure 5). All comparisons were made between samples run on the same gel and transferred simultaneously to the same piece of nitrocellulose. In Figure 5, lanes 1–8 were from the same gel/transfer with one intervening lane cut out between lanes 5 and 6. In Figure 5, lanes 9 and 10 were from another gel/transfer.

We have identified one prominent MPA binding glycoprotein (185 kd) in freshly isolated ATII cells (lanes 3 and 7, arrow) that was not seen in morphologically "dedifferentiated" ATII cells grown on tissue culture plasticware (lane 1). Isolated ATII cells were analyzed after 2 (lane 6), 4, 6 (lane 5), and 10 days (lane 4) of culture as they dedifferentiated on tissue culture plasticware under conditions where no cell proliferation took place. The results showed a decrease in detectable 185 kd MPA binding glycoprotein over time. As seen in lane 4, after 10 days in tissue culture, the ATII cells no longer contained a detectable significant 185 kd MPA binding glycoprotein. Instead they contained a minor MPA binding glycoprotein with a slightly lower molecular weight. The 185 kd MPA binding glycoprotein was not detected in secondary rat lung fibroblasts prepared as described above (lane 8) or in rat pulmonary alveolar macrophages analyzed immediately after lavage or after two days of culture on tissue culture plasticware (lane 2). BHK cells, an established cell line grown under the same conditions as the "dedifferentiated" ATII cells, was also negative (data not shown). Rat red blood cell ghosts were analyzed for MPA binding glycoproteins (data not shown) and found not to contain the 185 kd protein. The possibility that the MPA binding glycoproteins observed in isolated ATII cells were absorbed from sera (fetal calf, goat, or rat) during isolation or culturing was considered by identifying MPA binding glycoproteins in these sera after their albumin content was partially

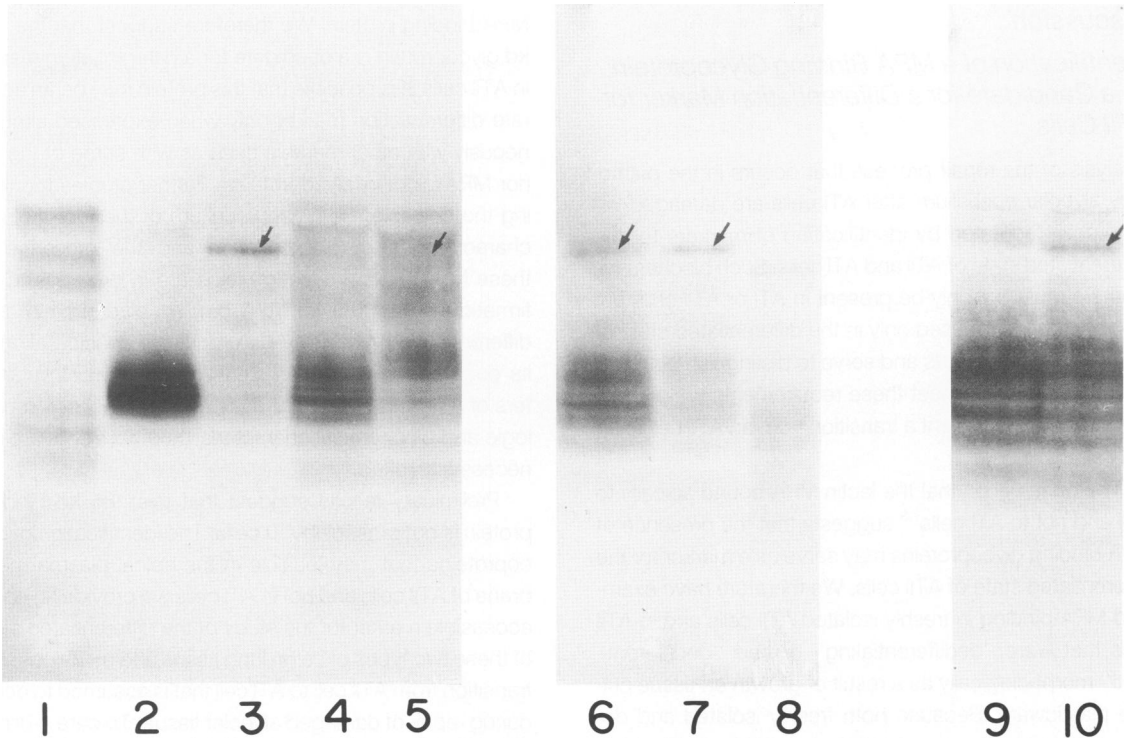

Figure 5. *Identification of MPA binding glycoproteins in "differentiated" and "dedifferentiated" ATII cells. Peroxidase-conjugated MPA was used as described in the text to identify MPA binding glycoproteins in freshly isolated ATII cells (lanes 3 and 7) and in ATII cells grown on tissue culture plasticware for 2 days (lane 6), 6 days (lane 5), 10 days (lane 4) and 10+ months (lane 1). Rat lung fibroblasts (lane 8) and rat lung alveolar macrophages (lane 2) were also analyzed for MPA binding glycoproteins. The MPA binding proteins detected in freshly isolated ATII cells (lanes 3 and 7) were compared with those detected in the other preparations to determine their uniqueness. The 185 kd band seen in ATII cells was found in "differentiated" freshly isolated ATII cells (lanes 3 and 7, arrow) but not in ATII cells which had "dedifferentiated" after growth on tissue culture plasticware (lane 1). Analysis of ATII cells as they "dedifferentiated" in tissue culture revealed that the 185 kd MPA binding band was still present in ATII cells which had been cultured for 2 days (lane 6) but was significantly diminished or absent in ATII cells that had been cultured for 6 days (lane 5, arrow). After 10 days in culture on tissue culture plasticware, the 185 kd MPA binding band could not be detected; instead a minor MPA binding band of a slightly lower molecular weight (lane 4) was seen. These results suggest that the 185 kd MPA binding band was lost as a result of ATII cell dedifferentiation in tissue culture. The 185 kd MPA binding component seen in freshly isolated ATII cells (lane 10, arrow) could not be detected in freshly isolated ATI cells (lane 9). The 185 kd band appears to run just below 2 bands shared by both ATII and ATI cells. The 185 kd MPA binding band also could not be detected in rat lung fibroblasts (lane 8) or in rat pulmonary alveolar macrophages (lane 2). Lanes 1 to 8 were from one gel/transfer and lanes 9 and 10 were from another gel/transfer. Variability between lots of MPA affected the extent to which minor bands could be detected; for example, minor components in lane 3 are more easily seen in lane 10. Within one gel/transfer/MPA staining, detection of components is consistent and, therefore, comparison between lanes is valid.*

depleted to allow better detection of minor glycoproteins (data not shown). There was no apparent similarity between MPA binding glycoproteins found in sera and those identified in ATII cells. In summary, the results showed that the prominent 185 kd MPA binding glycoprotein found in freshly isolated ATII cells was found in decreasing amounts of ATII cells as they "dedifferentiated" in tissue culture. This 185 kd MPA binding band was not detected in "dedifferentiated" ATII cells, rat lung fibroblasts, rat pulmonary alveolar macrophages, rat red blood cell ghosts, or BHK cells, nor was it found in the examined sera. In addition, preliminary analysis of freshly isolated ATI cells (Figure 5, lane 9) suggests that the 185 kd band was not found in ATI cells whereas it was detected in ATII cells (Figure 5, lane 10) just below MPA binding components common to both ATI and ATII cells. A 185 kd MPA binding band was also not detected (data not shown) in extracellular matrix secreted by ATII cells although, as suggested by cytochemistry (Figure 4B), the extracellular matrix secreted by ATII cells in culture did contain MPA binding components.

The question of whether the 185 kd band seen in freshly isolated ATII cells was generated as a result of the enzymatic digestion during isolation was approached by exposing the ATII cell "derivatives" to the enzyme mixture used in isolations. These enzyme-treated cells were then harvested and analyzed for MPA binding glycoproteins. On the resulting lectin blots some of the bands appeared less prominent, but no new bands were generated. A 185 kd MPA binding band had not been created.

Discussion

Identification of a MPA Binding Glycoprotein as a Candidate for a Differentiation Marker for ATII Cells

Analysis of the repair process that occurs in the pulmonary alveolar epithelium after ATI cells are damaged will be greatly facilitated by identification of markers for the differentiated state of ATII and ATI cells. Such biochemical markers must not only be present in ATI or ATII cells but also must be expressed only in the differentiated state of these two types of cells and serve to distinguish between them. Markers that meet these requirements will provide the means to document a transition from an ATII cell to an ATI cell.

The observation that the lectin MPA bound apically to ATII and not to ATI cells[7,8] suggests that the presence of MPA binding glycoproteins may serve as a marker for the differentiated state of ATII cells. We therefore have examined MPA binding in freshly isolated ATII cells and in ATII cells that were "dedifferentiating" or had "dedifferentiated" morphologically as a result of growth on tissue culture plasticware. Because both freshly isolated and dedifferentiated ATII cells bound peroxidase-conjugated MPA on their surfaces, we concluded that scoring alveolar epithelial cells plus or minus for MPA binding was not adequate for identifying the differentiated state of an ATII cell. We therefore identified and compared the individual MPA binding glycoproteins in the "differentiated," "dedifferentiating," and "dedifferentiated" ATII cells. A prominent MPA binding glycoprotein with an approximate molecular weight of 185 kd was found in freshly isolated ATII cells and not in ATII cells which had been cultured on tissue culture plasticware for 10 or more days. When ATII cells were analyzed as they "dedifferentiated" after 2, 4, 6, and 10 days of culture on tissue culture plasticware, decreasing amounts of the 185 kd MPA binding bands were detected. Because no cell proliferation occurred during this 10-day time period and because our cultures were at least 94% ATII cells, we can conclude that the decrease in the amount of detectable 185 kd MPA binding glycoprotein resulted from a change in the ATII cells and not from a proliferation of another cell type. Because our cytochemical results (Figure 4) suggested that the extracellular matrix secreted by ATII cells bound MPA, we considered whether the 185 kd MPA binding glycoprotein was a component of the extracellular matrix rather than being associated with ATII cells themselves. Even though there were many MPA binding glycoproteins associated with the extracellular matrix secreted by dedifferentiated ATII cells, we could not detect one with a molecular weight of 185 kd. During preliminary analysis of glycoproteins in ATI cells, we have not been able to detect the 185 kd MPA binding protein. We therefore suggest that the 185 kd glycoprotein is a candidate for a differentiation marker in ATII cells. It is possible that this protein may be an accurate differentiation marker only when expressed simultaneously with other markers such as with some of the minor MPA binding glycoproteins. Further studies monitoring the coexistence in tissue culture of the morphologic characteristics of differentiation and the presence of these MPA binding glycoproteins are in progress. Confirmation of the 185 kd MPA binding glycoprotein as a differentiation marker will also require a consideration of its coexistence with characteristic biochemical parameters of ATII cells because it has been shown that morphologic and biochemical characteristics of ATII cells are not necessarily linked.[9,19]

Preliminary results suggest that this 185 kd binding protein is not present in ATI cells. The identification of glycoproteins that may localize in the apical plasma membrane of ATII cells and not in ATI cells will provide an easily accessible marker for the study of the differentiated state of these two types of cell in lung tissue and of the possible transition from ATII cell to ATI cell that is assumed to occur during repair of damaged alveolar tissue. To date ATII and ATI cells can be distinguished on the basis of shape and the ability to package and secrete surfactant in lamellar bodies. Until the synthetic capacity of ATI cells is characterized, the biochemistry associated with surfactant in ATII cells can not be assumed to be unique to ATII cells. Ideally, a study of the possible transition of an ATII cell to an ATI cell will involve cell and differentiation specific antigens as well as biosynthetic capacity and morphology. In this respect the identification of the 185 kd MPA binding glycoprotein that is potentially differentiation specific for ATII cells, and may not be found in ATI cells, is important because such a glycoprotein may serve as a cell- and differentiation-specific antigen.

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