Characterization of a 36,000-dalton Protein from the Surface of Madin-Darby Canine Kidney Cells Involved in Cell Attachment and Spreading

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ABSTRACT We have identified and immunochemically characterized a 36,000-dalton membrane glycoprotein from Madin-Darby canine kidney cells. This protein is surface-labeled by lactoperoxidase-mediated iodination and metabolically labeled by [^{35}S]methionine. It binds to Concanavalin A and incorporates 2-D-^{3}H-mannose residues, thus indicating it is a glycoprotein. Rabbit polyclonal antibodies against this protein evenly decorate the external surface of trypsinized, unpolarized cells. The external apical surface of confluent monolayers, grown under culture conditions in which the tight junctions are closed and the cells have acquired polarity, is also evenly stained. The basolateral aspects of the external surface are stained only when the tight junctions are opened by removal of Ca^{++} or when the antibody has access to the monolayer from the basal side, which indicates an even distribution of this antigen on the surface of polarized cells. The antibody has no inhibitory effect on the opening and resealing of tight junctions in dense cultures, but does inhibit the attachment and spreading of cells on a substrate, which then blocks the establishment of a confluent functional monolayer.

Epithelial cells display a striking structural and functional polarity where the existence of two domains, apical and basolateral, on the cell surface is clearly established. The apical surface, covered by microvilli, is involved in absorptive and secretory processes, whereas the basolateral face, free of microvilli, is the site of high water permeability and of active transport of Na^+ through an Na^+K^+ ATPase. The two surfaces have different protein composition and are separated from one another by junctional complexes including the zonula adherens, the zonula occludens or tight junction, and desmosomes. The tight junction acts as a barrier to limit the penetration of macromolecules into the intercellular space (1) and to prevent lateral diffusion of proteins and some lipids from one surface domain to the other (2), thereby maintaining the polarity and unique composition of epithelial surface domains (3, 4, 5).

Experiments done with the Madin-Darby canine kidney (MDCK)[1] epithelial cell line by Leighton et al. (6), and later by Misfeldt et al. (7) and Cereijido et al. (8), suggested that the cell surface in these monolayers had polarity and that tight junctions were directly involved in maintenance of this polarity (9). We later demonstrated that Ca^{++} and components of the cytoskeleton, such as actin microfilaments, modulate the sealing capacity of tight junctions (10, 11). Additional evidence on the structural and functional polarity of MDCK cells has been provided more recently by several groups (12, 13, 14, 15).

In this report we analyze a surface protein from MDCK cells that is released by non-ionic detergents, characterize it as a glycoprotein, and determine its location through use of polyclonal antibodies and immunoelectron microscopy. The protein appears on the apical surface of closed monolayers. After Ca^{++} removal, which causes the opening of tight junctions, or trypsinization, we can locate the protein also on the basolateral surfaces or evenly distributed on the surface of rounded detached cells. The limited localization of the antibody to the apical surface in closed monolayers is due to its inability to cross the closed tight junctions. The basolateral surface can be labeled to a certain extent, in closed monolayers, if the antibody is given access to this surface. This result indicates that gp36 is distributed on all the surfaces of non-

[1] *Abbreviations used in this paper:* Con A, Concanavalin A; MDCK, Madin-Darby canine kidney; PMSF, phenylmethylsulfonyl fluoride.

polarized as well as polarized cells and does not redistribute, at least in a very marked way, when cells detach from the substrate or when the tight junctions are opened. The antibody shows an inhibitory effect on the attachment and spreading processes that lead to the formation of a confluent monolayer but shows no inhibitory effect on the opening or resealing of tight junctions.

MATERIALS AND METHODS

Cell Culturing

MDCK cells, obtained from M. Cereijido, Centro de Investigación y Estudios Avanzados, Mexico, were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics in a 5% CO_2 atmosphere at 37°C. Cells were plated on 35-mm petri or 125-ml Falcon dishes at 2 × 10^6 cells/ml. After 1 h, the nonattached cells were washed away and new medium was added to the already confluent monolayer. Monolayers plated in parallel on collagen-coated nylon disks began to show electrical resistance after 6 h in culture and reached a steady state value of ±300 ohms cm^2 at 22–24 h. The monolayers used in this work were 2 d old.

Labeling of the Cells

For the identification of surface proteins, cells were labeled in the culture dishes after washing several times with phosphate-buffered saline (PBS) (9.3 mM Na_2HPO_4, 4.2 mM KH_2PO_4, 0.132 M NaCl containing 2 mM phenylmethylsulfonyl fluoride [PMSF], [pH 7.4]) according to the procedure of Hubbard and Cohn (16) using lactoperoxidase, and 250 μCi/ml of ^{125}I (Amersham Corp., Arlington Heights, IL). Labeling with methionine was done by adding 10 μCi/ml of [^{35}S]methionine (727.58 Ci/mmol) to the cultures for 18–20 h.

Preparation of Membrane-enriched Fractions

10^8–10^{10} cells cultured in Falcon flasks were detached from the culture dishes with a rubber policeman and washed by centrifugation at 1,000 rpm for 10 min with PBS containing 10% sucrose. The pellet was then mixed with a small pellet of cells previously labeled with ^{125}I as indicated above. The pooled pellets were resuspended in 10 ml of lysis buffer, 15 mM iodoacetate, 2% dimethylsulfoxide, 10 mM Tris-HCl (pH 8.0), 2 mM PMSF according to the procedure of Brunette and Till (17). After standing in ice for 10 min, cells were homogenized in a glass homogenizer with 100–200 strokes. Breakage of the cells was checked under the microscope. Big sheets of membranes were obtained with the first homogenization. The membranes were pelleted by centrifugation at 4,000 *g* for 20 min, and the pellet was resuspended and mixed thoroughly in the two-phase system formed by 2.3 ml of 20% Dextran T_{500}, 1.3 ml of 30% polyethylene glycol 6000, 4.2 ml of 0.22 M sodium phosphate (pH 6.6), 10 μl of 1 M NaCl and 2 ml of H_2O. The pellet was then centrifuged at 12,000 *g* for 15 min at 4°C. The membranes concentrated at the interphase of the two-phase gradient formed by centrifugation. They were recovered, pelleted, and resuspended in 10 mM Tris buffer (pH 7.4) mixed with an equal volume of 50% sucrose in buffer A (20 mM Tris-HCl [pH 7.4], 50 mM KCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 1 mM PMSF). The resuspended membranes were layered on top of a 50–65% sucrose gradient made in buffer A (18) and centrifuged at 17,000 rpm in a Spinco SW27.1 rotor for 1 h. The membranes floating in the boundary between 50 and 65% sucrose were collected, diluted threefold with 10 mM Tris-HCl (pH 7.4), 2 mM PMSF, and recovered by centrifugation at 10,000 *g* for 20 min. Membrane fractions were processed for gel electrophoresis or stored at −20°C.

Gel Electrophoresis

One-dimensional gels were run according to the procedure of Laemmli (19), stained with 0.25% Coomassie Blue in a methanol–acetic acid–water (5:1:1) mixture, and then destained in 10% acetic acid. When required, gels were processed for fluorography as indicated by Bonner and Laskey (20).

Characterization of gp36 As A Glycoprotein

CONCANAVALIN A BINDING EXPERIMENTS: Concanavalin A (Con A) binding to ^{125}I-labeled MDCK cells was accomplished by incubating monolayers in the presence of the lectin (2.2 mg/ml) in 19 mM KH_2PO_4 (pH 7.2), 0.27 M NaCl, 10 mM $MgCl_2$ for 30 min. Cells were washed repeatedly with PBS containing 2 mM PMSF, 1 mM leupeptin, and incubated with a rabbit serum against Con A (1:4 dilution) for 45 min. Unreacted antibody was removed by repeated washing of the cells with PBS. The cells were then incubated with a suspension of 10% formalin-fixed *Staphylococcus aureus* (Cowan 1) in 0.5% Nonidet P-40, 2 mM methionine, 5 mM KI in PBS (21). The mixture was incubated at 4°C for 8–10 h and the pellet recovered by centrifugation. After several washes of the pellet with the Nonidet P-40–KI–methionine buffer, the antibody-antigen complexes were released from the bacteria with 7 M urea and analyzed by SDS gel electrophoresis. Labeled peptides were identified by fluorography.

LABELING WITH D-2-^{3}H-MANNOSE: Plated cells were labeled after 3 h in culture with 20 μCi/ml of D-2-^{3}H mannose (Amersham Corp.) for 4 h. Total proteins and membrane proteins were analyzed by gel electrophoresis and fluorography.

Preparation of Antibodies against gp36

MDCK cells growing on petri dishes and membrane-enriched fractions were treated with 1 ml or an equal volume of 0.5% Triton X-100 in 10 mM Tris (pH 7.4) containing 2 mM PMSF for 15 to 90 min at 4°C. The supernatant containing the detergent-solubilized components was recovered after centrifugation at 15,000 *g* and separated on 10% SDS acrylamide preparative gels. The most prominent stained band with a molecular mass of 36,000 was cut out, minced into small pieces, and mixed with Freund's adjuvant and PBS to make a homogeneous suspension. Approximately 175 μg of protein were injected intraperitoneally into female rabbits. After 4–5 injections, the sera were tested using immunoblots. The highest titer sera were fractionated and the IgGs separated by DEAE chromatography (22), concentrated, and stored at −20°C at 5–7 mg/ml. Immunoblots of SDS polyacrylamide gels containing cell extracts, membranes, and Triton-soluble or -insoluble fractions were done using 10% SDS gels as described by Towbin et al. (23). ^{125}I-Protein A was used as a second step to localize the previously bound anti-gp36. The purified anti-gp36 IgG was used as the first step antibody at 50–75 μg/ml and was incubated with the nitrocellulose filters for 3 h at 37°C.

Immunolocalization of gp36

Monolayers of MDCK cells, cultured on petri dishes, were examined in two physiological states, either closed or with their junctions opened by removal of Ca^{++}. Alternatively, we have examined trypsinized pelleted cells. Each set of cells was washed with PBS at 4°C, fixed, incubated with first and second step antibodies, and reacted with the peroxidase substrate as described by Courtoy et al. (24). Specifically, cells were fixed in 4% formaldehyde, 0.2% glutaraldehyde for 3 min, then washed with 0.1 M glycine, 0.2% gelatin for 30 min, and incubated with the first step rabbit polyclonal antibody (1:4 dilution) in PBS plus 0.1% BSA for 2 h at 4°C. In the controls, preimmune IgGs were used. Cells were then washed with PBS plus 0.1% BSA for 30 min and incubated with goat anti-rabbit immunoglobulin coupled to peroxidase at a 1:500 dilution for 2 h. After three washes with PBS, the peroxidase was developed with diamino benzidine (1 mg/ml) and 0.01% H_2O_2 in 0.05 M Tris-HCl (pH 7.2). Cells were then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate for 60 min and postfixed with 1% OsO_4, dehydrated, and block embedded in Epon 812. Thin sections were viewed in a Zeiss M-10 electron microscope. To gain access to the basal surface of closed monolayers, we fixed cell monolayers and gently lifted them from petri dishes as described previously (2). The continuous cell layer was then transferred to the antibody solution and incubated for 2 h.

Effects of Anti-gp36 on MDCK Cell Function

TESTS FOR INHIBITION OF ATTACHMENT AND SPREADING: Tests for anti-gp36 effects on attachment and spreading were done according to the experimental procedures described by Hahn and Yamada (25). Cells in suspension, at ~2 × 10^5 per milliliter, were incubated for 30 min with 500 μl of antibody and diluted 1:4 in growth medium. Control cells were incubated with preimmune IgGs. The cells were counted in a Neubauer chamber. 2 × 10^5 cells were plated and cultured in 35-mm petri dishes in normal medium for 2 h. In this time and at this cell density, control monolayers would be close to confluence (8, 12). Cells that remained unattached after 2 h were recovered by centrifugation and counted. The dishes with attached cells were incubated for one additional hour. The culture medium from this additional incubation and a final PBS wash were processed to determine the number of cells that detached during the incubation. Finally, one group of cells was washed extensively with PBS after antibody incubation, and then plated, incubated, and processed as indicated above to determine if the antibody effects were reversible. The number of cells attached to the substrate in both controls, and experiments using antibody, were calculated after trypsinization and counting of the cells.

Cells in the petri dishes were photographed with a Leitz inverted microscope using phase-contrast optics. Photographs were taken with Kodak Tri-X film.

TRANSEPITHELIAL ELECTRICAL RESISTANCE MEASUREMENTS: Confluent monolayers were prepared on nylon collagen-coated disks and the transepithelial electrical resistance measured as indicated by Cereijido et al. (8). To open tight junctions, we treated monolayers with 2–3 mM EGTA in medium without Ca^{++} for 20 min. Immune or preimmune IgGs were added to the closed monolayers for 30 min to see their effect on the opening of the monolayers. The recovery of the electrical resistance in the presence or absence of antibody was measured at different times after transferring the disks to culture medium containing normal levels of Ca^{++} and either preimmune or immune antibodies.

RESULTS

A Glycoprotein of 36,000 Daltons (gp36) Is A Cell Surface Component

Lactoperoxidase-catalyzed iodination of the surface of MDCK cells labels several proteins of diverse molecular mass. As seen in Fig. 1 (*C* and *D*), a main group includes proteins of high molecular mass (82,000–30,000 D). Prominent bands are those of 150,000, 82,000, 56,000, 40,000, and 36,000 D. Some of these labeled proteins are also present in enriched membrane preparations obtained from cells cultured as confluent monolayers (Fig. 1, *B* and *D*). One of the surface proteins labeled by iodine, the 36,000 dalton protein, is easily removed by Triton X-100 or Nonidet P-40 treatment at 4°C. More than 80% of it is released into the supernatant by a 15-min detergent treatment of MDCK cells or membrane-enriched preparations. (Fig. 1, *E* and *F*).

This protein is glycosylated as indicated by its binding to Con A and its immunoprecipitation as a complex with Con A by antibodies directed against Con A (Fig. 2, *A* and *B*). When the Con A–coimmunoprecipitated peptides were analyzed, a main band was recognized by anti-gp36 (Fig. 2*A*). ^{3}H-Mannose is incorporated into gp36 as shown in Fig. 2*C*. A comparison of the Coomassie Blue–stained proteins shows that other proteins appear to be more strongly labeled, suggesting a low degree of glycosylation of gp36 under the culture conditions used (Fig. 2*C*). [^{35}S]Methionine is also incorporated into gp36 (data not shown).

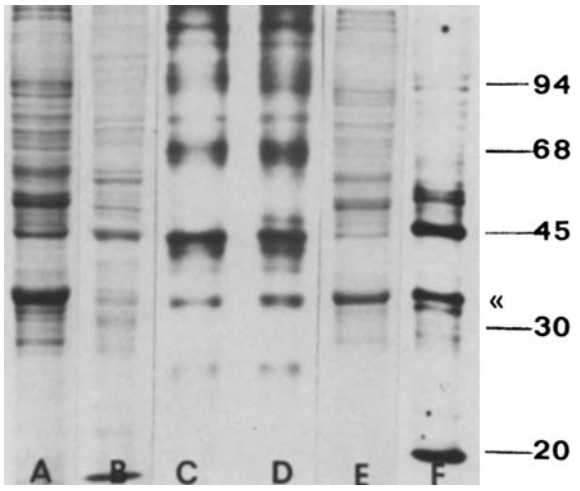

FIGURE 1 ^{125}I labeling of surface peptides in MDCK cell monolayers. After labeling with ^{125}I in a lactoperoxidase-catalyzed reaction, peptides were analyzed by gel electrophoresis and fluorography. (*A* and *B*) Total cell homogenate and membrane-enriched preparations stained with Coomassie Blue. (*C* and *D*) The iodine-labeled peptides from similar preparations. (*E*) Coomassie Blue–stained peptides solubilized by a 15-min detergent treatment and (*F*) the corresponding iodine-labeled peptides released by detergent. gp36 is almost completely released into the soluble fraction (see arrow).

Immunological Specificity of The Antibodies to gp36

Antibodies prepared in rabbits against gp36 were tested by immunoblotting cell extracts, isolated membranes, or detergent-soluble and -insoluble fractions. Fig. 3 shows that the antibody reacted specifically with one band at 36,000 in immunoblots of MDCK cell membranes or total cell extracts (lanes *A* and *B*). As gp36 is released into the Triton-soluble fraction, very little protein is detected by the antibody in the Triton-insoluble fraction after a 35-min extraction of the cells with detergent (lanes *C* and *D*).

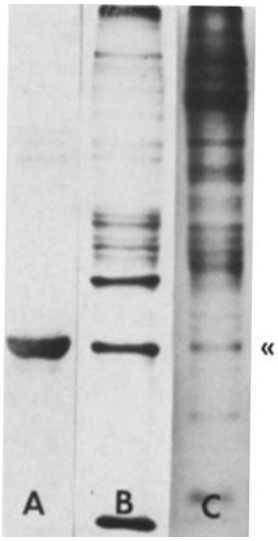

FIGURE 2 Binding to Con A and incorporation of ^{3}H-mannose. (*A*) Peptides immunoprecipitated by antibodies against Con A were transferred to nitrocellulose filters, reacted first with antibody against gp36, then with a second step antibody, goat anti-rabbit IgG labeled with horseradish peroxidase. Finally, aminobenzidine was used as the peroxidase substrate. (*B*) Peptides from monolayers surface-labeled with ^{125}I and Con A were immunoprecipitated using antibodies against Con A. (*C*) 2-D^{3}H-Mannose-labeled peptides from MDCK cells.

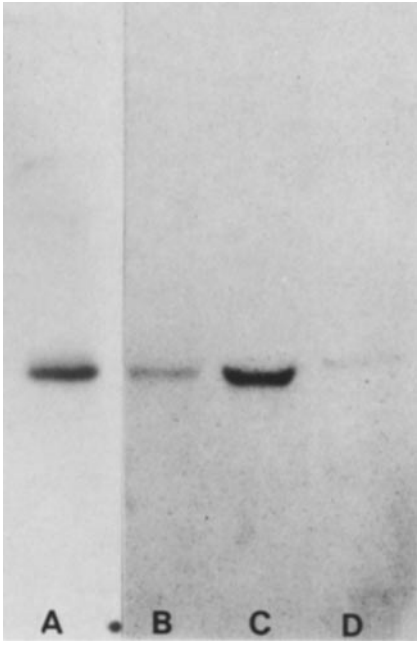

FIGURE 3 Immunoprecipitated analysis of MDCK cell fractions using antibody against gp36. Fluorographs are shown of (*A*) enriched membrane fraction, 50 μg; (*B*) MDCK cell extract, 50 μg; (C) detergent-soluble fraction, 50 μg; and (*D*) detergent-insoluble fraction, 32.5 μg.

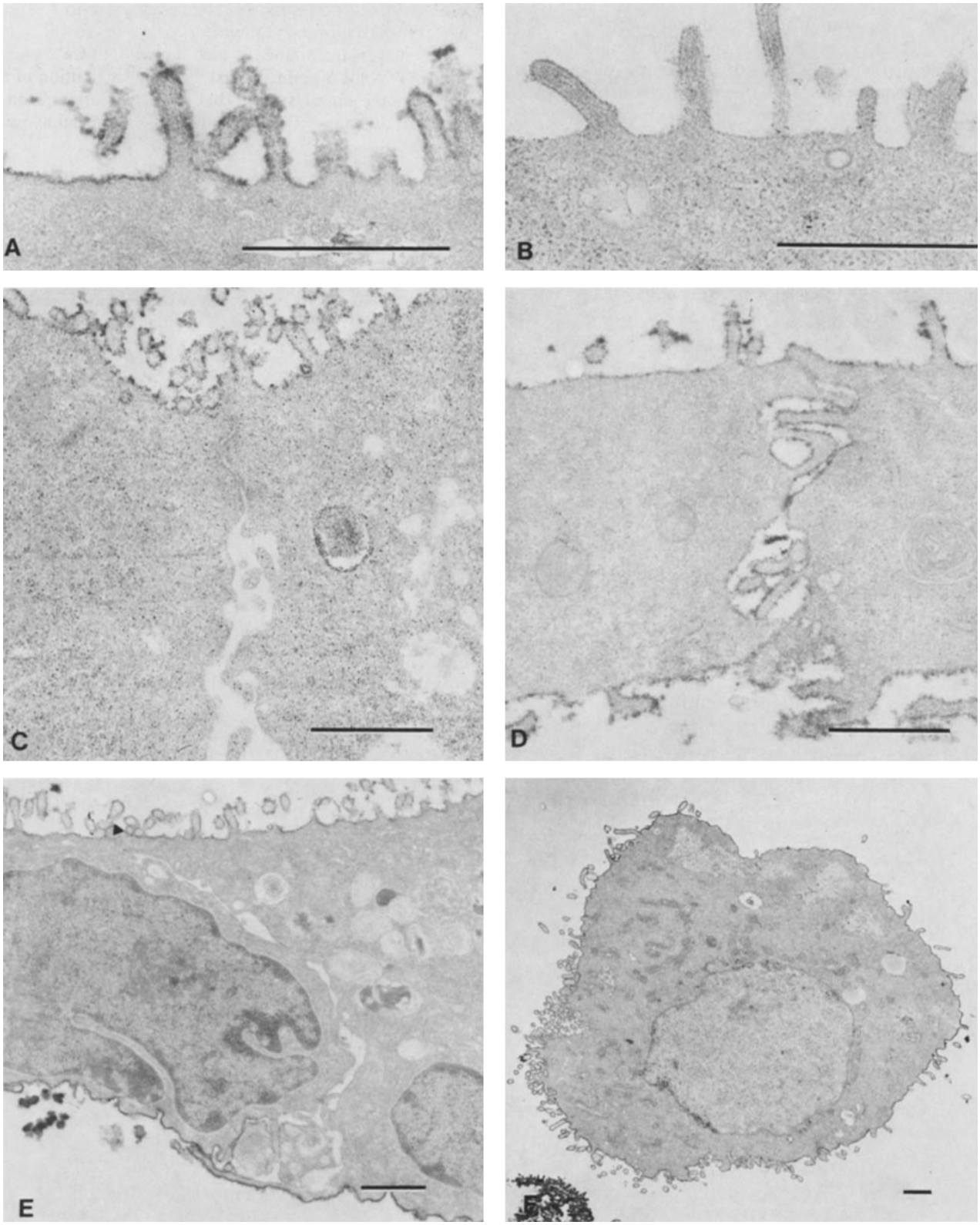

FIGURE 4 Binding of antibody against gp36 to MDCK cells. Prefixed MDCK cells were incubated sequentially with IgGs against gp36, with goat anti-rabbit immunoglobulins coupled to peroxidase, then with aminobenzidine. After the enzyme reaction, cells were fixed for electron microscopy, embedded, and sectioned. (*A*) Positive reaction as seen on the surface of the cells. (*B*) Negative reaction after incubation of cells with preimmune IgGs. (*C*) Antibody labeling of apical surfaces in adjacent cells in a closed monolayer. (*D*) Antibody labeling of apical and basolateral surfaces in cells treated with EGTA in Ca-free medium. (*E*) Antibody labeling of closed monolayers lifted from the substrate to permit antibody access to the basal side. (*F*) Surface labeling of trypsinized suspended cells with the antibody. Bar, 1 μm.

Immunolocalization and Antigen Distribution

The cells, trypsinized or as confluent monolayers, were briefly fixed with formaldehyde, stained with antibody against gp36 followed by a second antibody coupled to horseradish peroxidase, then reacted with the enzyme substrate and processed for electron microscopy. The micrographs in Fig. 4, *A* and *B*, show the peroxidase reaction product on the surface of MDCK cells after incubation with the specific antibody or with preimmune IgGs. Exposure to preimmune gamma-globulins gave no reaction on the surface of the cells as shown in panel *B*.

Monolayers grown in normal concentrations of Ca^{++} on petri dishes were labeled with the antibody. In these cells, the peroxidase reaction product was restricted to the apical region of the cells as shown in Fig. 4*C*. The tight junction region is visible and reaction product is seen evenly distributed on the apical surface following the profiles of the microvilli.

Monolayers maintained in medium without Ca^{++} plus 2–3 mM EGTA for 60 min showed an even distribution of antibody on the apical surface, but label also appears on the basolateral aspects of the cells. In Fig. 4*D*, apical as well as basolateral aspects in an open monolayer are shown. Heavy labeling can be seen all along the surface of adjacent cells. When confluent monolayers were first fixed, then gently lifted as a continuous sheet to expose the basal side of the cells to the antibody, labeling of the basal surface and parts of the basolateral aspects of the cells was also observed. In these cells, the tight junctions remained closed (Fig. 4*E*, arrow) and randomization or redistribution of surface components had not occurred; therefore, we think that gp36 is uniformly distributed over the surface of polarized cells. Moreover, in trypsinized cells, as shown in Fig. 4*F*, the antibody was distributed evenly all over the surface. In these rounded, nonpolarized cells, peroxidase-positive material could be identified along microvilli as well as smooth surfaces.

TABLE I

Effect of Anti-gp36 on the Attachment of MDCK Cells

Treatment	Cells attached	
	Second hour after plating	Third hour after plating
Control (*)	174,500 ± 4,500 (3)	187,200 ± 9,300 (4)
Antibody present (‡)	51,300 ± 10,800 (3)	50,300 ± 4,000 (4)
Antibody washed (§)	87,200 ± 12,500 (3)	89,300 ± 11,200 (4)

* 2×10^5 cells plated per petri dish in Dulbecco's modified Eagle's medium with 10% fetal calf serum as indicated in Materials and Methods after incubation with preimmune IgGs.

‡ Cells were incubated in suspension with the antibody, then 2×10^5 cells were plated in normal medium.

§ Cells treated with antibody as the second group were washed 4–6 times with PBS and then plated in normal medium.

Results are expressed as mean ± standard error.

Number in parentheses represents the number of observations.

Inhibitory Effects of Anti-gp36 on Cell Attachment and Spreading

When trypsinized, suspended cells were incubated with the antibody for 30 min and then plated at high cell density, the number of cells capable of attaching to the substrate decreased drastically. 36.5% of the cells treated with antibody adhered to the substrate as compared with the percentage of cells treated with preimmune gamma-globulins (Table I). The cells treated with antibody that did attach to the substrate under these conditions remained rounded and were unable to spread to form a normal monolayer after 3 h in culture (Fig. 5, *A*

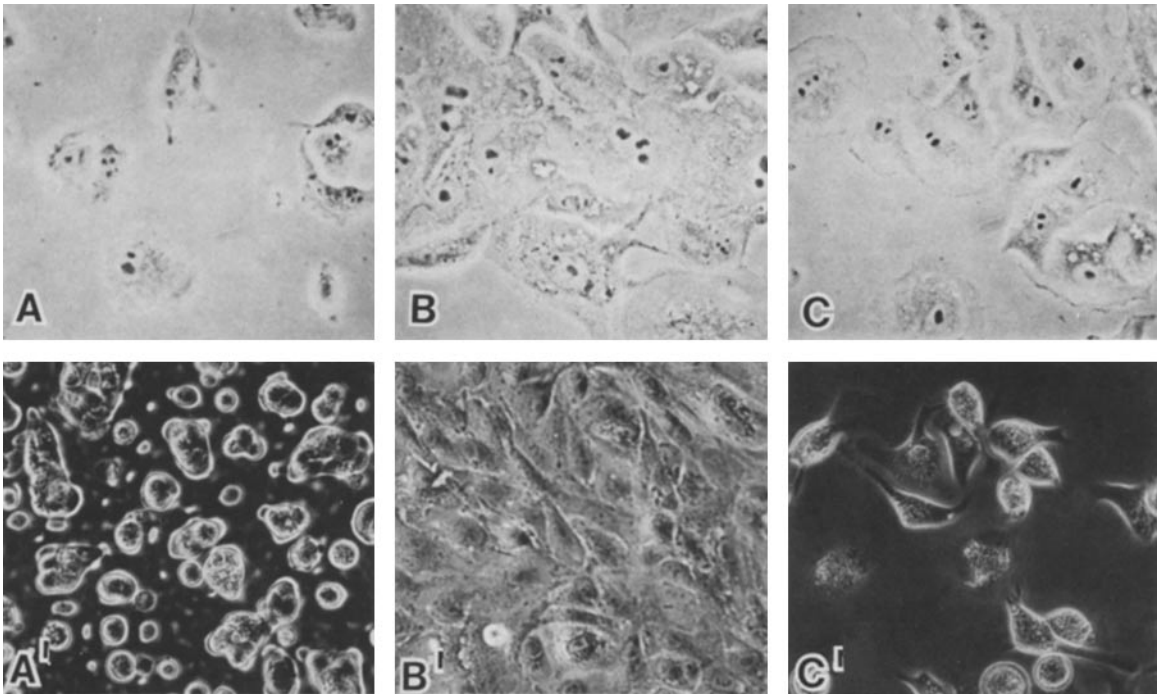

FIGURE 5 Phase-contrast microscopy of MDCK cells plated in the presence of antibody to gp36. Resuspended cells were incubated with antibody for 30 min. Control cells were incubated with preimmune IgGs. Cells were then plated in petri dishes and incubated in normal medium for 3 h. (*A* and *A'*) Cells incubated with antibody. (*B* and *B'*) Control cells. (*C* and *C'*) Cells treated with antibody as in *A*, but washed extensively before plating. Two different magnifications are shown (*A–C*, × 1,200; *A'–C'*, × 800).

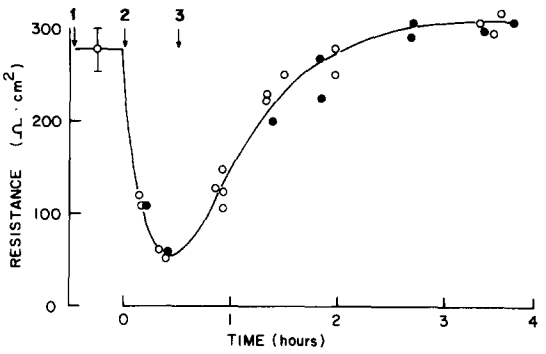

FIGURE 6 Effect of anti-gp36 on the opening and resealing of tight junctions. At arrow 1, antibody was added to a group of stable monolayers and incubated for 30 min. At arrow 2, disks were divided into four groups: (○) disks were transferred to Ca-free medium; (●) disks were transferred to Ca-free medium in the presence of antibody. At arrow 3, (○) disks were returned to normal Ca^{++}-containing medium; (●) disks were returned to normal Ca^{++}-containing medium in the presence of antibody.

and *A'*). Control monolayers on the other hand were almost confluent by this time as seen in *B* and *B'*.

Cells that were treated with the antibody under the same conditions, then washed extensively with normal medium before plating, seemed to recover partially from the inhibition caused by the antibody (Fig. 5, *C* and *C'* and Table I). The efficiency of attachment increased up to 50% after 2 h in normal medium, and the cells spread and touched forming small islands with several cells. The edges of these cells retained a ruffled appearance (*C*).

Anti-gp36 Does Not Interfere with The Opening or Resealing of Tight Junctions

Electrical resistance was measured in monolayers grown for 24 h on collagen-coated nylon disks in a Ussing chamber as described by Cereijido et al. (8). The average resistance was calculated after measuring six disks taken at random. An average value of ±300 ohms cm^2 was calculated (Fig. 6).

Closed monolayers were exposed to the antibody for 30 min and the electrical resistance recorded. No significant change was registered. A second set of six control monolayers were treated with 2–3 mM EGTA for 10–20 min. As seen in the graph, the depletion of Ca^{++} caused a fast drop in the electrical resistance caused by the opening of the tight junctions. This drop in resistance was not altered by the presence of antibody in the medium. Restoring Ca^{++} to the medium resealed the tight junctions and resulted in the recovery of the electrical resistance, in this case measured 1, 2, and 4 h after Ca^{++} restoration. The kinetics of recovery of the electrical resistance of open monolayers exposed to antibody was identical to that of control monolayers. The antibody was added at the same time as the EGTA and was present in the medium after the restoration of normal Ca^{++} levels (Fig. 6).

DISCUSSION

MDCK cells have been used as a model system to study several functions of natural epithelia. One of the most striking features of confluent monolayers formed by these cells is the establishment of a structural and functional polarity and the development of a transepithelial electrical resistance as tight junctions form and seal. The nature of the molecules and events involved in the establishment of a confluent polarized monolayer with functional junctional complexes are poorly understood. One approach to this problem has been the identification of specific proteins that can be localized in the apical and/or basolateral domains as well as those that could be associated with the membrane junctions (for review see reference 14).

In this study we report the identification and immunochemical characterization of an MDCK cell surface glycoprotein with a molecular mass of 36,000, termed gp36, which seems to be involved in substrate attachment and spreading. This protein is accessible to surface iodination, remains in the cell surface after trypsinization, but is easily solubilized with nonionic detergents. We have confirmed the location of this glycoprotein at the cell surface by immunolabeling fixed cells with a specific antibody raised against gp36. We have established that gp36 is evenly distributed on the surface of trypsinized cells. In polarized monolayers with the tight junctions sealed, the distribution of gp36 is uniform on the apical surface. Treatment of monolayers with EGTA, which causes opening of the tight junctions, results in the labeling of apical and basolateral surfaces; this labeling suggests the redistribution of gp36. However, labeling closed monolayers from underneath indicates that gp36 is also present in the basal faces and in part of the lateral aspects when polarity is maintained.

The redistribution of surface proteins in epithelial cells by their physical separation or simply by opening the tight junctions by removal of Ca^{++} or treatment with colchicine or the cytochalasins has been reported (26–29). In MDCK cells, polarization occurs when suspended cells touch the substrate and each other. Formation and sealing of the zonula occludens seems to be necessary for the polarization of at least some cell surface components and viral proteins. Changes in the sealing capacity of the tight junctions elicits protein redistribution (12, 13, 30). Herzlinger and Ojakian identified a 35,000-D MDCK cell surface protein with a monoclonal antibody that does redistribute in this manner (31). This protein is reported to move from the basolateral to the apical surface when tight junctions are opened and move back as a function of time after the junctions have resealed. Since this latter redistribution takes place after the junctions are resealed, it is not clear how the protein crosses the tight junction barrier. On the other hand, in mammary epithelium, Dulbecco et al. (32) reported the nonpolar distribution of surface antigens and suggest that formation of the zonula occludens does not cause polarization. Two other proteins from the MDCK cell surface have been recently identified by Imhoff et al. (33). These are restricted to the apical face but apparently participate in cell-cell contact phenomena and tight junction stability.

The 36,000 D glycoprotein described here does not redistribute to a particular surface. We think that its apparent restricted localization to the apical surface of closed monolayers, as seen by electron microscopy using anti-gp36, is due to the inability of the antibody molecules to cross the closed junctions. When closed monolayers were labeled from the basal side, label was found extending along the basolateral aspects of the cells. When monolayers are exposed to EGTA, and the tight junctions are opened, several morphological changes occur in the cells that produce a wider intercellular space (8, 11). Therefore, antibody added to the apical side of these monolayers penetrated more easily, resulting in evenly labeled lateral surfaces. In the closed monolayers, steric hind-

erance would be expected to slow antibody diffusion along the lateral surface even when the antibody was applied from the basal side.

Binding of anti-gp36 to trypsinized cells decreased the efficiency of attachment and markedly affected the normal spreading of those cells that did attach. Washing to remove excess antibody bound to the cells before plating, allowed more cells to attach and regain a normal appearance, spread, interact, and form groups. This would indicate that at least part of the bound antibody is either released, so cells are capable of spreading, or the antibody/antigen complexes cap and are moved away from the perspective contact regions. In either case, cells could then spread on the substrate and make contacts. Experiments using FITC-labeled antibodies against gp36 showed no evidence for capping so we assume washing may be removing bound antibody.

Richardson and Simmons have shown that several proteins on the MDCK cell surface bind to Con A (4) and that the bound lectin has inhibitory effects on the formation of MDCK monolayers and development of the electrical resistance (34). The protein characterized here, gp36, binds to Con A, and antibodies against it inhibit processes of cell contact that lead to the formation of a confluent monolayer. Many molecules involved in cell adhesion and cell-cell contact have been recently characterized in several cell systems (for review see reference 35). In MDCK cells, Imhoff et al. (33) have identified two peptides of 30,000- and 140,000-D that have a role in cell-cell interactions, even when localized on the apical surface. Addition of antibodies against these peptides to MDCK monolayers causes a disruption of membrane junctions. Binding of anti-gp36 to monolayers with the tight junctions closed does not affect the electrical resistance, and the presence of the antibody has no effect on the kinetics of recovery of the steady-state electrical values. This indicates that the antibody does not perturb specific cell-cell interactions necessary for the functionality of the tight junction and suggests that gp36 plays a role in cell-substrate interactions.

The authors thank Dr. M. Cereijido for his continuous interest and support for this work and Dr. J. Bryan for critically reviewing the manuscript.

This research was supported by grants 5220 and 1508 from Consejo Nacional de Ciencia y Tecnologia, from which M. Sabanero was a predoctoral fellow.

Received for publication 30 July 1984, and in revised form 7 March 1985.

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