Dissociation of Madin-Darby Canine Kidney Epithelial Cells by the Monoclonal Antibody Anti-Arc-1: Mechanistic Aspects and Identification of the Antigen as a Component Related to Uvomorulin

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ABSTRACT It has previously been shown that the monoclonal antibody anti-Arc-1 dissociates Madin-Darby canine kidney (MDCK) epithelial cells and changes their morphology in vitro (Imhof, B. A., H. P. Vollmers, S. L. Goodman, and W. Birchmeier, 1983, Cell, 35:667–675). In this article we demonstrate that the anti-Arc-1 antibody recognizes an uvomorulin-like molecule on MDCK cells, i.e., it immunoprecipitates an 84-kD protein fragment from a tryptic digest of cell surfaces in the presence of Ca²⁺ (as does anti-uvomorulin antiserum). Furthermore, anti-uvomorulin antiserum prevents the binding of anti-Arc-1 to MDCK cells. The distribution of the Arc-1 antigen is also quite similar to that of uvomorulin: it is enriched at the cell–cell contacts both of MDCK cells and of cells in various canine tissues. In the intestinal epithelium the antigen could be further localized in the region of the junctional complex.

To study the mechanism of action of the dissociating antibody, MDCK cells grown on Nuclepore filters in Boyden chambers were exposed to anti-Arc-1 from either the upper or lower compartment. It could be shown that the antibody interfered with cell adhesion only from the basolateral but not from the apical cell surface. Antibody action was inhibited in the presence of colchicine but not cytochalasin B. Furthermore, cell dissociation was prevented when the cellular cAMP level was raised. These findings indicate that the anti-Arc-1 antibody acts on a target below the tight junctions (possibly on the antigen located in the junctional complex), and they confirm that cytoskeleton and metabolic factors are actively involved in the maintenance of junctional integrity.

In recent years the process of cell adhesion has been extensively studied. In one line of research, antibodies that specifically inhibit cell–cell contacts have been used to identify the components involved (1–6). The neural cell adhesion molecule (N-CAM) has been so identified, and it seems to form direct bridges between neural cells (1, 7, 8). Different types of adhesion components have been discovered in nonneuronal cells (liver cell adhesion molecule [L-CAM] in chickens [9]; uvomorulin and cadherin in the mouse [2, 10, 11]; and cell adhesion molecule 120/80 in humans [12]). Their biochemical characteristics and their distribution in different tissues indicate that these adhesion components may all be closely related. Thus, L-CAM/uvomorulin-like adhesion molecules are found on cell surfaces and have molecular weights of ~120,000, from which 84,000-mol-wt fragments can be cleaved by trypsin in the presence of Ca²⁺ (2, 13). They are enriched at cell contact areas in epithelial tissues (9), and uvomorulin has been localized by immunoelectron microscopy at the intermediate junctions of the intestinal epithelium.
MATERIALS AND METHODS

Immunofluorescence of Cells and Frozen Sections: MDCK cells were cultured to ~70% confluence on glass coverslips overnight (5), fixed with 3% formaldehyde in phosphate-buffered saline (pH 8.3) for 15 min, and permeabilized with 1% Triton X-100 for 5 min. They were then stained with 70 µg/ml purified anti-Arc-1 antibody (5), and diluted 1 µg/ml of p-phosphoryldiamine (to reduce fluorescence quenching) and examined on a Leitz Orthoplan photomicroscope.

Cryostat sections were cut to thicknesses of 0.5 or 4-7 µm on a Sorvall ultramicrotome MT-2B (DuPont Instruments-Sorvall Biomedical Div., Wilmingon, DE) equipped with a frozen thin sectioning system or a Reichert-Jung Frgocut model 2700 (Jung, Nussloch, FRG). Sections were fixed with formaldehyde on gelatin-coated glass coverslips and prepared for immunofluorescence as described above.

Immunoprecipitation and Binding Experiments: MDCK cells were grown on 15-cm plates (Falcon Labware, Oxnard, CA) and labeled with [35S]methionine as previously described (15). The cells were scraped off the plates into 1 ml L-CAM assay buffer (13) containing Ca++ and 0.2 mg/ml trypsin (type XI, Sigma Chemical Co., St. Louis, MO) and incubated for 40 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min in an Eppendoff microfuge. The supernatants were precleared four times on ice with 50 µl of a Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min in an Eppendoff microfuge. The supernatants were precleared four times on ice with 50 µl of a Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 min at 37°C. Egg-white trypsin inhibitor (0.8 rag, type II-O, Sigma Chemical Co.) was then added, and the cells were centrifuged for 5 mi...
FIGURE 1 Immunolocalization of the Arc-1 antigen on MDCK cells in tissue culture and on sections of the canine small intestine. (a) Immunofluorescence of fixed and permeabilized MDCK cells. (b) Corresponding phase-contrast image. (c) Immunofluorescence of the canine intestinal epithelium. (d) Corresponding phase-contrast image. The section was 0.5 μm thick. e, epithelial cell layer. lp, lamina propria. g, goblet cell. A control antibody (anti-FC-1, which does not react with MDCK cells; reference 16) showed no staining at the cell borders. (a and b) x 600. (c and d) x 770.

and in cell binding experiments. Uvomorulin can be specifically cleaved from the cell surface by trypsin in the presence of Ca\(^{2+}\), yielding a characteristic 84-kD glycoprotein fragment (2). Such a fragment could be immunoprecipitated from trypsin-treated MDCK cells by both anti-Arc-1 monoclonal antibody and a rabbit anti-uvomorulin serum (Fig. 3A). The 84-kD trypsin fragment of uvomorulin is a Con A-binding protein (2); we were also able to immunoprecipitate the 84-kD protein by the use of both antibodies from an eluant of a Con A column. In addition, anti-uvomorulin serum competed in a concentration-dependent manner with \(^{125}\)I-anti-Arc-1 binding to fixed and permeabilized MDCK cells (Fig. 3B), whereas preimmune serum did not. These findings suggest that anti-Arc-1 recognizes an uvomorulin-like molecule in the canine epithelial cells.

Anti-Arc-1 Antibody Acts on the Basolateral but Not the Apical Side of MDCK Cells

The localization of the Arc-1 antigen at the junctional complex of the intestinal epithelium raised the question of from which surface the dissociating antibody might reach the functional target. The anti-Arc-1 antibody was therefore presented to either the upper or the lower surface of confluent MDCK monolayers growing on Nuclepore filters in Boyden chambers. Cell dissociation occurred only when the antibody was delivered to the basolateral side of the cells (Fig. 4, compare a with b). The average number of extended contacts of individual cells to their neighbors was 1.5 or 3.8, when the antibody was added to the lower or upper side, respectively (40 cells counted in representative fields). Apparently the tight junctions prevented the access of the antibody to its functional target when it was applied to the apical surface. This hypothesis was confirmed by immunofluorescence experiments using confluent, live MDCK cells in the Boyden chamber. The anti-Arc-1 antibody bound to the cell contact areas of living cells only when it was presented from the lower chamber (Fig. 4, compare c with d).

Modulation of Anti-Arc-1 Action by Drugs that Affect the Cytoskeleton and the cAMP System

The anti-Arc-1 antibody dissociates MDCK cell monolayers and thus induces pronounced morphological changes in the cells (Fig. 5, a and b; the average number of contacts between individual cells and their neighbors dropped from 4.2 to 1.4; see also above). Cytoskeletal components or metabolic events may contribute to these effects. To examine the involvement of the cytoskeleton, the influence of colchicine and cytochalasins on anti-Arc-1 activity was tested. In the presence of colchicine, disruption of the MDCK monolayer by anti-Arc-1 was inhibited (Fig. 5c; the average number of cell contacts was 3.9). Cytochalasin B did not inhibit but rather slightly promoted antibody action (Fig. 5d; the average number of contacts was 1.1; cytochalasin D had a similar effect). Apparently, an intact microtubular network is necessary for antibody activity whereas the microfilamentous network is less important.

We also tested the effect of various drugs that influence cell metabolism. Antibody action was prevented when cellular
cAMP was raised (Fig. 5, e and f; contact numbers 3.5 and 3.7). This was accomplished by the addition of isobutyl methylxanthine (which inhibits cyclic nucleotide phosphodiesterase [18]), or forskolin (which activates adenylate cyclase [19]; see also Materials and Methods), or dibutyryl cAMP. Compounds that affect other metabolic processes such as cycloheximide, calmidazolium (an inhibitor of calmodulin action [21]), the Ca$^{2+}$ ionophore A 23187 (20), and serum (0–20%) did not interfere with the antibody effect (data not shown).

The anti-Arc-1 antibody also greatly reduces communication of MDCK cells through gap junctions, as assessed in dye coupling experiments (5). Most MDCK cells are coupled, and antibody treatment decreases the amount of coupling (Fig. 6, compare a with b). As observed for the morphological effects of the antibody, colchicine and the cAMP-elevating drug isobutyl methylxanthine counteract the uncoupling effects of the antibody (Fig. 6, c, e, and f), whereas cytochalasin B has no effect (Fig. 6d). Under the conditions used, the drugs alone did not influence coupling.

**DISCUSSION**

In the present study we present evidence that suggests that the Arc-1 antigen of MDCK cells is an uvomorulin-like molecule. First, the anti-Arc-1 monoclonal antibody precipitates an 84-kD glycoprotein cleaved from the cell surface by trypsin in the presence of Ca$^{2+}$. This cleavage product is characteristic of the L-CAM/uvomorulin family of adhesion proteins (2, 13). Second, anti-uvomorulin antisem efficiently competes for the binding of anti-Arc-1 to its target on fixed and permeabilized MDCK cells. Third, both antigens are localized at the borders of epithelial cells in culture and in certain tissues. Last, Arc-1 and uvomorulin are restricted to the junctional complex of the intestinal epithelium. Taken together, these findings provide strong evidence for a structural and functional homology between Arc-1 and uvomorulin.

The intact epithelial cell adhesion molecules of the L-CAM/uvomorulin family are 120-kD proteins (3, 10, 13). In our earlier work (5) we identified Arc-1 as both 120- and 40-kD components by gel filtration of EDTA-cell extracts (uvomorulin can be extracted with EDTA from mouse teratocarcinoma cells [compare reference 10], although detergent extraction is currently preferred). Thus, the 120-kD Arc-1 might represent the intact adhesion molecule, whereas the 40-kD Arc-1 is probably a breakdown product. With the anti-Arc-1 monoclonal antibody we have so far been unable to immunoprecipitate the undigested 120-kD molecule from detergent extracts of MDCK cells. However, the 120-kD uvomorulin could be readily identified in MDCK cells after immunoprecipitation with anti-uvomorulin antisem (not shown).
The Arc-1 antigen could be detected in a variety of canine organs such as skin, intestine, kidney, and liver. Despite this relatively widespread occurrence, it is restricted to specialized cell types within single tissues. For instance, it was detected on distal, but less on proximal, tubules of the kidney, on external but not internal root sheath of the hair follicle, and on distinct layers of the epidermis. The localization of the antigen in the distal kidney tubules correlates with the possible source of the MDCK cells (23), against which the antibody was raised. For L-CAM and uvomorulin, similar locations in certain epithelia have been reported. For instance, both proteins are enriched in the germinal layers of the epidermis and in the epithelia of the kidney tubules, but less in the epithelia of the glomeruli (9). The reason for this restriction in only some epithelia is not at present clear. It might reflect a common property of the various cell layers such as similar mechanical stability or similar state of differentiation.

We have also shown here that the Arc-1 antigen is enriched in the junctional complex of the intestinal epithelium (Fig. 1, c and d). This agrees with the distribution of uvomorulin in the intestine, where higher resolution analysis revealed yet further restriction to the intermediate junctions (14). We therefore could hypothesize that the anti-Arc-1 antibody produces cell dissociation by first binding to a target in the area of the junctional complex, e.g., at the intermediate junctions, thereby disturbing critical cell-cell contacts. Our Boyden chamber experiments support this assumption, since the cell-dissociating anti-Arc-1 antibody acts only from the basolateral and not from the apical side, which indicates that its functional target lies below the tight junctions. This also explains earlier observations from our laboratory, namely that cell dissociation by anti-Arc-1 was reduced when the MDCK cells grown on tissue culture plates were fully confluent; presumably the antibody could not penetrate the monolayer and reach its target in this case. In our earlier article (5) we showed that the Arc-1 antigen could also be detected on the apical surface of MDCK cells by the extremely sensitive technique of immunoscopying electron microscopy. The apical Arc-1 may thus represent a subpopulation of the antigen below the detection limit of immunofluorescence, or may only exist before the cells become fully polarized. Other investigators (24) have shown that polarization of certain antigens in MDCK cells does not occur until full confluence of the monolayer is reached.

The MDCK cell is an excellent in vitro system with which to study cell adhesion phenomena since it forms tight monolayers, is well polarized, and expresses a series of characteristic cell-cell and cell-substrate junctions (5, 25, 26). The present work indicates that the uvomorulin-like Arc-1 antigen represents a central adhesion component in the junctional complex of these cells, i.e., the disturbance of one type of adhesion molecule induces complete dissociation of the monolayer. Anti-Arc-1 destroys cell-cell contacts but also seems to abolish epithelial cell polarity, i.e., the cells move over one another and elaborate microvilli on their lateral surfaces (5). In effect, the cells are functionally deprived of Arc-1, as they would be after a deletion of the Arc-1 gene, or a down-regulation of the Arc-1 surface molecule. This suggests that the expression of Arc-1 is necessary for a correctly polarized monolayer and may alone be sufficient to impose polarity on the MDCK cells. If this is a correct model, then differential activation of genes for Arc-1-like molecules could be a decisive controlling
FIGURE 4 Boyden chamber experiments. MDCK epithelial cells were grown to confluence on Nuclepore filters placed in Boyden chambers and exposed to the anti-Arc-1 antibody from either the lower or the upper compartment as described in Materials and Methods. The antibody dissociated the cells only when delivered to the basal (a), not to the apical (b) side. × 1,200. In c and d binding of the anti-Arc-1 antibody to living cells in the Boyden chamber is illustrated by immunofluorescence. After short-term incubation with anti-Arc-1 from the lower (c) or the upper (d) compartment, the cells were fixed and stained with fluorescently labeled second antibody. Bars, 10 μm. × 800.

factor during organogenesis, when numerous mesenchymal-epithelial transitions occur. Recently, a monoclonal antibody against mouse uvomorulin (DECMA-1) was obtained which also dissociates MDCK cells (Vestweber, D., and R. Kemler, manuscript submitted for publication). It will be interesting to find out whether this antibody also has free access to its target only from the basolateral cell surface, and whether other antibodies (e.g., anti-L-CAM) act in the same fashion on polarized epithelial cells.

How could the cytoskeleton- and metabolism-perturbing drugs interplay with the cell-dissociating antibody? It is clear that the dissociation and shape change of MDCK cells is a complex multistep process that shifts the cells from an epithelial form (Fig. 5, a and b). In the epithelial state the MDCK cells are adhesive, communicative, and polar. In contrast, the fibroblastic cells exhibit little communication, few intercellular junctions, and a strong anterior-posterior polarity (25, 27). We can hypothesize that any modulator that shifts the cells between these two extreme states might also affect the degree of anti-Arc-1 action. For instance, it is known that cAMP can increase cell communication in certain systems (28), and colchicine has been shown to destroy anterior-posterior polarity of fibroblasts (29). Thus, under the influence of both these effectors an epithelial form might be favored. By contrast, cytochalasin B itself can induce spindle-shaped MDCK cells at higher concentration (e.g., at 10 μg/ml) and might therefore potentiate anti-Arc-1 action. After having considered the morphological changes during cell dissociation, we will now look at what might happen at the molecular level. The steric interference of the antibody with Arc-1 at or near cell contacts may lead to a cascade of further events, e.g., a local influx of Ca2+ followed by closure of the gap junctions (5, 30). The cells, having now broken off communications with their neighbors (Fig. 6), might then change their morphology, a change requiring cytoskeletal rearrangement. cAMP could counteract this since it keeps the gap junctions open (28) or might interfere with the cytoskeleton through phosphorylation. The colchicine experiments also point toward a critical role of the microtubules in these secondary processes.

We should also consider that the anti-Arc-1 antibody could actually trigger a change of differentiation of the MDCK cells from the epithelial to a fibroblastic (mesenchymal) stage. For instance, when Madin-Darby bovine kidney epithelial cells...
FIGURE 5 Effect of the anti-Arc-1 antibody on cell dissociation: modulation by various drugs that affect cytoskeleton and cAMP metabolism. MDCK monolayers were incubated for 3 h with anti-Arc-1 antibody in the presence and absence of the drugs as described in Materials and Methods. Cells were used (a) without antibody and drugs, or with (b) anti-Arc-1 antibody alone, (c) anti-Arc-1 antibody plus colchicine, (d) anti-Arc-1 plus cytochalasin B, (e) anti-Arc-1 plus 3-isobutyl methylxanthine, or (f) anti-Arc-1 plus forskolin. Addition of dibutyryl cAMP had the same effect as in e and f. Cells were processed for scanning electron microscopy as described in reference 5. Bar, 10 μm. X 900.
are plated under conditions in which no intimate cell contacts are formed, they express a higher concentration of mesenchymal markers (31). Furthermore, the drugs we have used are known to interfere with such differentiation processes: colchicine and cytochalasin have opposite effects on chondrocyte differentiation (32), and cAMP partially restores the differentiated state of transformed Chinese hamster ovary cells (33). It has recently been shown that tumor promoters produce morphological changes of MDCK cells similar to those of the anti-Arc-1 antibody (23, 34). However, these changes seem to be different from the ones described in the present study, since cAMP does not interfere with the effect of the tumor promoters (34), but cytochalasin B does.

In conclusion, in this article we have further characterized the Arc-1 antigen as being localized at the cell borders (the junctional complex) of epithelial cells, and shown structural and functional homology of Arc-I to molecules of the L-CAM/uvmorulin family of adhesion components. How these molecules are involved in the adhesion of epithelial cells (e.g., whether homo- or heterotypic interaction is necessary), awaits further investigation.

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