The MDCK Epithelial Cell Line Expresses a Cell Surface Antigen of the Kidney Distal Tubule

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ABSTRACT Monoclonal antibodies were prepared against the Madin-Darby canine kidney (MDCK) cell line to identify epithelial cell surface macromolecules involved in renal function. Lymphocyte hybrids were generated by fusing P3U-1 myeloma cells with spleen cells from a C3H mouse immunized with MDCK cells. Hybridomas secreting anti-MDCK antibodies were obtained and clonal lines isolated in soft agarose. We are reporting on one hybridoma line that secretes a monoclonal antibody that binds to MDCK cells at levels 20-fold greater than background binding. Indirect immunofluorescence microscopy was utilized to study the distribution of antibody binding on MDCK cells and on frozen sections of dog kidney and several nonrenal tissues. In the kidney the fluorescence staining pattern demonstrates that the antibody recognizes an antigenic determinant that is expressed only on the epithelial cells of the thick ascending limb of Henle's loop and the distal convoluted tubule and appears to be localized on the basolateral plasma membrane. This antigen also has a unique distribution in non-renal tissues and can only be detected on cells known to be active in transepithelial ion movements. These results indicate the probable distal tubule origin of MDCK and suggest that the monoclonal antibody recognizes a cell surface antigen involved in physiological functions unique to the kidney distal tubule and transporting epithelia of nonrenal tissues.

The mammalian kidney is a complex organ containing a variety of epithelial cell types that exhibit unique morphological, biochemical, and physiological properties. Studies of renal function at the cellular and molecular levels have been hindered by the heterogeneity of epithelial cell types, and several experimental approaches have been utilized to circumvent these difficulties.

Studies on renal tubules isolated by microdissection have provided considerable information on both the functional segmentation and corresponding hormonal sensitivity of the nephron (1, 2); however, this technique does not provide sufficient quantities of discrete tubular segments for biochemical analysis. To overcome the paucity of homogeneous material, procedures have been developed for the separation of homogeneous cell populations from kidney cortex (3, 4) and, more recently, medulla (5). In addition, the development of a serum-free, defined medium for kidney epithelial cells (6) has allowed the establishment of long-term renal epithelial primary cell cultures free of fibroblasts (6, 7).

An alternative approach has been the utilization of kidney epithelial cell lines as models for transporting epithelia (8). The Madin-Darby canine kidney (MDCK) cell line, isolated from dog kidney cortex (9), has been studied extensively and shown to retain many of the differentiated properties associated with the kidney tubular epithelium. Morphologically, MDCK cells exhibit apical microvilli, junctional complexes, and lateral membrane infoldings (10–13) characteristic of transporting epithelia (14). Physiologically, MDCK cells transport both sodium and water in an apical-to-basal direction (10–12) and, when grown on permeable substrates, can generate a transepithelial electrical resistance (11–13) demonstrating the presence of functional tight junctions (15). Finally, MDCK cells have asymmetrically distributed plasma membrane polypeptides (16, 17) and support the polarized budding of lipid envelope viruses (18).

To study cell surface components characteristic of a renal transporting epithelium in a well-defined cell culture system, we have utilized the hybridoma technique developed by Kohler and Milstein (19, 20) to prepare monoclonal antibodies directed against the MDCK cell surface. This technique allows the generation of monospecific antibodies against both major and minor membrane components (21). Since these antibodies are homogeneous and recognize only a single antigenic determinant, they have been utilized in the structural, functional, and biochemical analysis of cell surface macromolecules (21–23). In this paper, we report on a monoclonal antibody that recog-

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nizes an MDCK cell surface antigenic determinant that also is expressed in adult dog kidney only on the epithelial cells of the thick ascending limb and distal convoluted tubule.

MATERIALS AND METHODS

Cell Culture

MDCK epithelial cells obtained from Dr. J. Leighton (Medical College of Pennsylvania) were grown in complete Eagle's minimal essential medium with Earle's salts (MEM), penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal calf serum (Gibco Laboratories, Grand Island, NY). Cultures were routinely passaged at confluence using 0.25% trypsin/2 mM EDTA for cell detachment. The myeloma cell line P3X63Ag8U1 (P3U-1) was obtained from Dr. J. Unkeless (The Rockefeller University). This cell line synthesizes κ light chains but not γ heavy chains, lacks hypoxanthine guanine phosphoribosyl activity, and will not grow in HAT medium (24). P3U-1 cells were grown as stationary suspension cultures in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/liter glucose, 3.7 g/liter $NaHCO_3$, and 10% fetal calf serum.

Hybridoma cell lines were maintained as described for myeloma cells in a culture medium (IM) that contained 66 vol of DMEM, 14 vol of an amino acid and vitamin solution described by Iscove and Melchers (25), and 20 vol of selected fetal calf serum.

Primary dog kidney fibroblast cultures were initiated by mincing dog kidney cortex in CLS collagenase (Worthington Biochemical Corp., Freehold, NJ) according to the protocol of Taub et al. (6). These cultures were grown in MEM with 10% fetal calf serum and passaged at least five times with 0.12% trypsin in phosphate-buffered saline (PBS) to ensure that they contained only fibroblasts. Primary cultures of rat embryo fibroblasts (Flow Laboratories, Inc., Rockville, MD) were grown in DMEM containing 10% fetal calf serum and passaged as described for MDCK cells.

Generation of Hybridoma Cell Lines

The fusion protocol of Gefter et al. (26) was utilized to produce hybridomas secreting anti-MDCK monoclonal antibodies. A C3H mouse was immunized by two intraperitoneal injections of 10^7 MDCK cells administered 3 wk apart. 3 d after the second injection, the spleen was removed and teased in DMEM containing 20 mM HEPES (DMEMH). Released splenocytes were centrifuged at 1,500 rpm, resuspended in 0.17 M NH_4Cl, and incubated for 10 min at 4°C to lyse erythrocytes. For cell fusion, 10^8 splenocytes were mixed with 2×10^7 P3U-1 myeloma cells, the cells were pelleted, resuspended in 0.2 ml of 30% polyethylene glycol 1000 (PEG) in DMEMH, and centrifuged again. After incubation in PEG for 8 min at 20°C, DMEMH was added and the pellet very gently resuspended. Cells were then collected, resuspended in 20 ml of HAT medium (IM containing 100 μM hypoxanthine, 10 μM aminopterin, and 30 μM thymidine), and seeded at 10^5 cells/well into 96 cluster microtiter plates (Flow Laboratories, Linbro Div., Hamden, CT). Culture supernates were screened for anti-MDCK activity 2 wk after the fusion, cells in positive wells were expanded and stock cultures were frozen in 5 vol of IM, 4 vol of fetal calf serum, and 1 vol of DMSO. Hybridomas were maintained in HAT medium for 1 mo, HT medium (HAT medium lacking aminopterin) for 2 wk, and thereafter in IM. Clonal cell lines were isolated by plating at low density (2,000 cells/100-mm plate) in IM with 0.55% agarose (Seaplaque; Seakem, Marine Colloids Div., Rockland, ME) over a rat embryo fibroblast feeder layer. Cells were grown for 1 wk and individual colonies were picked and grown in liquid culture as described.

Indirect Radioimmunoassay (RIA)

An indirect RIA was utilized to detect monoclonal antibody binding to externally exposed MDCK antigenic determinants. Preparation of ^{125}I-labeled goat anti-mouse IgG (GAM) follows the protocol of Herzenberg and Herzenberg (27) as modified in our laboratory. GAM (Cappel Laboratories Inc., Cochranville, PA) was applied to an affinity column of mouse IgG (Cappel Laboratories, Inc.) conjugated to cyanogen bromide–activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ), and GAM iodination catalyzed by chloramine-T (28) was performed on the column to protect antibody combining sites. After free ^{125}I had been washed from the column with PBS, iodinated GAM was eluted with 0.2 M glycine (pH 2.3) containing 1% bovine serum albumin (BSA) into an equal volume of 0.2 M Tris-HCl containing 1% BSA (pH 8.6). ^{125}I-GAM fractions were pooled, and aggregates were removed by gel filtration on a column of Sephadex G-200 (Pharmacia Fine Chemicals, Inc.) and stored at −20°C. The specific activity of the ^{125}I-GAM was 5–15 μCi/μg protein.

For the RIA, MDCK cells were plated in 96 cluster microliter wells at 5×10^3 cells/well and grown for 48 h to a density of 2×10^4 cells/well. This subconfluent density ensures access of antibody reagents to the entire MDCK cell surface. Cells (MDCK and fibroblasts) were washed with PBS containing 2% BSA, incubated with 50 μl of hybridoma supernatant for 1 h at 4°C, then extensively washed with PBS-BSA and incubated with 50 μl of ^{125}I-GAM (10^5 cpm/well) for 1 h at 4°C. The cells were washed, solubilized in 30 μl of 2% Triton X-100, swabbed from the wells, and counted in a Beckman gamma counter. To determine the binding of monoclonal antibody to Na^+,K^+-ATPase, purified ATPase (29) prepared from adult dog kidney medulla (provided by B. Forbush) was dried in polyvinyl chloride microtiter plates (Dynatech Laboratories Inc., Alexandria, VA) at 0.2 μg of protein/well. The Na^+,K^+-ATPase was incubated with antibodies as described above, individual wells were cut from the plate, and the bound radioactivity was determined.

Preparation of Kidney Frozen Sections

Kidneys were removed from mongrel dogs (30–50 lbs) (anesthetized with pentobarbitol sodium, 30 mg/kg) and immersed in cold PBS. Kidney cortex, medulla, and papilla were dissected, cut into 2-mm cubes, and immersed in 1% paraformaldehyde/0.1% glutaraldehyde in PBS for 1 h at 4°C. Tissue blocks were rinsed three times in PBS, incubated overnight at 4°C in PBS containing 30% sucrose and 25 mg/ml lysine, then frozen for 20 min in isopentane cooled over liquid N_2, and stored at −80°C. To prepare frozen sections, tissue blocks were mounted on aluminum chucks in Tissue-Tek, (Lab-Tek Div., Miles Laboratories Inc., Naperville, IL) and 8-μm sections were cut on a Slee cryostat (Slee International Inc., New York) at −20°C.

Indirect Immunofluorescence

All antibody reagents were purchased from Cappel Laboratories, Inc. and affinity-purified on an IgG–Sepharose 4B column as described above. MDCK cells were grown on 18-mm glass cover slips for 48 h, fixed with 2% paraformaldehyde for 30 min at 4°C, rinsed in PBS containing 2% BSA, and incubated at 4°C for 45 min each in (*a*) hybridoma supernatant, (*b*) rabbit anti-mouse IgG (RAM), and (*c*) rhodamine-labeled goat anti-rabbit IgG (rhodamine-GAR). Before and after each incubation, the cells were thoroughly washed in PBS/BSA. Specimens were then postfixed in 4% paraformaldehyde-PBS and mounted on slides in 90% glycerol. Indirect immunofluorescence on dog kidney frozen sections was performed with hybridoma supernatant as the first antibody, followed by fluorescein-labeled goat anti-mouse IgG (FITC-GAM). All samples were viewed with epifluorescence optics (Zeiss and Leitz) and photographed on Tri-X film (Kodak).

Characterization of Monoclonal Antibody

METABOLIC LABELING OF ANTIBODY: Hybridoma cells growing in logarithmic phase were adjusted to a concentration of 10^6 cells/ml in PBS and incubated for 30 min at 37°C. Cells were centrifuged at low speed and resuspended at the same cell concentration in MEM containing low methionine (3 μg/ml), 10 μCi/ml [^{35}S]methionine (New England Nuclear, Boston, MA) and 10% dialyzed fetal calf serum. After a 24-h incubation at 37°C, supernatant was harvested and stored at −20°C.

TWO-DIMENSIONAL PAGE: Labeled, secreted antibody was analyzed by two-dimensional (2D) PAGE with isoelectric focusing (IEF) in the first dimension and SDS PAGE in the second dimension (30).

For IEF, hybridoma supernatant was added to an equal volume of sample buffer (SB), which included 3.5% Nonidet P 40, 9% β-mercaptoethanol, 9 M urea, and 8% ampholytes (2% pH range of 3–7, 4% 7–9; 2% 3.5–10; LKB Instruments Inc., Rockville, MD). The sample was boiled for 1 min, and a 2-μl aliquot was applied to a tube gel (length 75 mm, diameter 1.1 mm) of 7% acrylamide, 0.16% *bis*-acrylamide, 8% Nonidet P-40, 9 M urea, and 8% carrier ampholytes in the same ratio as in the SB. The sample was overlayed with SB containing 2 M urea, gel mounted with electrolyte solutions (upper chamber cathode, 20 mM NaOH; bottom chamber annode, 20 mM phosphoric acid), and focused for 18 h at 150 V and 30 min at 400 V. Gels from capillary tubes were rinsed and overlaid onto a 12.5% SDS polyacrylamide slab gel and run for 2 h at 5 mV (31), incubated in 20% TCA, stained in 0.4% Coomassie Blue, and prepared for fluorography (32). Dried gels were then exposed to Kodak XR1 x-ray film for 48 h at −80°C.

RESULTS

Production and Characterization of Monoclonal Antibodies

Hybridomas were produced by fusing P3U-1 myeloma cells with spleen cells from a mouse immunized with MDCK cells. 92 of the 96 initial microtiter wells containing hybridomas

secreted anti-MDCK cell surface antibodies when assayed by RIA on live MDCK cells. Some of the hybridomas from positive wells were cloned in soft agarose and frozen for future use.

In this study we report exclusively on hybridoma cell line 11B8, a clone which secretes a monoclonal antibody that recognizes a cell surface antigenic determinant on both MDCK and selected dog kidney epithelial cells. Cell line 11B8 has been cloned four times in soft agarose and has been stable for >1 yr in culture. Monoclonal antibody 11B8 (designated antibody 11B8) binds to MDCK cells at levels 20-fold greater than binding to either rat embryo fibroblasts or primary dog kidney fibroblasts (Table I). The binding of this antibody to the two fibroblast cell types is comparable to that of the controls determined by *a*) the binding of an anti-skeletal muscle myosin monoclonal antibody (kindly provided by Donald A. Fischman) or *b*) the omission of first antibody (Table I). The specificity of antibody 11B8 is also supported by the observation that saturation binding of antibody 11B8 to MDCK cells can be demonstrated at up to 10-fold dilutions of hybridoma culture medium (data not shown).

Antibody 11B8 has been characterized as IgG_1 containing κ light chains by Ouchterlony gel diffusion (data not shown). To demonstrate that hybridoma line 11B8 was monoclonal, cells were grown in medium containing [^{35}S]methionine, and the labeled, secreted product was analyzed by 2D PAGE. When fluorograms of the gels were examined, a single light chain and two major and one minor heavy chain spots were observed (Fig. 1). This result is compatible with that expected for a monoclonal antibody since the heavy chain is subject to post-translational modifications which can give rise to heterogeneity in its isoelectric point (33, 34).

Antigen Localization on MDCK Cells

Indirect immunofluorescence microscopy demonstrates that antibody 11B8 binds to the surface of all the MDCK cells in our subconfluent cultures (Fig. 2). Because immunofluorescence staining of MDCK cells using only antibody 11B8 and FITC-GAM was very faint, an intermediate RAM labeling step was used to amplify the fluorescent image. MDCK cells were sequentially labeled with antibody 11B8, unlabeled RAM, and then rhodamine-GAR. No fluorescence staining was detected on control samples which included incubation of cells in *a*) an anti-myosin monoclonal antibody, RAM, and rhodamine-GAR, and *b*) antibody 11B8 and rhodamine-GAR. When immunofluorescence localization was performed at 20°C on live cells, the cell surface fluorescence staining pattern was very patchy (data not shown), indicating that antigen 11B8 can move laterally in the membrane plane.

TABLE I

Binding of Antibody 11B8 Determined by Indirect RIA

Cell type	First incubation		
	11B8	Anti-myosin	PBS
MDCK	3,685* ± 306	354 ± 20	150 ± 8
Rat embryo fibroblasts	119 ± 18	129 ± 7	—
Dog kidney primary fibroblasts	169 ± 6	173 ± 10	147 ± 4

2×10^4 cells in microtiter wells were incubated with 11B8 hybridoma supernatant for 1 h at 4°C, washed, and incubated with ^{125}I-GAM (10^5 cpm) as described in Materials and Methods. Cells were solubilized and the bound radioactivity was determined. As controls, an anti-myosin monoclonal antibody or washing buffer (PBS) was substituted for antibody 11B8.

* Cell-associated radioactivity was determined for six replicate samples and expressed as the average cpm bound ± the standard error of the mean.

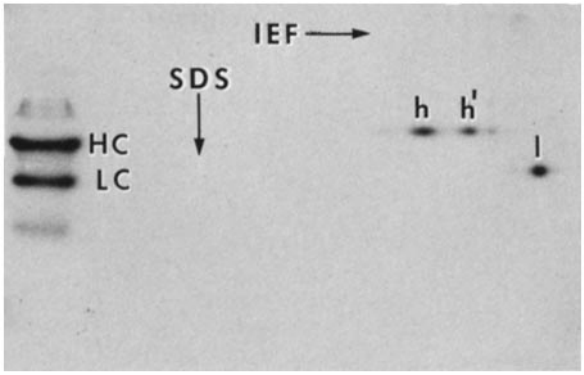

FIGURE 1 Analysis of antibody 11B8 by 2-D PAGE. Hybridoma line 11B8 was suspended in medium containing [^{35}S]methionine (10 μCi/ml), and the supernatant was collected after 24 h. The supernatant was diluted with an equal volume of SB, heated to 100° for 1 min, and a 2-μl aliquot was analyzed by IEF (pH 3.5-10) followed by SDS-PAGE in a 12.5% gel as described in Materials and Methods. The acidic end of the IEF gel is at the right. In an adjacent lane at the left of the 2-D gel, an aliquot of 11B8 supernatant was electrophoresed in one dimension. Polypeptides identified by 2-D PAGE of antibody 11B8 include two major spots (*h, h'*) and one minor spot corresponding to the IgG heavy chain (*HC*) in SDS PAGE, and a single spot (*l*) corresponding to the IgG light chain (*LC*).

Antigen Distribution in Dog Kidney

Since MDCK cells were originally derived from dog kidney cortex (9), indirect immunofluorescence microscopy of dog kidney frozen sections was performed to determine whether antigen 11B8 was expressed in vivo. Sections of kidney cortex, medulla, and papilla were screened for this antigen utilizing hybridoma culture supernatant as first antibody and FITC-GAM as the fluorescent tracer. When selected fields were examined with both phase-contrast and epifluorescence optics, fluorescent staining could only be detected on a small proportion of cortical tubules and not in glomeruli, renal vasculature, or interstitium (Fig. 3). The cortical tubules that bind antibody 11B8 are of two distinct morphologies: tubules with a low cuboidal epithelium characteristic of the thick ascending limb (TAL) are present in both medullary rays and the cortical labyrinth, and tubules present only in the cortical labyrinth with a higher cuboidal epithelium characteristic of distal convoluted tubules (DCT). The proximal convoluted tubule (PR), which is characterized by a high epithelium with an irregular apical cell border that obscures the tubule lumen, lacks fluorescent staining (Fig. 3).

To verify the phase-contrast identification of renal tubules, adjacent serial frozen sections were processed for immunofluorescence and conventional hematoxylin and eosin (H/E) staining, respectively. Sections were examined with both phase and epifluorescence optics and then the identical fields were found on adjacent sections stained with H/E. In the H/E sections the most eosinophilic tubules with the high irregular epithelium correspond to the PR, whereas tubules with a cuboidal epithelium correspond to the TAL and DCT (Fig. 4*a*). Tubules that stain with the fluorescent antibody are the TAL and DCT (Fig. 4*b*), confirming our previous observations with phase-contrast microscopy (Fig. 3). At higher magnification, antigen 11B8 appears to be distributed on only the basolateral aspect and could not be detected on the apical

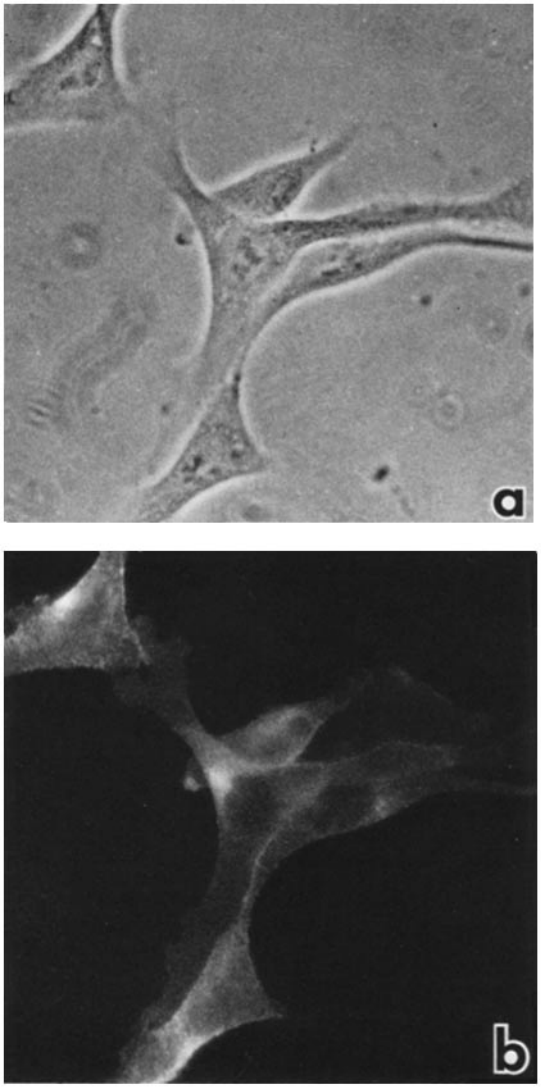

FIGURE 2 Antibody 11B8 binding to MDCK cells. Prefixed MDCK cells were incubated sequentially in hybridoma supernatant, rabbit anti-mouse IgG, and rhodamine-conjugated goat anti-rabbit IgG. Phase-contrast (*a*) and corresponding immunofluorescence (*b*) micrographs of a low-density culture demonstrate that antibody 11B8 binds to all cells of the MDCK population. X 650.

surface with the immunofluorescence technique (Fig. 5). In preliminary ultrastructural studies, we have utilized horseradish peroxidase coupled to GAM to demonstrate that antigen 11B8 is localized on the basolateral infoldings of the plasma membrane in TAL and DCT and that the antigen could not be detected within the cytoplasm of these tubules (unpublished observations). Fluorescent staining was observed on the tightly packed cells of the macula densa contacting the glomerular vascular pole (Fig. 6). Some cells of the cortical collecting tubule (CCT) bind antibody 11B8 but, unlike the TAL and DCT, the CCT also has a population of cells on which antibody binding could not be detected (data not shown). In sections through the outer medulla near the cortical medullary junction, antigen 11B8 is present on cells of the TAL but absent from thick descending limbs and medullary collecting tubules (Fig. 7). As was observed for tubules in the cortex, antigen 11B8 is distributed only on the basolateral membrane and not on the apical cell surface (Fig. 7). Deeper in the medulla, antigen 11B8 can still be observed on epithelial cells of the TAL but is absent from collecting tubules, thin segments of Henle's loop and the vasa recta (Fig. 8). On many of the TAL, the immunofluorescence staining is arranged in linear arrays that run parallel to the lateral cell borders (Fig. 8), suggesting that the antigen is present on basal infoldings of the plasma membrane. The inner medulla containing large papillary collecting ducts was completely negative for antibody 11B8 staining, as were control sections of cortex and medulla incubated with three different anti-myosin monoclonal antibodies (subclass IgG_1) and FITC-GAM. Because the immunofluorescence detection of antibody 11B8 binding on MDCK required amplification by the addition of an intermediate unlabeled RAM and a subsequent incubation in rhodamine-GAR, the same procedures were used on kidney frozen sections to determine whether antibody 11B8 binding could be detected on proximal tubules or medullary collecting tubules. Results of these experiments, however, were identical to those obtained when antibody 11B8

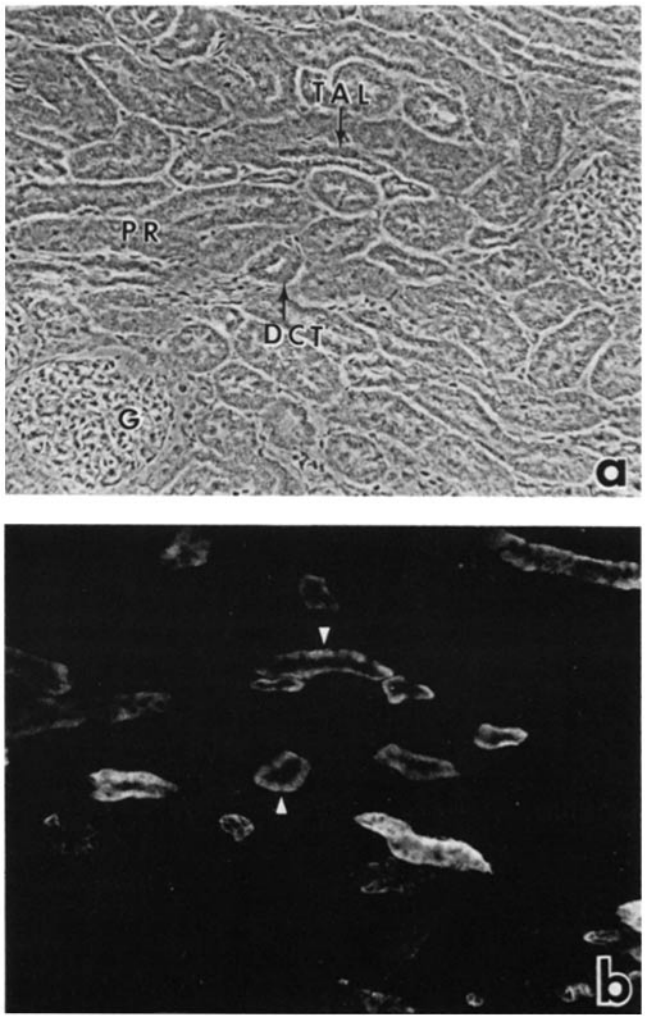

FIGURE 3 The distribution of antigen 11B8 in dog kidney cortex. Frozen sections of dog kidney cortex were incubated in hybridoma 11B8 supernatant followed by FITC-GAM. Nephron segments identified in phase-contrast microscopy (*a*) include glomeruli (*G*), proximal tubules (*PR*), thick ascending limbs (*TAL*), and distal convoluted tubules (*DCT*). Immunofluorescence microscopy (*b*) of the same field demonstrates that only the epithelial cells of the thick ascending limb and distal convoluted tubule bind antibody 11B8 (white arrows). × 150.

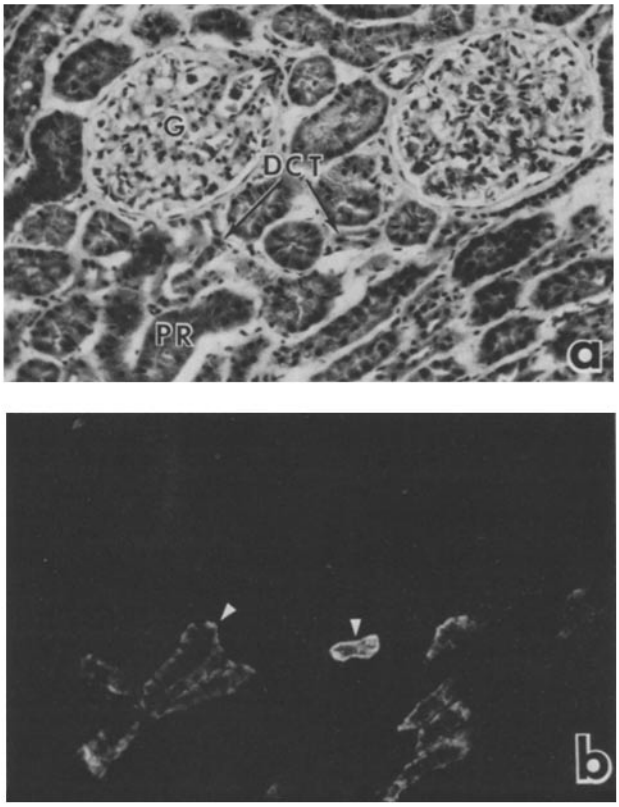

FIGURE 4 The identification of dog kidney cortical tubules with phase-contrast microscopy was verified by hematoxylin-eosin (H/E) staining. Adjacent frozen serial sections were processed for either H/E (*a*) or immunofluorescence (*b*) microscopy. Renal glomeruli (*G*), proximal convoluted (*PR*) and distal convoluted tubules (*DCT*) were identified by H/E staining (see Results for a complete discussion) and compared with their corresponding phase-contrast and immunofluorescence images on the adjacent serial section. The distal convoluted tubules have a cuboidal epithelium and bind antibody 11B8 (white arrows), whereas the other nephron segments do not. × 175.

binding is visualized by FITC-GAM, with no fluorescence being observed on tubules other than the TAL and DCT.

Binding of Monoclonal Antibody to Na^+,K^+-ATPase

Since Na^+,K^+-ATPase is one of the major kidney medulla membrane polypeptides (29), the binding of antibody 11B8 to the purified ATPase was analyzed by RIA to determine whether we had obtained a monoclonal antibody to this protein. Utilizing an antiserum to the ATPase as a positive control, we have determined that antibody 11B8 does not bind to kidney medulla Na^+,K^+-ATPase (Table II).

Antigen Distribution in Nonrenal Tissues

As the selective expression of antigen 11B8 in the kidney suggested a functional significance, the transporting epithelia of several nonrenal tissues were surveyed for antigen expression by indirect immunofluorescence microscopy. Frozen sections of submandibular and parotid salivary glands and dog pancreas were incubated in 11B8 hybridoma supernatant and FITC-GAM. In the pancreas, antibody 11B8 binds only to cells of the intralobular and interlobular ducts and not on the acinar cells (Fig. 9) while in the salivary glands the antigen is present on the plasma membranes of the secretory end pieces as well as the striated and excretory ducts (data not shown). Experiments are currently in progress to characterize the cell surface component(s) that bind antibody 11B8 in MDCK cells and renal and nonrenal tissues. Until these studies are completed, we cannot exclude the possibility that antibody 11B8 is binding to an antigenic determinant that is common to different cell surface molecules (35).

DISCUSSION

Utilizing a monoclonal antibody generated against MDCK cells, we have detected a cell surface antigenic determinant present on both the MDCK cell line and the epithelial cells of the dog kidney distal tubule including both the thick ascending limb and distal convoluted tubule. This antigen is not present on cells of the glomeruli, proximal tubules, thin segments, vasculature, or interstitium. These results provide the first direct evidence for the existence of a nephron segment-specific cell surface antigen and its expression on MDCK cells.

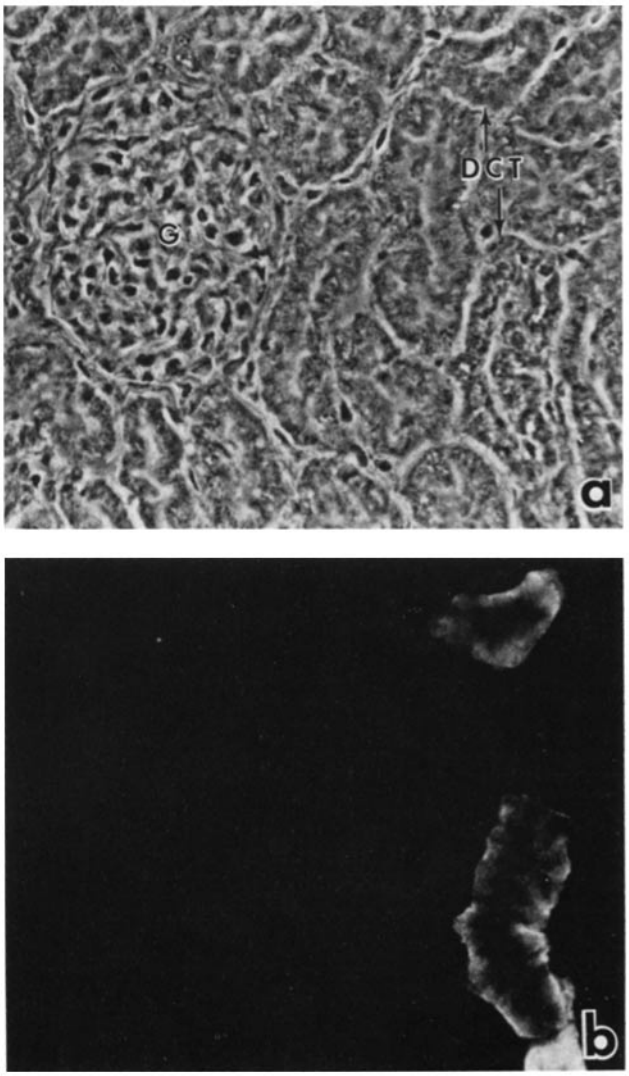

FIGURE 5 The basolateral distribution of antigen 11B8 on cortical tubules. Phase-contrast (*a*) and corresponding immunofluorescence micrographs (*b*) of dog kidney cortex frozen sections. The two distal convoluted tubules (*DCT*) in the field express antigen 11B8 on the basolateral (but not apical) region of the cell. A glomerulus (*G*) is unstained. × 300.

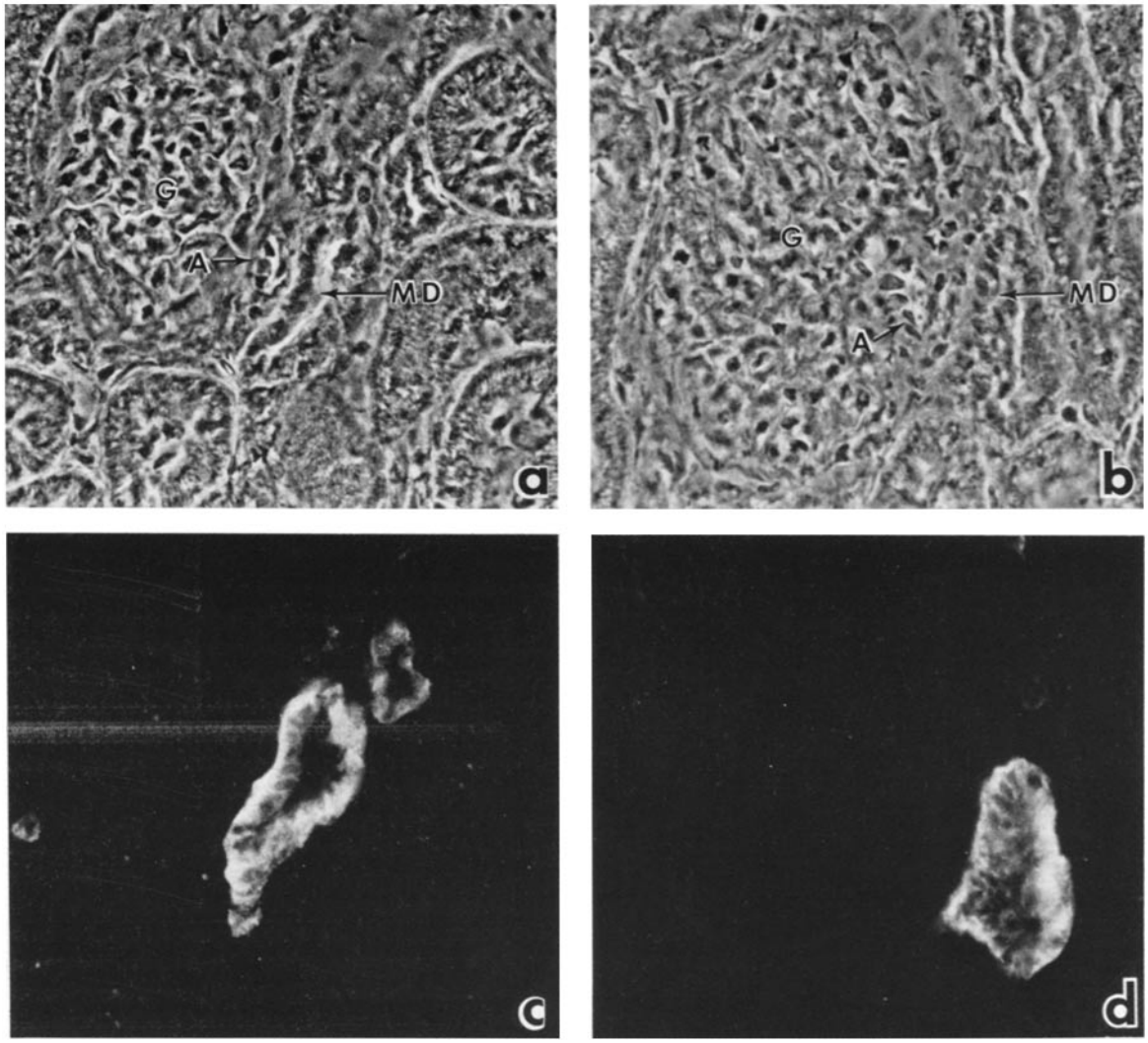

FIGURE 6 Localization of antigen 11B8 in the macula densa. Phase-contrast (*a* and *b*) and corresponding immunofluorescence (*c* and *d*) micrographs of dog kidney cortical regions that have macula densa (*MD*) adjacent to an accompanying arteriole (*A*) at the glomerular (*G*) vascular pole. Immunofluorescence staining demonstrates that the MD segment of the thick ascending limb expresses antigen 11B8. (*a* and *c*) × 300; (*b* and *d*) × 350.

Since their initial isolation by Madin and Darby in 1958 and their characterization by Gausch et al. (9), MDCK cells have been used extensively as a model for a transporting renal epithelium, although their nephron segment origin (or origins) has never been clearly defined. MDCK cells have retained certain differentiated properties in vitro, including the ability to reform tubule-like structures after their injection into baby nude mice (36), some of the ultrastructural characteristics of the distal tubule (10–12), and transepithelial resistances (11–13, 37) that approximate those measured for the distal tubule (38). These values are considerably higher than those recorded for isolated TAL segments (39). MDCK cells produce elevated levels of cyclic AMP in the presence of vasopressin (36, 40), and this observation has been used to support a distal tubule origin. However, this hormonal response is also common to the collecting duct in all species examined (2).

It is not clear whether nephron segment-specific properties reflecting the origin of MDCK have been faithfully retained or modulated during growth in culture, or whether the various MDCK sublines in use are essentially identical or even homogeneous. For example, recent observations indicate that low-passage MDCK monolayers display much higher electrical resistances (41) than those cited previously (11–13, 37) and possess morphological characteristics of both DCT and collecting tubule (42). Similarly, LLC-PK_1, another well-studied renal epithelial cell line, expresses characteristics associated with a variety of renal tubule segments (8). Our data suggest that the high-passage MDCK cell line used in this study is derived from either the DCT or TAL, and that these cells are homogeneous for expression of a segment-specific cell surface antigen. This tentative identification is based on antigen 11B8 expression and should be treated with some caution until other segment-specific antigens can be identified and compared with 11B8.

Although medullary and papillary collecting ducts do not bind detectable levels of antibody 11B8, a definitive identification of the most distal cortical nephron segment containing 11B8 antigen is more difficult. The transition from DCT to collecting tubule (CT) in well-studied examples varies considerably among species. In the rabbit, an abrupt transition from the distal to the connecting tubule has been characterized morphologically (43). A more gradual transition with the intermingling of different cell types has been observed by electron microscopy in the rat (44), human (45), and desert rodent (46).

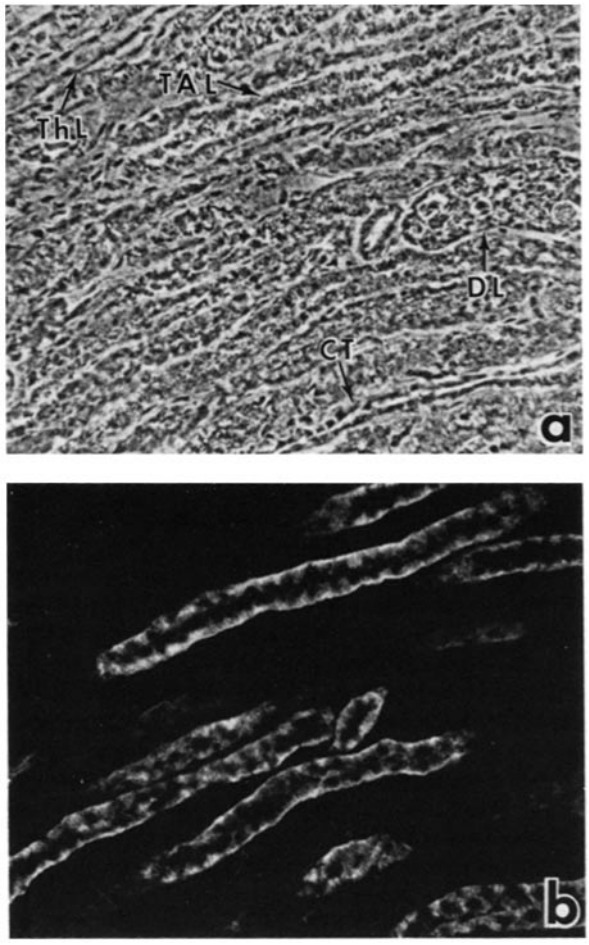

FIGURE 7 The distribution of antigen 11B8 in the outer medulla. Phase-contrast micrograph (*a*) of a frozen section through the cortical-medullary junction that includes thin segments (*ThL*), descending limb of the proximal tubule (*DL*), thick ascending limbs (*TAL*), and a collecting tubule (*CT*). The corresponding fluorescence micrograph (*b*) demonstrates that only the thick ascending limb expresses antigen 11B8. As demonstrated previously (Fig. 5), this antigen appears localized on only the basolateral region of the cell. × 200.

The dog distal segment has been reported to most closely resemble the human distal segment in its morphology (47). Preliminary immunofluorescence results suggest that antigen 11B8 is only expressed on some cells of the cortical tubules that correspond to this transition zone between DCT and medullary CT. High-resolution immunoelectron microscope studies of antigen 11B8 distribution now in progress should allow a definitive identification of the cell type (or types) present in this zone that express the antigen.

The antigen recognized by antibody 11B8 has not yet been isolated and characterized; however, our unpublished observations that *a*) this antigen cannot be extracted from dog kidney medulla membranes (29) with a mixture of chloroform and methanol (2:1, vol/vol) and that (*b*) treatment of MDCK cells with glutaraldehyde, a bifunctional cross-linking agent, reduces antigenicity suggest that the antigen is probably a protein.

On the basis of the selective distribution of antigen 11B8 in the kidney, we have been able to rule out the possibility that this antigen is one of several previously identified renal proteins. Histocompatibility antigens are expressed on both fibroblasts and lymphoid tissue (48), and Thy-1 antigen has been detected in kidney, brain, and fibroblasts (49). However, antigen 11B8 has not been detected on dog fibroblasts (Table I) or in frozen sections of either lymph node or brain (data not shown). Tamm-Horsfall glycoprotein has been localized on the

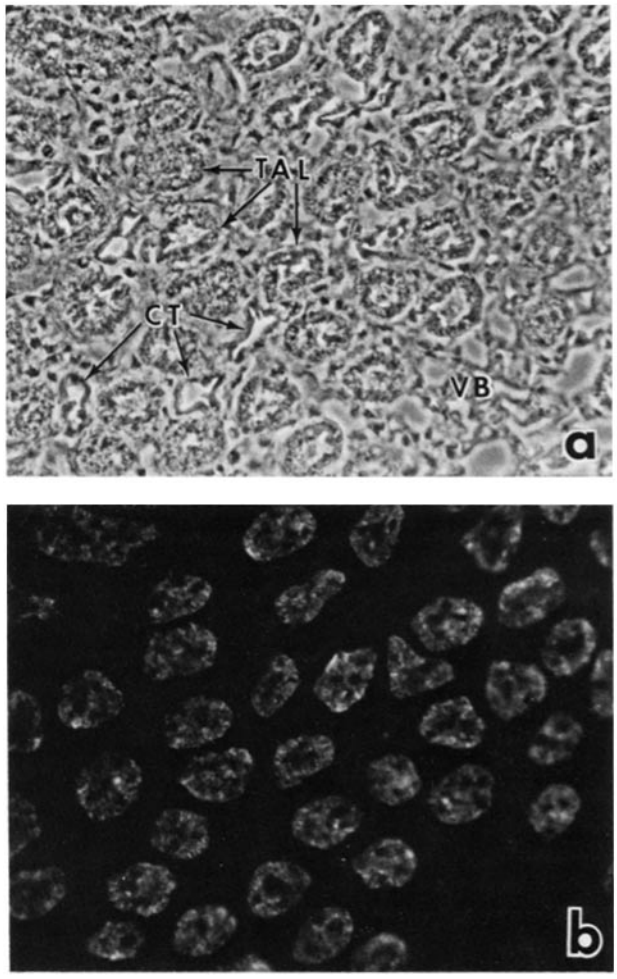

FIGURE 8 The distribution of antigen 11B8 in the medulla. Frozen cross section of kidney deeper in the medulla. In phase contrast (*a*), well-developed vascular bundles (*VB*) containing thin segments and vasa recta, thick ascending limbs (*TAL*), and collecting tubules (*CT*) can be observed. Immunofluorescence microscopy (*b*) demonstrates that only the thick ascending limb expresses antigen 11B8. The linear staining pattern on many of the tubules suggests that the antigen is localized on the basal infoldings of the plasma membrane. × 250.

TABLE II

Binding of Antibody 11B8 to Purified Na^+,K^+-ATPase

Antibody	Antibody binding
	cpm
Anti-Na^+,K^+-ATPase	6,238
11B8	358
Anti-myosin	404

Na^+,K^+-ATPase purified from dog kidney medulla was dried in polyvinyl chloride microtiter plates. The ATPase was then incubated with either a 1:100 dilution of rabbit antiserum against dog kidney medulla Na^+,K^+-ATPase (provided by D. Biemesderfer), 11B8 hybridoma supernatant or an antimyosin monoclonal antibody (subclass IgG_1), washed, and then incubated in ^{125}I-GAM under the same conditions described for MDCK cells in Materials and Methods. Standard errors were <10%.

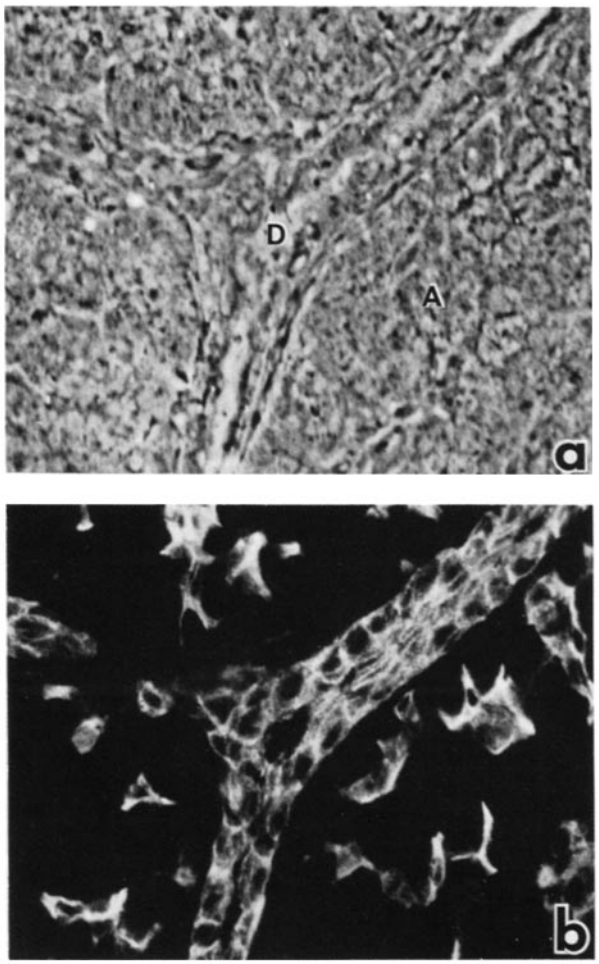

FIGURE 9 Indirect immunofluorescence localization of antigen 11B8 in dog pancreas. Frozen sections of pancreas were incubated in hybridoma 11B8 supernatant followed by FITC-GAM. Antigen 11B8 was detected only on the cells of the interlobular ducts (*D*) and the smaller intralobular ducts and centroacinar cells which are closely associated with the secretory acini (*A*). Antigen was not detected on the acinar cells. × 600.

TAL and the luminal surfaces of DCT, macula densa, and CT (50, 51) while, in contrast, antigen 11B8 is distributed only on the basolateral cell surfaces of the TAL and DCT. Na^+,K^+-ATPase is a major basolateral cell surface component found on both MDCK cells (16, 52, 53) and the distal tubule (54, 55). The possibility that we have an antibody against the Na^+,K^+-ATPase appears unlikely since antigen 11B8 has a different distribution in the kidney. Na^+,K^+-ATPase is found on PR, DCT, and TAL in similar concentrations (56) and is on the plasma membrane of the proximal tubule (57), a nephron segment that does not express antigen 11B8. In addition, antibody 11B8 has been detected in the macula densa at levels qualitatively comparable to that of the distal tubule. The observation that Na^+,K^+-ATPase has been localized histochemically in the distal tubule but not in the macula densa (55) would support our proposal that antigen 11B8 is not a Na^+,K^+-ATPase. However, this histochemical technique does not detect the presence of Na^+,K^+-ATPase in the proximal tubule (55), a nephron segment known to have this enzyme (56, 57). We have also determined that antibody 11B8 does not bind to purified kidney medulla Na^+,K^+-ATPase; however, as a cautionary note, it should be pointed out that the isolation and purification of the medullary Na^+,K^+-ATPase utilized SDS (29) and it is possible that the detergent treatment may have prevented the binding of the monoclonal antibody but not the antiserum. Despite these objections, there is the possibility that isoenzymes of the Na^+,K^+-ATPase exist in the kidney and that we are localizing a distal tubule form due to the high specificity of our monoclonal antibody. Although two forms of Na^+,K^+-ATPase have been identified in brain (58), the known distribution of antigen 11B8 in the nephron and its absence from brain argue against this conjecture.

The tubular specificity of antibody 11B8 binding in the kidney suggests that this antibody recognizes an antigen which may be involved in functions unique to the TAL and DCT. For example, physiological studies have demonstrated that the distal tubule is impermeable to water while the proximal tubule and thin loop segments are not (1) and that isolated individual nephron segments are responsive to different hormones (2). The TAL is known to be integral to urine concentration, actively reabsorbing salts by a proposed electrogenic Cl^- pump which appears to be unique to this nephron segment since it is the only renal tubule in which consistant positive lumen potential differences have been recorded (38, 59). Further evidence supporting a possible transport role for antigen 11B8 is its distribution in nonrenal tissues. Numerous studies have demonstrated that pancreatic ducts and salivary gland acini and ducts are active in transepithelial ion transport (60–62), and our immunofluorescence studies have demonstrated that antibody 11B8 binds to these epithelial cells.

In conclusion, the renal tubule specificity and basolateral distribution of antigen 11B8 suggests that this cell surface component may play a role in either the transepithelial transport or hormonal sensitivities unique to TAL and DCT. This possibility is supported further by the observation that this antigen has only been localized on the transporting epithelia of nonrenal tissues.

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