Abstract. In patients with Sjögren's syndrome and asecretory-defect distal renal tubular acidosis (dRTA), absence of vacuolar \( \text{H}^+ \)-ATPase from collecting duct intercalated cells has been reported. The \( \text{H}^+ \)-ATPase was examined in two patients with lupus nephritis and hyperkalemic (presumed voltage defect) dRTA. Both patients had a positive urine anion gap, alkaline urine despite acidemia, no rise in urine \( \text{PCO}_2 \) with alkaluria, a urine pH > 5.5, and urine potassium excretion rate not significantly increased after 80 mg of intravenous furosemide. In both patients, immunocytochemistry of renal biopsy frozen sections with an anti-W-ATPase monoclonal antibody showed bright staining of the proximal tubule brush border and collecting duct intercalated cells. In one patient, routine immunofluorescence analysis of a frozen section of her kidney biopsy with antihuman IgG showed staining of the collecting duct, indicative of autoantibodies to this segment. Moreover, neither patient's sera reacted with affinity-purified bovine \( \text{H}^+ \)-ATPase or with lysates from 293 cell fibroblasts in which either of both isoforms of the human \( \text{H}^+ \)-ATPase B subunit (56 kD) were expressed. These findings demonstrate that the spectrum of dRTA includes the preservation of \( \text{H}^+ \)-ATPase in intercalated cells, in patients with presumed voltage defect dRTA. Moreover, some patients may have autoantibodies to the intercalated cells that are not directed to subunits of the \( \text{H}^+ \)-ATPase.

Distal renal tubular acidosis (dRTA) is characterized by hyperchloremic metabolic acidosis, defective net acid excretion, and an inappropriately alkaline urine (pH > 5.5) in the setting of systemic acidemia (pH < 7.35). Normal distal urinary acidification requires an impermeant luminal membrane capable of sustaining large hydrogen ion gradients, an adequate rate of hydrogen ion secretion by the intercalated cells of the cortical and medullary collecting ducts, a lumen-negative potential difference in the cortical collecting duct supporting both hydrogen ion and potassium secretion in this nephron segment, and delivery of sufficient urinary buffers to offset the development of a limiting pH gradient (1). In principle, a defect in the rate of distal hydrogen ion secretion may arise from a reduction in the number of intercalated cells, a reduction in the quantity of \( \text{H}^+ \)-ATPase in the intercalated cells, an abnormal distribution of the \( \text{H}^+ \)-ATPase with a reduced quantity at the luminal membrane, or an abnormally slow rate of enzymatic activity of the \( \text{H}^+ \)-ATPase, resulting either from alterations intrinsic to the \( \text{H}^+ \)-ATPase or derangements in the regulatory proteins that can inhibit or activate the enzyme (2). We showed previously that vacuolar \( \text{H}^+ \)-ATPase was absent from the intercalated cells in renal biopsies from patients with Sjögren's syndrome and a classic dRTA, with hypokalemia and a secretory-type acidification defect (3-5).

In hyperkalemic dRTA arising presumably from a voltage defect, decreased sodium reabsorption and a consequent reduction in the lumen-negative potential of the cortical collecting duct are thought to be responsible for the insufficient excretion of potassium and the acidification deficiency. Patients with hyperkalemic dRTA cannot lower the urine pH to < 5.5 either during acidemia or after maneuvers that normally stimulate sodium-dependent distal acidification, such as administration of furosemide or infusion of sodium sulfate (6,7). These patients also fail to show an appropriate rise in urinary potassium excretion rate in response to the same maneuvers (6,7). Thus, in theory, patients with hyperkalemic dRTA could exhibit an acidification defect even with a normal quantity of vacuolar \( \text{H}^+ \)-ATPases. In previous reports, we had shown that total absence of the \( \text{H}^+ \)-ATPase pump in the intercalated cells was responsible for the classic hyperkalemic (secretory-defect)
dRTA complicating Sjögren's syndrome (3-5). Our findings were unique in that for the first time we had shown direct evidence that the absence of the H^+-ATPase pump was responsible for the distal acidification defect. To test the hypothesis that patients with hyperkalemic dRTA may have a different pathophysiologic basis underlying their distal acidification defect and that the H^+-ATPase pump may be present, but nonfunctional, in these patients, the present studies were undertaken. In this report, we present two patients with lupus nephritis and hyperkalemic (presumed voltage defect) dRTA whose kidney biopsies showed prominent staining for H^+-ATPase in the intercalated cells. Serum from one patient contained IgG that reacted with her own collecting duct cells and with intercalated cells along the collecting duct of the rat kidney. Although sera from both patients reacted with an approximately 56-kD polypeptide in the human kidney microsomal protein, neither reacted with an immunoaffinity-purified bovine H^+-ATPase or with the recombinant B1 ("kidney") and B2 ("brain") isoforms of the B subunit expressed in embryonal human cell line 293. Thus, in contrast to our previous observations in patients with Sjögren's syndrome and secretory-defect dRTA who lacked H^+-ATPase pump expression in their intercalated cells, in the patients with hyperkalemic dRTA presumably due to voltage defect, the distal acidification defect occurs in spite of the presence of the H^+-ATPase pumps in the intercalated cells. Our findings support the hypothesis that lack of an appropriate negative voltage may have contributed to the patients' distal acidification defect even in the presence of the H^+-ATPase pumps. Moreover, our findings suggest that autoantibodies to some components of the intercalated cells may play a role in this distal acidification defect.

Case Report 1

A 31-yr-old black woman with a 10-yr history of systemic lupus erythematosus who underwent two kidney biopsies 7 and 3 yr earlier showing diffuse proliferative glomerulonephritis presented with nephrotic syndrome and hyperchloremic metabolic acidosis. She had received immunosuppressive agents (prednisone and azathioprine or cyclophosphamide) intermittently in the past. Her blood chemistries revealed: Na, 139 mEq/L; K, 4.6 to 5.1 mEq/L; Cl, 114 to 117 mEq/L; CO_2, 16 to 20 mEq/L; creatinine, 1.5 mg/dl; albumin, 2.3 g/dl; and cholesterol, 344 mg/dl. Her other laboratory data included: venous blood pH, 7.33; urine pH, 5.8 to 6.5; antinuclear antibody titer, 1:160; anti-single-stranded DNA, 207 (normal, <10); total hemolytic complement, 16% (normal, 51 to 150%); and normal levels of C3 and C4. A 24-h urine collection revealed a creatinine clearance rate of 82 ml/min and 12.7 g of protein.

A repeat percutaneous renal biopsy showed advanced membranous glomerulonephritis with high chronicity (3 of 4) and low activity (1 of 4) indexes. Routine immunofluorescence staining with FITC-labeled antihuman IgG showed labeling of a majority of collecting duct cells (Figure 1).

Figure 1. Immunofluorescence analysis in frozen section of renal biopsy from patient 1, stained with FITC-labeled antihuman-IgG. A majority of the collecting duct (C) cells were labeled, indicative of autoantibodies to the collecting duct cells.

RTA Evaluation

The urine anion gap was +50 (urine Na, 54 mEq/L; urine K, 46 mEq/L; urine Cl, 50 mEq/L) with a venous blood pH of 7.33, venous blood HCO_3 of 19.5 mEq/L, and venous blood PCO_2 of 37.8 mmHg. Urine tests were negative for the presence of aminoaciduria or glucosuria. After intravenous bicarbonate infusion, the laboratory results were: venous blood pH, 7.49; venous blood HCO_3, 38.5 mEq/L; venous blood PCO_2, 50 mmHg; urine pH, 8.0; urine Pco_2, 46 mmHg; and urine minus blood Pco_2 difference, -4 mmHg. Three hours after 80 mg of intravenous furosemide, her urine pH changed from 5.9 to 6.1 U, the urine sodium excretion rate from 52 to 217 μEq/min, the fractional excretion of sodium from 0.6 to 3%, the urine potassium excretion rate (U_KV) from 17 to 26 μEq/min, and the fractional excretion of potassium from 8% to 14%.

Treatment and Subsequent Course

The patient was treated with oral prednisone 50 mg/d, which was gradually tapered over the next few months. She also received intravenous cyclophosphamide (1000 mg per treat-
ment) every other month for six doses and then every 3 to 4 mo for another six treatments.

Six months after initiation of treatment, her laboratory evaluation showed: tCO₂, 22 mEq/L; Cl, 111 mEq/L; K, 4.5 mEq/L; creatinine, 1.5 mg/dl; albumin, 2.5 g/dl; and cholesterol, 344 mg/dl. A 24-h urine collection had 5 g of protein.

Two years after initiation of treatment, her laboratory evaluation showed: tCO₂, 20 to 22 mEq/L; Cl, 111 mEq/L; K, 4.7 mEq/L; creatinine, 1.5 to 1.7 mg/dl; albumin, 3.8 g/dl; cholesterol, 204 mg/dl; <1 g of protein in 24-h urine collection; upright plasma renin activity 6.3 ng/ml per h; and upright plasma aldosterone, 4 ng/dl (normal, 4 to 31 ng/dl).

Case Report 2

A 21-yr-old white woman with a 5-yr history of systemic lupus erythematosus and a baseline serum creatinine rate of 0.8 mg/dl was admitted after she was found to have an elevation in her serum creatinine level and significant proteinuria by dipstick. She had been taking 15 mg of prednisone and 200 mg of hydroxychloroquine sulfate per day. Her admission laboratory values were: Na, 141 mEq/L; K, 4.8 to 6.0 mEq/L; Cl, 121 mEq/L; tCO₂, 13 to 17 mEq/L; blood urea nitrogen, 44 mg/dl; creatinine, 2.5 mg/dl; albumin, 1.9 g/dl; cholesterol, 276 mg/dl; hemoglobin, 11.5 g/dl; hematocrit, 34%; platelet count, 167,000/mm³; C3, 30 mg/dl (normal, 55 to 120 mg/dl); C4, 14 mg/dl (normal, 20 to 50 mg/dl); erythrocyte sedimentation rate, 59 mm/h; anti-single-stranded DNA (Crithidia), 1:128; and a low positive anti-cardiolipin IgG of 12.9 IgG phospholipid/ml. Arterial blood gas: pH, 7.31; Pco₂, 24 mmHg; HCO₃, 12 mEq/L; PO₂, 106 mmHg. Urinalysis: pH, 6.0; specific gravity, 1.012; protein, >300 mg/dl; glucose, negative; Microscopy: 42 erythrocytes and eight leukocytes per high-power field. A 24-h urine collection revealed 11 g of protein.

The patient underwent a percutaneous renal biopsy, which showed severe diffuse proliferative glomerulonephritis (World Health Organization class IV) with high activity (4 of 4) and moderate chronicity (2 of 4) indexes. Her medical course was complicated with an episode of generalized grand mal seizure secondary to lupus cerebritis, rapidly progressive renal failure, and mild-to-moderate congestive heart failure, with evidence on a two-dimensional echocardiogram of mild, global left ventricular hypokinesis and a small-to-moderately sized pericardial effusion. She developed moderately severe hypertension, Coombs positive hemolytic anemia, thrombocytopenia, a pleural effusion, and bullous lupus erythematosus on the extremities.

**RTA Evaluation**

Three days after the renal biopsy, her laboratory data revealed: creatinine, 3.5 mg/dl; Na, 143 mEq/L; K, 5.3 mEq/L; Cl, 122 mEq/L; tCO₂, 13 mEq/L; venous blood pH, 7.31; venous blood Pco₂, 28 mmHg; venous blood HCO₃, 14 mEq/L; urine pH, 5.84; urine Na, 64 mEq/L; urine K, 22 mEq/L; urine Cl, 47 mEq/L; and a urine anion gap of +39 mEq/L. After bicarbonate supplementation, her serum bicarbonate concentration increased to 23 mEq/L, with a fractional excretion of HCO₃ of 2%.

One month after the biopsy, her serum creatinine had increased to 5.6 mg/dl. A bicarbonate infusion test resulted in: venous blood pH, 7.62; venous blood Pco₂, 49.5 mmHg; venous blood HCO₃, 52 mEq/L; urine pH, 7.8; urine Pco₂, 45.5 mmHg; and urine minus blood Pco₂ difference, −4 mmHg. After a 100-mg dose of intravenous furosemide, her urine pH changed from 6.3 to 6.7. Ure sodium excretion rate increased from 48 to 192 μEq/min, fractional excretion of sodium from 3.4 to 14.1%, UₖV from 16.2 to 27.8 μEq/min, UₖV/creatinine clearance rate from 0.81 to 1.39; fractional excretion of potassium from 31 to 56.5%.

**Treatment and Subsequent Course**

The patient was treated with intravenous methylprednisolone (500 mg/d) for 3 consecutive d, followed by 20 mg of oral prednisone every 6 h, which was gradually tapered over the next few months. She also received intravenous cyclophosphamide (1000 mg) on a monthly basis for the first 6 mo and then every other month for six more doses.

Eight months after initiation of therapy, her serum creatinine level had decreased to 2.1 mg/dl with no evidence of hyperchloremic metabolic acidosis. She had an upright plasma renin activity of 20.5 ng/ml per h, and a plasma aldosterone level of 3 ng/dl with a concomitant serum potassium of 5.1 mEq/L.

**Materials and Methods**

**Immunocytochemistry**

Four-micrometer-thick frozen sections from human kidney biopsies were serially incubated in acetone for 5 min, phosphate-buffered saline (PBS) for 10 min, hematoxylin counter stain for 20 s, and blocking solution (10%) calf serum in blocking solution for 15 min to block nonspecific binding. Sections were then incubated for 2 h with undiluted supernatant from hybridoma E11, a monoclonal antibody to the approximately 31-kD subunit of the vacuolar H-ATPase (8,9) and rinsed in PBS. Bound antibody was detected with FITC-labeled goat anti-mouse IgG diluted 1:50 (vol/vol) in the blocking solution. The sections were mounted in a fresh mixture of 2 mg/ml paraphenylenediamine in 50% glycerol (vol/vol in PBS). The slides were viewed using a Nikon Optiphot-2 mercury epifluorescent microscope (Nikon, Inc., Instrument Group, Melville, NY).

In the evaluation for anti-intercalated cell antibodies, rat kidney biopsies were fixed in B5 fixative (0.22 M MgCl₂, 90 mM sodium acetate, and 3.7% formaldehyde) and embedded in paraffin, and 4-μm sections were prepared and incubated sequentially in xylene, decreasing concentrations of ethanol, Lugol's iodine solution, 5% sodium thiosulfate, and finally PBS. The procedure for antibody staining was similar to that used for frozen sections, except that double-label immunocytochemistry was performed using a mixture of monoclonal antibody E11 and the patient serum diluted 1:50 in blocking solution as primary antibody, and a mixture of FITC-labeled goat antihuman IgG and Texas red-labeled goat anti-mouse IgG as secondary antibody.

**Immunoblot Analysis**

Normal human kidney microsomes were prepared by methods identical to those used for the preparation of bovine kidney microsomes (10). Affinity-purified bovine kidney H⁺-ATPase was pre-
pared with a monoclonal antibody affinity column as described previously (10). Proteins (20 µg/lane) were resolved by electrophoresis on 11.25% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred electrophoretically to nitrocellulose membranes. Membrane strips were probed either with monoclonal antibody E11 (diluted 1:400 vol/vol in blocking solution), patient serum (diluted 1:10 to 1:50 vol/vol in blocking solution), control serum (diluted 1:10 to 1:50 vol/vol in blocking solution), or rabbit antisera against the H\(^+\)-ATPase B1 (“kidney”) isoform of the B subunit (diluted 1:200 diluted vol/vol in blocking solution) (11), using enhanced chemiluminescence for detection (enhanced chemiluminescence Western blotting, Amersham Life Sciences, Arlington Heights, IL) following the manufacturer’s recommended protocol.

In the evaluation for the presence of autoantibodies against the B1 and B2 isoforms of the human H\(^+\)-ATPase B subunit, the coding regions from the human B1 and B2 isoform cDNA were inserted into the pCEP expression vector (Invitrogen, San Diego, CA) as described (12). A total of 5 µg of each recombinant vector was used to transfect 293 cells (American Type Culture Collection number CRL1573, Rockville, MD), a human embryonic kidney fibroblast cell line, by calcium phosphate coprecipitation. Thirty-six hours after transfection, cells were lysed in PBS containing 0.1% SDS, 1 mM phenylmethylsulfonly fluoride, and 1 µg/ml each leupeptin, pepstatin A, antipain, and aprotinin. Protein from control (nontransfected) cells (40 µg), B2-transfected cells (40 µg), B1-transfected cells (10 µg), and human kidney microsomes (40 µg) were separated by SDS-polyacrylamide gel electrophoresis on 10% gels and transferred electrophoretically to Immobilon P (Millipore, Bedford, MA) membranes. The membranes were incubated overnight at 4°C in milk-blocking solution (5% dry milk in PBS with 0.01% Tween-20 and 1 mM azide). Primary antibodies, diluted (vol/vol) in milk-blocking solution and applied to the membranes at room temperature for 2 h, consisted of the following: patient serum, 1:50; rabbit anti-B2 antisera, 1:500; and rabbit anti-B1 antisera, 1:500. Membranes were washed with PBS + 0.01% Tween, reincubated in milk-blocking solution without azide, and incubated for 45 min with goat anti-human and goat anti-rabbit horseradish peroxidase-conjugated IgG (Southern Biotechnology Associates, Birmingham, AL) diluted 1:1000. Membranes were washed with PBS + 0.01% Tween and developed with the electrochemiluminescence reagents as above.

Results

Both patients fulfilled the clinical physiologic criteria for distal renal tubular acidosis with a presumable voltage-type defect, as described in each case history.

To determine whether the underlying H\(^+\) secretory defect was a result of absence of H\(^+\)-ATPase in the intercalated cells, we examined the distribution of vacuolar H\(^+\)-ATPase in frozen sections of kidney biopsy samples from both patients, using anti-H\(^+\)-ATPase monoclonal antibody E11. In prior studies using polyclonal antibodies (13,14) or monoclonal antibody E11 (3,9,15,16), we showed that the distribution of vacuolar H\(^+\)-ATPase in normal human kidneys was similar to that in rat kidney. In both species, intense H\(^+\)-ATPase staining was observed in the brush border microvilli and subvillar invaginations of the proximal convoluted tubules, and in the subvillar invaginations of the proximal straight tubules. In the cortical collecting ducts, the intercalated cells, comprising approximately 42% of the cells, stained intensely (9); principal cells had minimal or no detectable H\(^+\)-ATPase staining.

In the biopsy sections, staining of the brush border in the proximal tubules and intense staining of the intercalated cells in the cortical collecting ducts was observed (Figure 2, A and B). Because of the small size of the specimens, the percentage of collecting duct cells represented by intercalated cells could not be determined accurately.

To examine whether either patient was producing anti-intercalated cell antibodies, we performed double-label immunocytochemistry on rat kidney sections using patient serum, and anti-H\(^+\)-ATPase monoclonal antibody E11 to identify intercalated cells. In sections incubated with serum from patient 1, staining was observed in most of the intercalated cells in the cortical and medullary collecting ducts (Figure 3). No intercalated cell staining was observed in sections incubated with serum from patient 2 (data not shown).

To determine whether the patients were producing antibodies to the vacuolar H\(^+\)-ATPase or other human kidney proteins, we performed immunoblots on human kidney microsomal protein using serum from each patient (Figure 4). As controls, antibodies to several vacuolar H\(^+\)-ATPase subunits labeled polypeptides of the appropriate sizes in the microsomes (lanes 4 and 5). Serum from both patient 1 (lane 2) and patient 2 (lane 3) reacted with an approximately 56-kD polypeptide, which did not react with control human serum (lane 1). No immunoreactivity with affinity-purified bovine H\(^+\)-ATPase was found in serum samples from either patient (Figure 5, lanes 2 and 3).

To examine whether the immunoreactive, approximately 56-kD polypeptide in human microsomes was the B subunit of the vacuolar H\(^+\)-ATPase, we performed immunoblots on extracts of 293 cells expressing the recombinant B1 (58 kD) and B2 (56 kD) subunit isoforms using serum from both patients. In control experiments (Figure 6) in which samples were probed with rabbit anti-H\(^+\)-ATPase B2 subunit antibody, weak immunoreactivity was observed in control, untransfected 293 cells (lane 1); strong reactivity was present in cells expressing the recombinant B2 subunit (lane 2) and in human kidney microsomes (lane 4); and no reactivity was observed in 293 cells expressing the recombinant B1 subunit (lane 3). In samples probed with rabbit anti-H\(^+\)-ATPase B1 subunit antibody, no immunoreactivity was found in untransfected 293 cells (lane 5) or in cells expressing the recombinant B2 subunit (lane 6), but intense reactivity was observed in cells expressing the B1 subunit (lane 7) and in human kidney microsomal protein (lane 8). In immunoblots probed with serum from either patient, no reactivity was found with untransfected 293 cells (Figure 7, lanes 1 and 4), with cells expressing the B2 subunit (lanes 2 and 5), or with cells expressing the B1 subunit (lanes 3 and 6). Thus, although both patients produced antibodies to unidentified human kidney microsomal proteins, no antibodies to the vacuolar H\(^+\)-ATPase were detectable.

Discussion

In theory, hyperkalemic dRTA, which is thought to be due to a defect in distal sodium absorption and generation of the appropriate negative voltage in the lumen, could occur despite the presence of H\(^+\)-ATPase in the distal nephron. Our finding of significant H\(^+\)-ATPase staining in the intercalated cells of
two patients with lupus nephritis and hyperkalemic dRTA supports this hypothesis. The two patients described fulfilled clinical criteria for hyperkalemic (presumed voltage defect) dRTA (6,7). The urine was inappropriately alkaline, and the measured urinary cations exceeded the anions (a positive urinary net charge or anion gap) during spontaneous systemic acidemia, suggesting that the patients had a low urinary ammonium excretion rate (17,18). The patients had defects in distal tubular hydrogen ion secretion, established by a urine pH that did not change after furosemide infusion, and a urine minus blood Pco₂ that was abnormally low during bicarbonate administration. Both patients had a urinary potassium excretion rate that was abnormally low both before and after furosemide infusion, a defect found in voltage-type dRTA, but not in secretory-type dRTA (6,7). Both patients had evidence of selective aldosterone deficiency approximately 8 mo to 2 yr after the initial evaluation for dRTA. A concomitant aldosterone deficiency has been reported previously in patients with hyperkalemic (presumed voltage defect) dRTA (19). The fact that both patients had inappropriate response of urine pH and U₅V along with a low urine minus blood Pco₂ with alkaluria indicates that aldosterone deficiency (type IV RTA) did not play a major pathophysiologic role at the time of RTA work up. Nevertheless, the slight increase in urine potassium in patient 1, after furosemide administration, could suggest a component of aldosterone deficiency, although lack of urine pH and Pco₂ responses may negate this possibility. Moreover, because urine pH is affected by urine flow rate, it would have been more accurate to measure net acid excretion rather than urine pH in response to furosemide. Furthermore, our patient 2 was studied at the time of rising serum creatinine, which could have resulted in a hyperkalemic metabolic acido-
Figure 3. Double-label immunocytochemistry on rat kidney sections using mouse anti-H-ATPase monoclonal antibody with Texas red-labeled goat anti-mouse IgG and serum from patient 1 with FITC-labeled goat antihuman IgG. Panel A, Texas red fluorescence (H⁺-ATPase) showing labeling of brush border membrane in proximal tubules (P) and intense staining of intercalated cells in collecting ducts (C). Panel B, FITC fluorescence showing labeling of some collecting duct cells (arrows) corresponding to intercalated cells. Serum from patient 2 did not show any staining (not shown).

sis with a low urine ammonia excretion rate and a positive urine anion gap regardless of a concomitant distal acidification defect. However, the failure to increase urine PaCO₂ and to lower urine pH suggested a concomitant distal acidification defect as well.

In analyses of patients with systemic lupus erythematosus, several case reports (20–27), a few small surveys (23–25), and one large prospective study (27) have reported a high incidence of renal tubular acidification defects and hyperkalemia. In the largest series (27), 30 patients were studied within 1 mo of onset or 2 mo of an exacerbation of the disease. Eighteen patients (60%) had one or more defects in potassium, hydrogen ion, or sodium handling. Eight of these patients had an isolated proton secretory defect, and the remaining patients had either a voltage defect (hyperkalemic) dRTA (three patients), gradient defect dRTA (five patients), hyporenin-hypoaldosteronism with a type IV RTA (one patient), or a mixture of type IV and voltage defect dRTA (one patient). Some of the patients diagnosed with a gradient defect dRTA may have had dRTA as a result of a medullary proton secretory defect (6,7).

In the present study, both patients showed prominent staining for the vacuolar H⁺-ATPase in the intercalated cells. This result contrasts with our previous reports of three patients with Sjögren’s syndrome and secretory defect dRTA who were characterized by the absence of H⁺-ATPase in their intercalated cells (3–5). Our findings support the concept that in voltage defect dRTA, an inadequate lumen-negative voltage in the cortical collecting duct prevents the intercalated cells from secreting protons at normal rates despite the presence of seemingly adequate cellular H⁺-ATPase.

Serum from one of our patients (patient 1) had autoantibodies that reacted with rat kidney intercalated cells, identified by simultaneous staining with an anti-H⁺-ATPase monoclonal antibody. Kidney biopsy frozen sections from the same patient, stained with FITC-labeled antihuman IgG, also showed diffuse staining of collecting duct cells. Circulating autoantibodies against collecting duct cells have been implicated previously in the pathogenesis of dRTA (28–32). In three reports, patient serum yielded diffuse staining of all collecting duct cells (28–30). In one report (28), what were
Figure 4. In immunoblot analysis of human kidney microsomal protein (20 μg) probed with different antibodies and detected by electrochemiluminescence as described in Materials and Methods. Lane 1, control human serum (1:50); lane 2, serum from patient 1 (1:50); lane 3, serum from patient 2 (1:50); lane 4, rabbit anti-B2 subunit antiserum (1:200); lane 5, anti-H+ ATPase monoclonal antibody E11 (1:400 dilution of ascites). In lanes 2 and 3, immunoreactivity with an approximately 56-kD polypeptide was observed.

reported originally as loops of Henle were identified subsequently as collecting ducts (31).

In the other two studies, the autoantibodies stained a subpopulation of collecting duct cells, which, on the basis of light microscopy morphologic criteria (31) or lectin binding and Band-3 immunocytochemical staining (32), were considered intercalated cells. Thus, our results, together with the previous reports, suggest that autoantibodies to the intercalated cells, but not necessarily to the subunits of the H+ ATPase pump, may play a role in the pathogenesis of dRTA.

It is possible that autoantibodies to the intercalated cells or to all collecting duct cells could have a role in the pathogenesis of collecting duct dysfunction, contributing either to the defect in sodium transport or directly to a proton secretory defect in these patients. Schlüter et al. (19) suggested that some patients with hyperkalemic dRTA may have a primary defect in collecting tubule hydrogen ion secretion due to H+ ATPase dysfunction. At the present time, however, it is not known whether such autoantibodies alter collecting duct function.

Although serum antibodies from both of our patients reacted with an approximately 56-kD polypeptide in human kidney microsomal proteins, we found no detectable reactivity with the human or bovine H+ ATPase. It is possible that identifica-

Figure 5. Immunoblot analysis of affinity-purified bovine H+ ATPase (5 μg) probed with different antibodies. Methods were as described in Figure 4. Lane 1, control serum (1:10); lane 2, serum from patient 1 (1:10); lane 3, serum from patient 2 (1:10); lane 4, rabbit anti-B2 subunit antiserum (1:200); lane 5, anti-H+ ATPase monoclonal antibody E11 (1:400 dilution of ascites). Neither control nor patients' sera showed any immunoreactivity with the affinity-purified bovine H+ ATPase (lanes 1–3). Rabbit anti-B2 antiserum (lane 4) and monoclonal antibody E11 (lane 5) showed the appropriate bands.

tion of the immunoreactive proteins present in human kidney microsomes may provide insight into the pathophysiologic basis for voltage defect dRTA.

In summary, we have shown that the spectrum of dRTA expands from total absence of the H+ ATPase pumps in patients with isolated proton secretory defect (e.g., hypokalemic dRTA in Sjögren's syndrome) to the presence of H+ ATPase pumps in patients with hyperkalemic dRTA, thought to be due to a voltage defect. Our results also suggest that, at least in some patients, autoantibodies to the intercalated cells, but not to the subunits of the H+ ATPase, may play a pathogenic role in distal proton and potassium secretory defect.

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Figure 6. Immunoblot analysis of control and transfected 293 cell extracts and human kidney microsomes probed with rabbit anti-B2 subunit antiserum (lanes 1 through 4) or rabbit anti-B1 subunit antiserum (lanes 5 through 8) demonstrating B subunit isoform-specific immunoreactivity. Vectors expressing the recombinant B2 or B1 subunit were used to transfect 293 cells as described in Materials and Methods. Lanes 1 and 5, untransfected 293 cells (40 μg); lanes 2 and 6, 293 cells expressing the B2 (brain) subunit isoform (40 μg); lanes 3 and 7, 293 cells expressing the B1 (kidney) subunit isoform (10 μg); lanes 4 and 8, human kidney microsomal protein (50 μg). The faint 56-kD reactivity in lane 1 represents endogenous B2 subunit in 293 cells.

Figure 7. Immunoblot analysis of control and transfected 293 cell extracts probed with serum from patient 1 (lanes 1 through 3) and patient 2 (lanes 4 through 6). Lanes 1 and 4, untransfected 293 cells (40 μg); lanes 2 and 5, 293 cells expressing the B2 isoform (40 μg); lanes 3 and 6, 293 cells expressing the B1 isoform (10 μg). No immunoreactivity was observed with serum from either patient.

References


