AJKD

Distal Renal Tubular Acidosis With Multiorgan Autoimmunity: A Case Report

Maria J. van den Wildenberg, MD,[1,*] Ewout J. Hoorn, MD, PhD,[2,*]
Nilufar Mohebbi, MD,[3,4] Carsten A. Wagner, PhD,[4] Arend-Jan Woittiez, MD, PhD,[1]
Peter A.M. de Vries, MD, PhD,[1] and Gozewijn D. Laverman, MD, PhD[1]

A 61-year-old woman with a history of pernicious anemia presented with progressive muscle weakness and dysarthria. Hypokalemic paralysis (serum potassium, 1.4 mEq/L) due to distal renal tubular acidosis (dRTA) was diagnosed. After excluding several possible causes, dRTA was considered autoimmune. However, the patient did not meet criteria for any of the autoimmune disorders classically associated with dRTA. She had very high antibody titers against parietal cells, intrinsic factor, and thyroid peroxidase (despite normal thyroid function). The patient consented to a kidney biopsy, and acid-base transporters, anion exchanger type 1 (AE1), and pendrin were undetectable by immunofluorescence. Indirect immunofluorescence detected diminished abundance of AE1- and pendrin-expressing intercalated cells in the kidney, as well as staining by the patient's serum of normal human intercalated cells and parietal cells expressing the adenosine triphosphatase hydrogen/potassium pump (H^+/K^+-ATPase) in normal human gastric mucosa. The dRTA likely is caused by circulating autoantibodies against intercalated cells, with possible cross-reactivity against structures containing gastric H^+/K^+-ATPase. This case demonstrates that in patients with dRTA without a classic autoimmune disorder, autoimmunity may still be the underlying cause. The mechanisms involved in autoantibody development and how dRTA can be caused by highly specific autoantibodies against intercalated cells have yet to be determined.

Am J Kidney Dis. 65(4):607-610. © 2015 by the National Kidney Foundation, Inc.

INDEX WORDS: Distal renal tubular acidosis (dRTA); type I RTA; anion exchanger type 1 (AE1); antibodies; autoimmunity; hypokalemia; immunofluorescence; kidney biopsy; intercalated cells; stomach.

Distal renal tubular acidosis (dRTA, also called type I RTA) is caused by inability of the kidney collecting duct to acidify urine.[1] The metabolic acidosis in dRTA is characterized by a normal anion gap, urinary pH > 5.3, and low ammonium excretion. dRTA usually is caused by a proton secretion defect in the intercalated cells. This proton secretion defect explains why dRTA usually is accompanied by hypokalemia, because it indirectly causes renal potassium losses and cellular shifts of potassium. dRTA may be inherited or acquired by drugs, hypercalciuria, or an underlying autoimmune disorder. The condition has been reported in various autoimmune disorders, including Sjögren syndrome, primary biliary cirrhosis, autoimmune hepatitis, systemic lupus erythematosus, and rheumatoid arthritis.[2] How these autoimmune disorders cause dRTA is not well known. dRTA is a relatively common complication of Sjögren syndrome, and studies have suggested the presence of autoantibodies against acid-base transporters.[3-5] We report a case of dRTA associated with circulating antibodies against intercalated cells. Surprisingly, the patient did not have one of the systemic autoimmune disorders associated with dRTA, but had multiorgan autoimmunity involving the kidney, stomach, and thyroid glands.

CASE REPORT

A 61-year-old woman presented with progressive muscle weakness and dysarthria, which she experienced in the week leading up to admission. Her dietary habits were unremarkable and she did not have diarrhea. She took folic acid (5 mg/d) and vitamin B_{12} injections for pernicious anemia (her only medical history). She did not use H_2-receptor antagonists or proton pump inhibitors. Aside from paralysis (muscle strength, 2/5), physical examination findings, including that of the thyroid, were unremarkable. Laboratory tests revealed severe hypokalemia (serum potassium, 1.4 mEq/L) and non–anion gap metabolic acidosis (serum pH 7.14; bicarbonate, 9 mEq/L). Hypokalemia was due to renal potassium losses (urine potassium, 20 mEq/L; fractional excretion of potassium, 21%). The metabolic acidosis was characterized by an inappropriately high urine pH of 7.1 and positive urine anion gap (+21 mEq/L), suggesting RTA. Other laboratory findings were normal, including thyroid and kidney function. The patient had normal-sized kidneys without nephrocalcinosis. She was hospitalized for several weeks, initially at the intensive care unit, for intravenous potassium and

bicarbonate supplementation under telemetry. At the time of writing, the patient was continuing treatment with oral sodium bicarbonate (1,500 mg/d) and potassium citrate (1,800 mg/d), maintaining serum potassium (4.1 mEq/L) and bicarbonate (25 mEq/L) levels within reference ranges, and was being followed up as an outpatient for electrolyte and acid-base balance, as well as gastric and thyroid function.

After the acute phase, additional tests were performed to investigate the type and cause of RTA. An acidification test using fludrocortisone and furosemide was performed; the patient failed to reach urine pH < 5.3, achieving no lower than pH 5.5.[6] In addition, renal ammonium excretion was assessed by calculating the urine osmolal gap and found to be very low in view of the existing acidosis (11 mEq/L). These findings confirmed dRTA, although a fractional bicarbonate excretion test was not performed to formally exclude a proximal RTA. The diagnosis was supported further by the absence of other markers of proximal tubular dysfunction (hypouricemia, hypophosphatemia, proteinuria, and glucosuria) and because paraprotein and urinary light chains were undetectable. Although no previous measurements of serum potassium or bicarbonate were

available for comparison, a hereditary form of dRTA seemed unlikely for our patient given her lack of family history and late age of presentation. Repeat history and physical examination did not suggest a systemic autoimmune disorder. There was no hypercalciuria. Test results for antinuclear antibodies, antineutrophil cytoplasmic antibodies, and rheumatoid factor were negative. However, although tests for antibodies against SS-A, SS-B, and tissue transglutaminase were negative, those for antibodies against parietal cells and intrinsic factor (titers not available), as well as thyroid peroxidase (titer > 1,300 IU/mL; normal range < 60 IU/mL), were strongly positive.

With informed consent of the patient, a kidney biopsy was performed. Light microscopy showed normal glomeruli and an atrophic aspect of some of the proximal tubuli without other histologic changes (data not shown). Subsequently, we performed indirect immunofluorescence on the patient's kidney tissue and with the patient's serum on normal human kidney and stomach tissue. In the indirect immunofluorescence studies, we probed aquaporin 2 (AQP2) as a marker of principal cells, and 3 acid-base transporters in intercalated cells (including the a4 and B1 subunits of the adenosine triphosphatase proton pump [H^+-ATPase]), anion exchanger 1
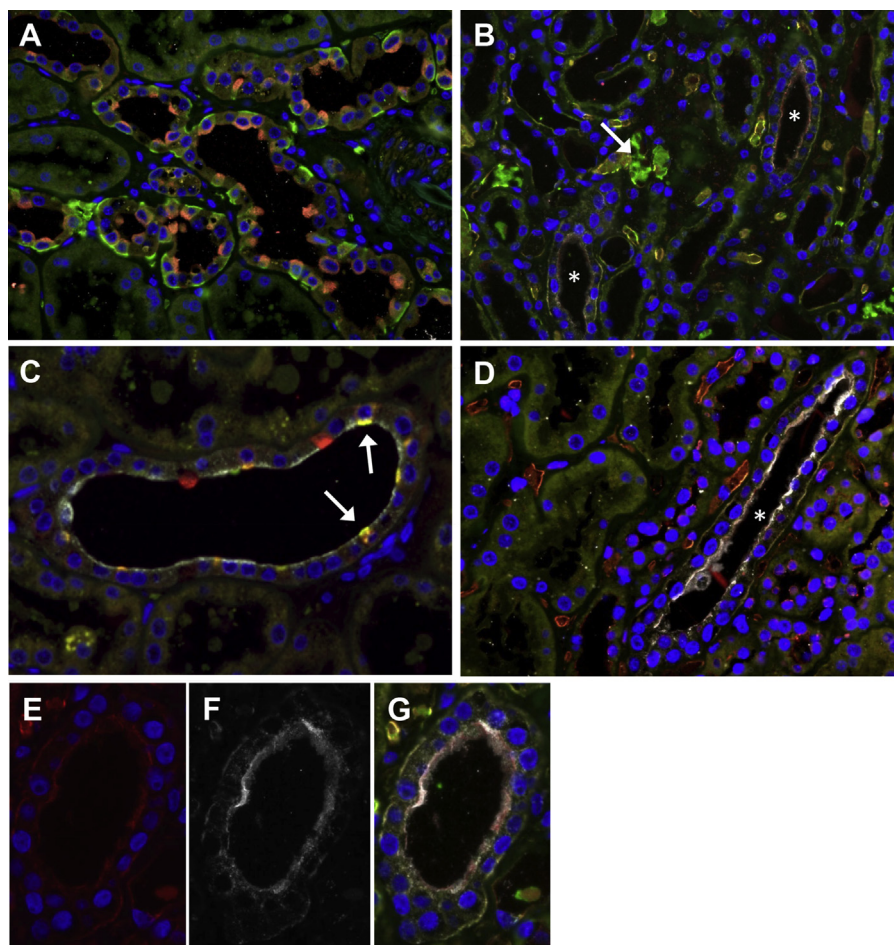

Figure 1. Loss of intercalated cells expressing anion exchanger type 1 (AE1) and pendrin in the patient's kidney. (A) Healthy human kidney was stained for AE1 (green), the B1 adenosine triphosphatase proton pump (H^+-ATPase) subunit (red), and cell nuclei (blue). (B) The patient's kidney shows no staining for AE1 in collecting ducts (*), but in red blood cells (arrow). (C) Healthy human kidney stained for pendrin (green), aquaporin 2 (AQP2; white), the a4 H^+-ATPase subunit (red), and cell nuclei (blue). In some cells, pendrin and the a4 H^+-ATPase subunit colocalize (arrow, yellow overlay). (D) Staining of patient's kidney for AE1 (red), AQP2 (white), and pendrin (green). Nuclei are stained in blue. No staining for pendrin was detectable in the patient's kidney, but all cells in the collecting duct (*) were positive for AQP2. (E-G) Patient's kidney stained for the (E) B1 H^+-ATPase subunit (red) and (F) AQP2 (white); (G) shows the overlay of all channels. All cells are positive for AQP2 but some cells also have a weak but distinct luminal red staining, suggesting colocalization of AQP2 and B1 in the same cells (original magnification, ×400-630).

(AE1, a marker for acid-secretory type A intercalated cells), and pendrin (a marker for bicarbonate-secretory type B intercalated cells; Fig 1). Remarkably, AE1 and pendrin were not detected in the patient's kidney sample. Although residual staining for the a4 and B1 subunits was present, these colocalized in the same cells with AQP2.

Incubating the patient's serum with normal human kidney tissue caused strong staining exclusively in both types of intercalated cells, which did not occur when testing control sera from 5 men and women aged 30 to 60 years (Fig 2). To confirm that the staining occurred in intercalated cells, we performed double labeling with AE1 and AQP2. These studies showed overlap of staining from the patient's serum with that of AE1, the marker of type A intercalated cells. Because the patient's serum also stained cells in which AE1 and AQP2 were not detected, we believe this additional staining occurred in type B intercalated cells. In gastric tissue, staining for the α-subunit of the ATPase hydrogen/potassium pump (H$^+$/K$^+$-ATPase), a marker of acid-secretory parietal cells in the gastric mucosa, revealed partial but substantial overlap with the signal from the patient's serum. This staining pattern suggests that the pump itself or associated proteins are part of the antigen. With serum from a healthy control, there was no detectable staining of cells expressing the α-subunit of the H$^+$/K$^+$-ATPase.

DISCUSSION

We report an unusual case of dRTA, which clearly was of autoimmune origin but could not be linked to any systemic autoimmune disorder known to be associated with dRTA. Indirect immunofluorescence showed diminished abundance of AE1- and pendrin-expressing intercalated cells in the kidney of the patient and positive staining by the patient's serum of normal human intercalated cells and parietal cells expressing the H$^+$/K$^+$-ATPase pump in normal human gastric mucosa. We conclude that dRTA likely is caused by circulating autoantibodies against intercalated cells with possible cross-reactivity against structures containing the gastric H$^+$/K$^+$-ATPase pump. The colocalization of H$^+$-ATPase with AQP2 may suggest one of 2 scenarios: intercalated cells have completely disappeared and principal cells express some markers of intercalated cells, or the differentiation process is affected by autoantibodies and cells coexpressing H$^+$-ATPases and AQP2 represents an intermediate cell type. The latter pathway has been reported in mice lacking the transcription factor FOXI1, which is required for normal intercalated cell differentiation.[7]

It is unclear whether one common epitope is present in the kidney, stomach, and possibly thyroid or multiple autoantibodies target different epitopes in these 3 tissues. The B1 subunit of H$^+$-ATPase is not expressed in stomach or thyroid glands, whereas the B2 subunit is present in every tissue and cell type. AE1 and pendrin also are not expressed in the stomach. In addition,
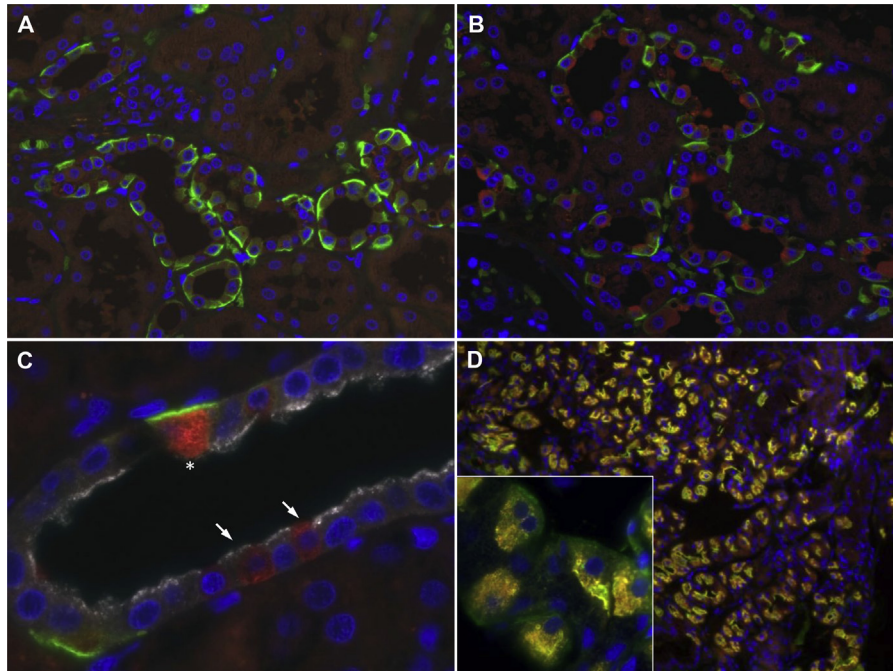

Figure 2. Autoantibodies against renal intercalated cells and gastric parietal cells. (A) Incubation of healthy human kidney with serum from an apparently healthy control (1:1,000 dilution of serum; secondary antibody stains red) and staining of type A intercalated cells with antibodies against anion exchanger type 1 (AE1; green). No specific red staining was detectable. Incubation of healthy human kidney with the patient's serum (1:1,000 dilution; secondary antibody stains red) and (B) antibodies against AE1 (green), demonstrating specific staining of type A intercalated cells, or (C) antibodies against AE1 (green) and aquaporin 2 (AQP2; white), show autoantibody-mediated staining of cells negative for AQP2 and AE1 positive (*type A intercalated cells) or negative (arrow: type B intercalated cells). (D) Incubation of healthy human stomach with patient's serum (red staining) and antibodies against gastric α-H$^+$/K$^+$-ATPase (green), a marker of acid-secretory parietal cells, shows partial colocalization of both signals indicated by yellow. Inset, higher magnification of parietal cells (original magnification, ×1,000). (Original magnification, ×400.)

cross-reactivity of the patient's serum with red blood cells would be expected in the case of AE1-reacting autoantibodies.[4] Moreover, patients with mutations in AE1 or H^+-ATPase subunits are not known to have thyroid or gastric disorders.[8] Autoantibodies against pendrin have been reported in patients with autoimmune thyroiditis and hypothyroidism.[9] Patients with mutations in pendrin present with deafness that can be accompanied by goiter and hypothyroidism; electrolyte and acid-base disorders have been reported in these patients only in the context of thiazide use or intercurrent illness.[9-11] The pathogenesis of dRTA is difficult to reconcile with dysfunctional pendrin because this transporter is involved in renal bicarbonate secretion and sodium chloride reabsorption. Another candidate protein would be SLC26A7, although we did not analyze this transporter in our immunolabeling studies. SLC26A7 is expressed in intercalated cells and stomach, and mice lacking SLC26A7 have RTA and reduced gastric acid secretion.[12] Of note, the association of dRTA with a constellation of autoantibodies against parietal cells, intrinsic factor, and thyroid peroxidase has been reported previously; this study of a series of 113 patients identified a complement-fixing autoantibody that reacted with the renal collecting duct.[13]

In conclusion, this unusual case demonstrates that to diagnose dRTA, it sometimes is necessary to think beyond the autoimmune disorders with which the condition is classically associated. After excluding the more typical autoimmune disorders and demonstrating autoantibodies against affected organs, the diagnosis can be made. The exact mechanisms involved in autoantibody development and how dRTA can be caused by highly specific autoantibodies against intercalated cells remain to be determined.

ACKNOWLEDGEMENTS

REFERENCES

1. Unwin RJ, Shirley DG, Capasso G. Urinary acidification and distal renal tubular acidosis. *J Nephrol*. 2002;15(suppl 5):S142-S150.

2. Rodriguez Soriano J. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol*. 2002;13(8):2160-2170.

3. Cohen EP, Bastani B, Cohen MR, Kolner S, Hemken P, Gluck SL. Absence of H(+)-ATPase in cortical collecting tubules of a patient with Sjogren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol*. 1992;3(2):264-271.

4. Walsh S, Turner CM, Toye A, et al. Immunohistochemical comparison of a case of inherited distal renal tubular acidosis (with a unique AE1 mutation) with an acquired case secondary to autoimmune disease. *Nephrol Dial Transplant*. 2007;22(3):807-812.

5. Han JS, Kim GH, Kim J, et al. Secretory-defect distal renal tubular acidosis is associated with transporter defect in H(+)-ATPase and anion exchanger-1. *J Am Soc Nephrol*. 2002;13(6):1425-1432.

6. Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int*. 2007;71(12):1310-1316.

7. Blomqvist SR, Vidarsson H, Fitzgerald S, et al. Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. *J Clin Invest*. 2004;113(11):1560-1570.

8. Batlle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant*. 2012;27(10):3691-3704.

9. Kandasamy N, Fugazzola L, Evans M, Chatterjee K, Karet F. Life-threatening metabolic alkalosis in Pendred syndrome. *Eur J Endocrinol*. 2011;165(1):167-170.

10. Wagner CA, Mohebbi N, Capasso G, Geibel JP. The anion exchanger pendrin (SLC26A4) and renal acid-base homeostasis. *Cell Physiol Biochem*. 2011;28(3):497-504.

11. Pela I, Bigozzi M, Bianchi B. Profound hypokalemia and hypochloremic metabolic alkalosis during thiazide therapy in a child with Pendred syndrome. *Clin Nephrol*. 2008;69(6):450-453.

12. Xu J, Song P, Nakamura S, et al. Deletion of the chloride transporter slc26a7 causes distal renal tubular acidosis and impairs gastric acid secretion. *J Biol Chem*. 2009;284(43):29470-29479.

13. Gaarder PI, Heier HE. A human autoantibody to renal collecting duct cells associated with thyroid and gastric autoimmunity and possibly renal tubular acidosis. *Clin Exp Immunol*. 1983;51(1):29-37.