Distinct α-intercalated cell morphology and its modification by acidosis define regions of the collecting duct

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Purkerson JM, Schwaderer AL, Nakamori A, Schwartz GJ. Distinct α-intercalated cell morphology and its modification by acidosis define regions of the collecting duct. Am J Physiol Renal Physiol 309: F464–F473, 2015. First published June 17, 2015; doi:10.1152/ajprenal.00161.2015.—During metabolic acidosis, the cortical collecting duct (CCD) of the rabbit reverses the polarity of bicarbonate flux from net secretion to net absorption, and this is accomplished by increasing the proton secretory rate by α-intercalated cells (ICs) and decreasing bicarbonate secretion by β-ICs. To better characterize dynamic changes in H⁺-secreting α-ICs, we examined their morphology in collecting ducts microdissected from kidneys of normal, acidic, and recovering rabbits. α-ICs in defined axial regions varied in number and basolateral anion exchanger (AE1) morphology, which likely reflects their relative activity and function along the collecting duct. Upon transition from CCD to outer medullary collecting duct from the outer stripe to the inner stripe, the number of α-ICs increases from 11.0 ± 2.0 to 15.4 ± 1.11 and to 32.0 ± 1.3 cells/200 μm, respectively. In the CCD, the basolateral structure defined by AE1 typically exhibited a pyramidal or conical shape, whereas in the medulla the morphology was elongated and shallow, resulting in a more rectangular shape. Furthermore, acidosis reversibly induced α-ICs in the CCD to acquire a more rectangular morphology concomitant with a transition from diffusely cytoplasmic to increased basolateral surface distribution of AE1 and apical polarization of B1-V-ATPase. The latter results are consistent with the supposition that morphological adaptation from the pyramidal to rectangular shape reflects a transition toward a more “active” configuration. In addition, α-ICs in the outer medullary collecting duct from the outer stripe exhibited cellular morphology strikingly similar to dendritic cells that may reflect a newly defined ancillary function in immune defense of the kidney.

DURING metabolic acidosis, there is extensive remodeling of the CD, including a hypertrophy of ICs and an increase in the relative number of α-ICs (5, 6, 19, 20, 38). A study (19) performed in our laboratory using kidney sections has also suggested that in the kidney cortex, acidosis induces a reversible redistribution of AE1 toward basolateral regions of α-ICs. An ultrastructural study by Verlander et al. (36) further revealed that cytoplasmic anion exchanger (AE1) was largely confined to multivesicular bodies, and there was a quantitative change in the distribution of AE1 toward basolateral membrane surfaces in response to acidosis. In this same study (36), it was also noted AE1-positive ICs in the inner CCD and outer stripe of the OMCD (OMCDo) often exhibited a stellate morphology. In the present study, we characterized the axial distribution of distinct α-IC morphologies along the rabbit CD and quantitated morphological adaptations in response to 3 days of metabolic acidosis (and also subsequent alkali loading) to enable definition of quiescent versus active cell configurations.

MATERIALS AND METHODS

Animals. Female New Zealand White rabbits weighing 1.5–3.0 kg were maintained on standard rabbit chow and tap water (18, 19). Acid loading was accomplished by providing 75–100 mM NH₄Cl-7.5% sucrose solution for 3 days with food intake limited to 30 g rabbit chow/day. For amelioration of acidosis, rabbits administered NH₄Cl in drinking water for 3 days were abruptly transitioned to 75–100 mM NaHCO₃-7.5% sucrose in drinking water for 12-18 h. The protocols for these animal experiments were submitted to and approved by the University Committee on Animal Rights of the University of Rochester Medical Center (UCAR-2005-199R). The pH and serum bicarbonate levels of blood samples taken from anesthetized animals as well as the pH of urine collected directly from the bladder immediately after euthanasia were measured with a Radiometer Blood Gas Analyzer (ABL5 model LB17493, Westlake, OH) and/or i-STAT using the G3⁺ cartridge (Abbott Point of Care, Abbott Park, IL).

Antibodies and immunohistochemistry. Rabbit kidneys were perfused with Dulbecco’s PBS (dPBS; Life Technologies, Grand Island, NY) followed by periodate lysine paraformaldehyde prepared as previously described (12). Kidney slices were cut perpendicular to the long axis (1- to 2-mm thickness) and immersion fixed in periodate lysine paraformaldehyde supplemented with 5% sucrose for 6–8 h at room temperature. Fixed tissues were embedded in paraffin, and 4- to 8-μm sections were baked onto positively charged glass slides by heating in an oven at 50–60°C for 30–60 min. After deparaffinization and gradual rehydration through graded ethanol, antigen retrieval was performed by heating to 90 ± 5°C for 5–10 min in high-pH antigen unmasking solution (H-3301, Vector Labs, Burlingame, CA) followed by blockade of nonspecific binding in 5% donkey serum (Jackson ImmunoResearch, Birmingham, AL) in PBS. Sections were then incubated with a 1:50–1:100 dilution of primary antibodies in PBS supplemented with 1% donkey serum. Primary antibodies included polyclonal goat anti-B1-V-ATPase (SC-21206), mouse monoclonal antibody (F-6) to V-ATPase B1/B2 (SC-55544), and mouse...
monoclonal antibody IVF12 directed against AE1 (7). Sections were overlaid with antibody solution, covered with a small rectangle of parafilm, and incubated in a humidified chamber for \( \sim 16 \) h at 4°C. After being washed, slides were then incubated with a 1:1,000 dilution of secondary antibodies (donkey anti-mouse FITC and donkey anti-gold goat Dylight 649, Jackson ImmunoResearch, West Grove, PA) or peanut agglutinin (PNA-FITC, Vector Labs) for 2–4 h and mounted in fluorescence mounting medium (VectaShield, H-1000, Vector Labs). Slides were analyzed for green and far red fluorescence using a Nikon E400 fluorescent microscope and photographed at \( \times 400 \) magnification using a Spot RT model 7.0 monochrome camera and Spot RT software (Diagnostic Instruments, Sterling Heights, MI).

**Immunofluorescent staining of microdissected CCDs.** CCDs were microdissected from medullary rays of the outer cortex of normal, acid-loaded, and 16- to 18-h recovery rabbits and fixed for 15–20 min in a 1:4 dilution of Prefer concentrate (Anatech) or periodate lysine paraformaldehyde [AE1 and zonula occludens 1 (ZO-1)]. Medullary CDs (MCDs) were also microdissected from OMCDs (deep to the glomeruli) and inner stripe of OMCD (OMCDi; deep to end proximal straight tubule) (8). After a wash in dPBS supplemented with 0.1% BSA, tubules were incubated overnight at 4°C with primary antibodies including polyclonal goat anti-B1-V-ATPase (N-20, SC-21206, Santa Cruz Biotechnology) or monoclonal anti-AE1 (7) (Iowa Hybridoma Bank, Iowa City, IA) in dPBS supplemented with 5% donkey serum and 0.1% Triton X-100 (Sigma-Aldrich). After a wash, CCDs were incubated 2–4 h with the following secondary antibodies: donkey anti-mouse or goat conjugated with either far red (Dylight 649 or Alexa 647) or green (FITC or Alexa 488) fluorochromes (Jackson ImmunoResearch or Life Technologies). Co-labeling with anti-ZO-1 antibody (Alexa 488-conjugated mouse monoclonal antibody clone 1A12, Molecular Probes) staining was accomplished with a tertiary incubation for 10–15 min at 4°C in dPBS supplemented with 5% donkey serum and 0.1% Triton X-100. CCDs were briefly washed and then mounted on slides by pipetting into mounting media (Prolong gold antifade reagent with 4',6-diamidino-2-phenylindole for staining nuclei, Molecular Probes). Secure seal spacers (13-mm diameter \( \times 0.12 \) mm thick, Electron Microscopy Sciences, Hatfield, PA) prevented compression of CDs under glass coverslips. We defined \( \alpha \)-ICs as being AE1 positive and \( \beta \)-ICs as being PNA positive or pendrin positive (18, 22, 24, 25).

Confocal microscopy and image analysis. Z-stack images of immunofluorescence-stained CDs were obtained by collecting 0.4- to 1-\( \mu \)-m optical sections using the \( \times 60 \) objective of an Olympus FV1000 laser scanning confocal microscope (Olympus, Center Valley, PA) as previously described (18, 19). The secure seal spacers increased the mounting media depth and caused some variation in the working distance, causing variable fluorescence quenching by the mounting media. Therefore, laser transmission and photomultiplier tube voltages were adjusted using the ctrl-H function of Fluoview software so that the fluorescence was just below saturation to maintain consistency of the morphometric analyses. As a result, fluorescence intensities for different experimental conditions were not directly comparable. Three-dimensional (3-D) reconstructions of individual ICs were performed in Fluoview FV1000 software, and specific cell orientations were saved as TIFF images for subsequent morphometric analysis (Fig. 1A). The fluorescence intensity (grayscale, uncalibrated optical densiometry) and areas (pixels) of structures in digital images were measured using the elliptical brush functions or polygon in ImageJ, respectively. For determination of cell shape, three width and length (i.e., depth) measurements spanning the \( \alpha \)-IC basolateral surface were made along the horizontal and vertical planes using the line function of ImageJ. The area of AE1 stained was measured using the

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**Fig. 1.** Axial distribution of \( \alpha \)-intercalated cell (IC) morphology as defined by anion exchanger (AE)1 expression. A: strategy for morphometric analysis of \( \alpha \)-ICs. The lateral view shows a three-dimensional (3-D) reconstruction of regions of interest containing individual \( \alpha \)-ICs rotated such that the apical boundary defined by zonula occludens 1 (ZO-1; green) was orthogonal to the viewer with the apical surface above and the basolateral region below. Basolateral morphology defined by AE1 staining (red) was determined by making measurements of length (depth) along the vertical axis and width along the horizontal axis as described in MATERIALS AND METHODS. The vertical view shows cells in the lateral perspective rotated 90° such that the ZO-1 boundary is facing the viewer. In this perspective, the basolateral area encompassing the nucleus (blue) and demarcated by AE1 was measured (dashed lines). B: images of microdissected rabbit collecting duct (CD) segments and 3-D reconstructions of individual \( \alpha \)-ICs from left to right: cortex [cortical CD (CCD)], outer stripe [outer medullary CD (OMCD)], and inner stripe [medullary CD (MCD)]. Top, 3-D reconstructions showing representative \( \alpha \)-IC shapes viewed from the lateral perspective where ZO-1 (green) demarcates basolateral expression of AE1 (red). Bottom, the vertical perspective (lateral view rotated 90° toward the reader) of the same \( \alpha \)-ICs. Blue circles (with the region of interest number in white) outline nuclear 4',6-di-amidino-2-phenylindole (DAPI) staining in some images.
Table 1. Summary of intercalated cell morphometric analysis

<table>
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<tr>
<th>Parameter</th>
<th>Acid/Base Status</th>
<th>Number of Rabbits</th>
<th>Number of CDs/Rabbit</th>
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<th>Number of Cells</th>
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CD, collecting duct; CCD, cortical CD; MCD, medullary CD; AE, anion exchanger.

RESULTS

Axial distribution of α-IC morphology along the rabbit CD. In the present study, we examined the 3-D structure of α-ICs in the rabbit CD to better understand the structure-function relationships that are associated with the regulation of acid-base homeostasis (3, 18, 19, 23, 36). Figure 1B shows CD segments stained for AE1 (red) from cortical (CCD), OMCD, and OMCDi of the rabbit kidney alongside 3-D reconstructions of representative individual cells observed within the respective CD segment. Figure 1B, top, shows α-ICs from the lateral perspective [cells were rotated such that the apical boundary defined by ZO-1 (green) was above AE1 on the horizontal axis and the widest portion of the cell was orthogonal to the viewer]. Figure 1B, bottom, show α-ICs viewed from a vertical perspective (from the lateral orientation, the apical side of α-IC was rotated 90° toward the viewer).

The CCD contained 11.0 ± 1.2 AE1-positive cells/200-μm CD length. When viewed from the lateral perspective, α-ICs in this region typically exhibited a roughly square or pyramidal/conical shape tapering toward an apical boundary defined by ZO-1 (Fig. 1B, top left). Upon transition to the OMCDi, AE1-positive cells increased in number to 15.4 ± 1.1/200 μm (CCD → OMCD: P < 0.004), and α-ICs in this region exhibited a more rectangular shape in the lateral perspective (Fig. 1B, top middle). AE1-positive cells in this region occasionally exhibited cytoplasmic lateral projections in the vertical perspective (Fig. 1B, middle bottom). Cell numbers in CDs from the inner stripe increased dramatically to 32.0 ± 1.3 cells/200 μm. The lateral α-IC shape was rectangular, and the prevalence of cytoplasmic lateral projections appeared to decrease with increased density of AE1-positive cells (Fig. 1B, right). The axial variation in α-IC morphology raises the possibility that cells in the cortex and medulla may have different cellular functions in acid-base homeostasis.

Metabolic acidosis reversibly alters the shape of α-ICs in the CCD. Since proton secretory flux is higher in the rabbit OMCD than in the CCD (10, 32, 35), we examined whether α-IC morphology was influenced by acid-base status by comparing AE1 morphology in CDs from normal rabbits (normal; urine pH: 8.31 ± 0.28 and HCO3−: 27.3 ± 4.5 mM) with those from rabbits administered NH4Cl for 3 days (acidosis; urine pH: 4.80 ± 0.48 and HCO3−: 18 ± 4.3 mM) and with those from rabbits given NH4Cl for 3 days and then abruptly transitioned to NaHCO3 for 12–18 h (recovery; urine pH: 8.16 ± 0.32 and HCO3−: 29.4 ± 5.6 mM) (18). The α-IC cell shape, defined by AE1 expression, was determined from images of 3-D reconstructions of individual cells with the specified orientation. For cells viewed from the lateral perspective (Fig. 2, A–C, G, and H), the line function of ImageJ was used to make three length and three width measurements spanning the cell shape, and a width-to-length ratio was calculated from the average of the respective measurements. As described above, in CDs from normal rabbits, the basolateral region of α-ICs defined by AE1 expression typically exhibited a roughly square to pyramidal or cone-like shape that tapered toward the apical boundary defined by ZO-1 (Fig. 2A); in this condition, the ratio of width to length was 1.3 ± 0.06 (Fig. 2K, top). However, in CDs from acidic rabbits, the basolateral shape was more

Statistics. Significance of changes in transporter morphology and distribution was assessed by Student’s t-test (two tails, two samples with equal variance). The Mann-Whitney U-test was also used to test statistical significance of B1-V-ATPase distribution in CDs from different acid-base conditions, as the assumption of a normal distribution was not necessarily valid in this case. When three comparisons were performed, statistical significance was established as P ≤ 0.02 using the Bonferroni correction of the 95% confidence interval.

ImageJ polygon function. Numbers of rabbits, sections, CDs, and cells analyzed for each parameter are shown in Table 1.

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Fig. 2. Acidosis alters basolateral α-IC shape in the CCD. Images of 3-D reconstructions of individual α-IC stained for AE1 (red) and ZO-1 (green) are shown. Cells that represent the respective acid/base condition are viewed from the lateral (CCD: A–C and MCD: G and H) or vertical perspective (CCD: D–F and MCD: I and J) and reveal IC shape changes or changes in AE1 distribution induced by acidosis, respectively. Yellow is an artifact of the two-dimensional image derived from the 3-D projection. K: α-ICs in the CCD adapt a more rectangular shape defined by AE1 expression in response to acidosis. Top, ratio of width (horizontal axis) to length (vertical axis). Bottom, ratio of AE1 area measured from the vertical perspective (as in D–F) to the depth (length) measured from the lateral view (as in A–C). The CCD results shown in K comprise 4–8 rabbits for each acid-base condition and 2–4 CCDs/rabbit, whereas the MCD results comprise 6–8 rabbits/condition and 3–6 MCDs/rabbit. See Table 1 for total numbers of cells analyzed for each acid-base condition. N, normal; A, acidic. For the CCD, *normal → acidosis: P < 0.0012; **acidosis → recovery: P < 0.01. ***CCD → MCD: P < 0.0001.

Modification of the basolateral shape of α-IC was also determined by measuring the AE1 area in images of cells viewed from the vertical perspective (Fig. 2, D–F, I, and J) using the polygon function in ImageJ. A ratio of area to length (depth, as determined from the lateral perspective) was calculated (area/depth; Fig. 2K, bottom). In the CCD, acidosis reduced depth without changing area (normal: 77,405 ± 8,723 pixel area vs. acidosis: 84,792 ± 9,076 pixel area; P > 0.5; thus, the area-to-depth ratio increased from 323 ± 35 to 313 ± 31 (normal → acidosis: P < 0.001; Fig. 2K, bottom)). In addition, during acidosis, AE1 staining appeared more polar-
ized to the basal (Fig. 2B) and lateral (Fig. 2E) membranes. Upon recovery, the area-to-depth ratio returned to essentially normal values (349 ± 17, normal → recovery: P > 0.5), confirming an overall change in basolateral α-IC shape, as defined by AE1 expression, in response to acid-base disturbances. Although acidosis reversibly altered basolateral morphology of α-ICs, cell volume (calculated by multiplying the cell area by cell depth) was unchanged (data not shown), indicating that the observed changes represented an alteration of α-IC shape rather than an increase in cell size.

In contrast to the CCD, the dimensions of AE1 expression in the OMCD were not influenced by acidosis. As shown in Figs. 1B and 2, G and H, in OMCDs from normal rabbits, the basolateral regions of α-ICs were generally more rectangular (width-to-length ratio: 2.5 ± 0.13) or shallow (area-to-depth ratio: 561 ± 28) compared with cells located in the CCD (CCD → MCD: P < 0.0001; Fig. 2K). Since the dimensions of α-ICs in the inner stripe versus outer stripe were indistinguishable, these cell populations were pooled together as the OMCD population in Fig. 2K. Volumes of AE1-positive surfaces in the CCD and OMCD were not significantly different (P > 0.1; data not shown), reaffirming that the cell shape of α-ICs, rather than cell size, was modified along the CD. Acidosis resulted in a statistically insignificant change in the basolateral AE1 dimensions of α-ICs of the OMCD (Fig. 2, G, H, and K), indicating that the morphological adaptations of α-ICs in response to acidosis are more extensive in the CCD than in the OMCD.

*Metabolic acidosis induces reversible radial redistribution of AE1 in α-ICs.* In addition to morphological adaptations, α-ICs redirect AE1 toward basolateral surfaces in response to acidosis. We have confirmed and extended our previous observations made in kidney sections regarding AE1 redistribution in response to acidosis (19), via 3-D morphometric analysis of AE1 distribution in microdissected CCDs. AE1 distribution was determined by measuring AE1 intensity in relation to the nucleus. When viewed from the vertical perspective (Fig. 3A), the outline of 4′,6-diamidino-2-phenylindole-stained nuclei (blue circle) defined cell regions adjacent to or overlapping the nuclear boundary. The intensity of AE1 staining along the outer cell boundary (OCB) versus staining overlapping the nucleus along the perinuclear boundary (PNB) was measured using the elliptical brush selection tool in ImageJ. As shown in Fig. 3B, the OCB-to-PNB ratio was 1.1 ± 0.3 in α-ICs from normal rabbits, indicating that AE1 is evenly distributed throughout the cytoplasm under conditions in which bicarbonate secretion predominates in the rabbit CCD (25, 32). However, during acidosis, when the rabbit CCD switches from net bicarbonate secretion to net proton secretion (25, 32), AE1 staining intensity increased on the outer edge of the cell such that the OCB-to-PNB ratio increased to 1.6 ± 0.1 (normal → acidosis: P < 0.001; Fig. 3B). Upon recovery from acidosis, the OCB-to-PNB ratio returned to near normal (1.2 ± 0.04, acidosis → recovery: P < 0.005 and normal → recovery: P > 0.5), confirming the contribution of acid-base status to AE1 distribution in α-ICs. Although morphological adaptation of the basolateral region of α-ICs in the OMCD was not observed, redistribution of AE1 did occur (Fig. 2, compare I and J); the OCB-to-PNB ratio in normal MCDs was higher in the OMCD than in the CCD (normal CCD → normal MCD: P < 0.001; Fig. 3B), indicating that AE1 is more laterally distributed in the distal OMCD under normal conditions. Acidosis further increased the lateral distribution of AE1 in the OMCD to 1.8 ± 0.1 (normal → acidosis: P < 0.001), suggesting that medullary α-ICs are also adapting to acidosis.

**Apical projection of B1-V-ATPase during metabolic acidosis.** The results described above suggest that changes in the basolateral morphology of cortical α-ICs reflect modifications of cell structure to facilitate H⁺/HCO₃⁻ flux in response to acidosis. In the next set of experiments, we sought to characterize any change in the apical B1 subunit of V-ATPase that results concomitant with modification of basolateral AE1 morphology. In initial experiments, we investigated B1 subunit distribution in rabbit kidney sections. Immunofluorescence staining of B1-V-ATPase revealed a reversible apical projection of the B1 subunit in AE1-negative and AE1-positive IC populations during acidosis. As shown in Fig. 4, A and B, we measured the intensity of B1-V-ATPase staining in cells along the vertical axis (yellow parallel lines) using the line function of ImageJ (top) to generate plots of fluorescent intensity (uncalibrated optical density) versus distance (pixels) and then calculated apical-to-basolateral ratios from peak intensities and the integrated area under the curve (Fig. 4, C and D).
Figure 4, A and C, shows the B1 subunit distribution most frequently associated with ICs from normal rabbit CCDs, where the apical-to-basolateral ratio was >2 regardless of IC subtype; this ratio results from the diffuse cytoplasmic distribution of the B1 subunit in rabbit ICs coupled with the relative size of the apical versus basolateral regions (see explanation below). This contrasts with rodent species where the B1 subunit clearly exhibits a predominantly apical or basolateral distribution (1). This is shown in Fig. 4A, where the distribution was predominantly cytoplasmic; however, since the apical region was larger than the basolateral space, the dimensional analysis gave an apical-to-basolateral ratio of >1, as shown in Fig. 4C. Because the B1-V-ATPase distribution in rabbit is diffusely cytoplasmic, it is not possible to define IC subtypes in the rabbit by B1-V-ATPase polarization. Figure 4, B and D, shows the increase in the apical-to-basolateral ratio indicative of a shift in B1 subunit distribution toward the apical pole (i.e., increased apical-to-basolateral ratio) that was generally observed in CCDs obtained from acidic rabbits. Acidosis induced apical redistribution in both AE1-positive and AE1-negative IC subtypes (normal → acidosis: P < 0.001; Fig. 4E), and recovery from acidosis restored the B1 subunit distribution to essentially normal (acidosis → recovery: P < 0.01 and normal → recovery: P > 0.15). Similar results were obtained for PNA-positive cells (β-ICs), where the proportion of cells with an apical-to-basolateral peak intensity ratio of >3 was as follows: normal = 28%, acidosis = 81%, and recovery = 15% (see Table 1 for numbers of rabbits, sections, and cells analyzed).

We confirmed the results obtained in kidney sections with 3-D morphometric analysis of B1-V-ATPase distribution in ICs from microdissected CCDs. The apical-to-basolateral distribution of B1-V-ATPase was measured in 3-D reconstructed individual cells from Z-stack images of CCDs obtained by
confocal microscopy. As described for analysis of anion exchangers, ICs were rotated to the lateral viewpoint, such that the apical boundary defined by ZO-1 was in the horizontal plane (green line; Fig. 5, A–C). The distribution of B1-V-ATPase along the vertical plane of intercalated cells (B1-positive cells) was quantitated using ImageJ as described for kidney sections above. As shown in Fig. 5G, acidosis increased the proportion of cells with predominantly apical B1 subunit distribution (apical-to-basolateral ratio ≥10) to 62% compared with 10% and 6% in normal and recovery rabbits, respectively (normal → acidosis and acidosis → recovery: P < 0.001; normal → recovery: P > 0.5; by Mann-Whitney U-test). B1 subunit redistribution occurred in both IC subtypes; both pendrin-positive and pendrin-negative cells revealed similar changes in B1 subunit distribution in response to acidosis, and B1-V-ATPase redistribution coincided with changes in pendrin expression in β-IC, as we have previously reported (18, 19). Figure 5 shows lateral view of representative α-ICs from the CCDs of normal (D), acidic (E), and recovery (F) rabbits. Note that the transition of the basolateral region defined by AE1 (green) from a pyramidal or conical shape to a more rectangular shape during acidosis was accompanied by apical projection of B1-V-ATPase (red).

α-ICs from the OMCDo have unusual shapes and processes that resemble dendritic cells. Figure 6 shows AE1 staining of the basolateral processes of several α-ICs from the OMCDo taken from normal rabbit kidneys from a vertical perspective. The morphology of these cytoplasmic projections was variable. Some cytoplasmic projections had a stellate appearance (Fig. 6, left) similar to what has been previously noted in the OMCD of acid-loaded rabbits (36). In other α-ICs, the cytoplasmic projections branched, resulting in a dendritic cell-like morphology (Fig. 6, middle and right) (31). Note that dendritic cells are antigen-presenting cells that initiate protective T cell responses and participate in the immune surveillance system (31). Such an appearance was rarely noted in the OMCDi or inner stripe, where the density of α-ICs was much higher. In the OMCD in regions in which there were <25 ICs/200 μm, 53 ± 1% of cells had at least one lateral projection. In regions in which there were >25 ICs/200 μm, only 11 ± 1% exhibited lateral projections. A stellate appearance had been previously noted in the OMCD of acid-loaded rabbits (36), but we observed dendritic cell-like morphology predominantly in OMCDo segments from normal rabbits. Metabolic acidosis did not overtly affect the morphology of α-ICs in the OMCDo (not shown).

![Fig. 5. Apical projection of B1-V-ATPase accompanies basolateral shape change in α-ICs. A–C: images of B1-V-ATPase distribution (lateral view) in ICs associated with normal (A), acidic (B), and recovery rabbits (C). Green lines denote the apical boundary defined by ZO-1, and blue circle outline DAPI-stained nuclei. D–F: images of α-ICs with B1-V-ATPase (red) and AE1 (green) distributions associated with normal (D), acidic (E), and recovery rabbits (F). G: B1-V-ATPase distribution in microdissected CCDs including both IC subtypes. Normal → acidosis and acidosis → recovery. ***p < 0.0005.](http://ajprenal.physiology.org/doi/10.1152/ajprenal.00161.2015/figure/F5)
DISCUSSION

Data from microdissected CDs showed that there were progressively more AE1-positive cells as one moved from the cortex to the outer stripe to the inner stripe of the outer medulla. The shape of the α-ICs changed as well, from a pyramidal/conical shape in the cortex to a more rectangular shape in the outer medulla. The prevalence of dendritic cell-like lateral projections was maximal in the outer stripe and diminished with increased density of α-ICs in the inner stripe. Such axial variation in number and shape suggest the possibility of different functional activities along the CD. Metabolic acidosis (for 3 days) stimulates proton secretion by α-ICs, and this was associated with a transition from a pyramidal to a more rectangular shape of the α-IC in the CCD. Such changes in α-IC shape were reversible with recovery from acidosis, which occurred over 16 h. Note, there were no changes in IC size induced by acidosis, even though there were changes in IC distribution of AE1 (see below). Interestingly, acidosis did not induce a change in IC shape in the OMCD. Thus, acidosis induces in the CCD a “medullarization” of α-ICs, which greatly enhances net proton secretion across the epithelium. Medullary α-ICs did not appear to undergo such changes.

Within the α-ICs, there was a major change in AE1 distribution during metabolic acidosis; the intensity of AE1 staining along the outer edge of the cell (basolateral membrane) increased compared with AE1 staining over the perinuclear region in the CCD and OMCD. Such findings have been previously noted ultrastructurally by Verlander et al. (37). Such polarization would facilitate increased basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange and transepithelial proton secretion by creating a larger surface area dedicated to electrochemical transport, as has been demonstrated in both CCD and OMCD segments (25, 32, 33, 35). Our study shows that such a redistribution of transporters and change of shape to α-ICs in the CCD occurs rather quickly (within a few days or less) and is reversible within 16 h. The speed of such adaptation suggests that vesicle trafficking perhaps along cytoskeletal fibrils may contribute to the cellular changes. Additional studies examining the endosomal trafficking of AEs in ICs will be necessary to determine which vesicular pathways are essential for this form of adaptation. Indeed, for β-ICs, adaptation to acidosis involved a reduction in the static volume of apical recycling endosomes containing pendrin (18); the endosomal compartments through which AE1 redistribution occurs has yet to be established.

Concomitant with modification of basolateral α-IC morphology and AE1 subcellular distribution, acidosis also induces an increase in the apical polarization of B1-V-ATPase; the B1 subunit appeared to be more apically polarized during acidosis compared with baseline, and this was true for both AE1-positive as well as AE1-negative ICs (Figs. 4 and 5). Apical redistribution of V-ATPase was also observed by ultrastructure at the cellular organelle level by Verlander and colleagues (36). Apical polarization would favor higher rates of proton secretion in α-ICs, but in β-ICs, such apical polarization would lead to a reduction in bicarbonate secretion, as previously found (21, 19). We and others have shown that pendrin protein and mRNA abundance of the apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger in β-ICs is reversibly downregulated during acidosis (18, 19), further inhibiting offsetting bicarbonate secretion from the net transepithelial flux. Whether or not this apparent redistribution of the B1 subunit to the apical region is due to increased trafficking or an increase in apical surface area containing B1-V-ATPase (in microvilli) has yet to be determined. Our analysis did not have sufficient resolution to quantify microvilli, but it

Fig. 6. α-ICs in the outer stripe of the OMCD frequently exhibit dendritic cell-like morphology. A vertical view of individual α-ICs (3-D reconstructions) with lateral cytoplasmic projections is shown. Blue circles (region of interest number in white) outline nuclear DAPI staining. In some α-ICs, the cytoplasmic projections begin to branch (arrows).
is possible that α-IC apical microvilli may have grown under the influence of the low pH, much as has been shown to result from cellular cAMP generation (17). The growth of apical microvilli containing V-ATPase would likely increase apical surface area and thereby facilitate exocytic insertion of H+ pumps.

Examination of α-IC morphology also revealed striking cell shapes (Fig. 6) located primarily in the OMCDo that are uncharacteristic of a prototypical epithelial cell, rather bearing a resemblance to tissue dendritic cells (2, 13) and microglial cells of the central nervous system (9, 40). Dendritic cells are professional antigen-presenting cells that bridge innate and adaptive immune responses to infection by pathogens (2, 13, 31). This raises the question as to whether the dendritic-like α-IC structure is indicative of an ancillary function for these cells in the distal nephron. Chassín et al. (4) demonstrated that uropathogenic Escherichia coli preferentially adhere to the luminal surface of ICs. Subsequently, ICs initiate the renal immune response to bacteria by several distinct signaling pathways, including Toll-like receptor 4-dependent and -independent pathways. Further α-ICs help defend the kidney against uropathogenic E. coli bacteria by secreting bactericidal antimicrobial peptides and bacteriostatic nutrient metal scavengers (16, 27–30). Because they are located in the CD, ICs are ideally positioned to recognize the threat of ascending pathogens. It is intriguing to speculate that the dendritic cell-like structural phenotype may use these appendages for antigen presentation and/or constitutive macropinocytosis of bacteria (15).

In conclusion, α-ICs exhibit structural diversity depending on location and acid-base status. We have demonstrated that acidosis results in α-IC reconfiguring from a pyramidal to rectangular shape, redirection of AE1 to the basolateral membrane, and apical projection of B1-V-ATPase. These structural phenotypes varied depending on location (CCD vs. OMCDi), indicating that in addition to IC subtype (α-IC vs. β-IC), location needs to be a factor when evaluating IC structure and function. Additionally, we have identified a subset of α-ICs with branching lateral cytoplasmic projections that exhibit a dendritic cell structural phenotype. Whereas rabbit ICs may have differences compared with ICs from humans and other model systems, this subset of α-ICs is clearly of interest. Further investigation into the endosomal compartments responsible for AE1 distribution and surface receptor expression, which may involve antigen presentation, macropinocytosis of bacteria, and adaptive immune response, would be warranted.

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DISCLOSURES

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