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Human intestinal anion exchanger isoforms: expression, distribution, and membrane localization

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Abstract

A family of anion exchangers (AEs) including AE1, AE2 and AE3 has been described. AE3 gene has been shown to encode two alternatively spliced isoforms termed as bAE3 (brain subtype) and cAE3 (cardiac subtype). The identity of the AE(s) involved in the human intestinal NaCl absorption is not fully understood. Current studies were undertaken to identify the AE isoforms expressed in the human intestine, to define their regional and vertical axis (crypt vs. surface cells) distribution, and to elucidate their membrane localization in the epithelial cells along the entire length of the human intestine. Our studies utilizing reverse transcription (RT)-PCR with total RNA extracted from pinch biopsies from various regions of the human intestine demonstrate that AE2 and bAE3 but not AE1 or cAE3 were expressed in all the regions of the human intestine. Utilizing in situ RT-PCR, we demonstrated that the message of AE2 was expressed throughout the vertical surface–crypt axis of the colon. Our Western blotting studies demonstrated that AE2 and bAE3 are localized to the basolateral but not the apical membranes of the intestinal epithelial cells from the human ileum and colon. In conclusion, our results demonstrated that in the human intestine, AE2 and bAE3, but not AE1 or cAE3, are expressed throughout the tract with the highest expression in the colon compared to the ileum and jejunum. Both the isoforms were found to be localized to the basolateral but not the apical membranes of the epithelial cells. We speculate that, in the human intestine, AE2 and bAE3 may be the 'housekeeping' isoforms, and the apical AE, the potential candidate for chloride absorption, remains to be identified.

Keywords: Human colon; Cl^-/HCO_3^- exchanger; Immunoblotting; In situ reverse transcription-PCR

1. Introduction

The major mechanism of NaCl absorption in the human intestine has been suggested to be through the operation of dual ion exchangers of Na^+/H^+ and Cl^-/HCO_3^- [1]. Sodium–hydrogen exchangers (NHEs) and anion exchangers (AEs) are plasma membrane proteins that mediate in eukaryotes the electroneutral exchange of extracellular Na^+ and Cl^- with intracellular H^+ and HCO_3^-, respectively, with a stoichiometry of 1:1. These exchange activities are nearly ubiquitous among vertebrate cells. Although NHE isoforms and their involvement in intestinal vectorial Na^+ transport are currently under intense investigation by many investigators including our laboratory, the identity of the human intestinal

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AE isoforms and their role in the intestinal chloride absorption has not been defined.

AEs have been shown to contribute, in a cell-specific manner, to the maintenance of intracellular pH_i, intracellular [Cl^-], volume regulation, and vectorial bicarbonate and chloride transport [2]. Recent studies have identified a family of at least three structurally and functionally related genes for AE, termed as AE1, AE2 and AE3 [2]. The cDNA and genes for these isoforms have been cloned from different species including human [2].

AE1 or band-3 AE in the erythrocytes was the first described AE. Outside the erythrocytes the predominant site of AE1 expression is the kidney, where immunocytochemical localization studies have found its polypeptide restricted to the basolateral membrane of type-A acid secreting intercalated cells [2]. AE2 mRNAs are widely expressed in epithelial and mesenchymal tissues [2]. Membrane localization studies revealed that AE2 polypeptide was disposed to the basolateral membranes of alveolar epithelial cell monolayers in the rat [3], choroid plexus epithelium [4], mouse small intestinal epithelium [5] and gastric parietal cells in the human [6]. Previous studies, however, have revealed that membrane localization of the AE2 isoform in the mammalian intestine is controversial. For example, in the rabbit ileum, Chow et al. demonstrated that AE2 was localized to the apical membranes [7]. In contrast, Rossman et al. showed that AE2 was localized to the basolateral membranes of rabbit ileal enterocytes [8]. Of the above three isoforms, AE3 gene has been shown to code at least two transcripts and two protein isoforms, bAE3 (brain subtype) and cAE3 (cardiac subtype) which are believed to be alternatively spliced products of the same gene. The two proteins differ in their 42 amino acid residues at the N-terminal. Adult hearts of rat, rabbit, chicken and human express both bAE3 and cAE3 at roughly equal levels [9]. To date, very little is known about expression of the AE3 isoforms in epithelial tissues.

Previous studies from our laboratory, utilizing organ donor colonic apical and basolateral membrane vesicles, have shown the presence of kinetically distinct Cl^-/HCO_3^- exchange activities in the apical and basolateral membranes of the human colon [10–12]. Therefore, the goals of the current studies were to identify the AE isoforms expressed along the entire length of the human intestine, to compare their relative abundance in various regions of the tract, to characterize the apical vs. basolateral membrane localization and the vertical axis distribution of the isoforms.

Our results demonstrated that: (i) AE2 and bAE3 isoform mRNAs were present along the entire length of the human intestine, whereas AE1 and cAE3 mRNAs were not detected; (ii) AE2 mRNA was expressed along the entire vertical axis of the colonic crypt including the surface cells; and (iii) the protein products of AE2 and bAE3 were localized predominantly to the basolateral but not the apical membranes with a higher expression in the colon compared to the ileum and jejunum.

2. Materials and methods

2.1. Isolation of RNA

Human tissue samples were either obtained from pinch biopsies or surgically removed and immediately suspended in RNAzol solution for total RNA extraction. Collection of these specimens was performed according to approved protocols by the Institutional Review Board of the University of Illinois at Chicago and by the Human Investigations Subcommittee of the West Side VA Medical Center. Total RNA was extracted from various tissue sources by the method of Chomczynski and Sacchi [13] using RNAzol solution supplied by the manufacturer (Tel-Test Inc., Friendswood, TX, USA) and essentially using the manufacturer's protocol.

2.2. Designing of PCR primer sets for AE1, AE2, bAE3 and cAE3

The unique PCR primer sets for AE1, AE2, bAE3 and cAE3 were designed from the amino-terminal regions (region exhibiting maximal diversity among the isoforms) of the published human sequences utilizing Gene Works software (Intelligenetics, Mountain View, CA, USA). The primer sequences are given below: AE1: 5′-primer: 5′-CCAGACTCCAGCTTCTACAAGG-3′ (position 1174–1195), 3′-primer: 5′-GGAAGGAGAAGATCTCCTGG-3′ (position 1674–1693) (length of amplified region 520 residues,

M27819) [14]; AE2: 5′-primer: 5′-GAAGATTCCT-GAGAATGCCG-3′ (position 1698–1717), 3′-primer: 5′-GTCCATGTTGGCACTACTCG-3′ (position 1859–1878) (length of amplified region 181 residues, U62531) [15]; AE3: 5′-primer: 5′-GTCCTCATCCT-GATCTTCATGG-3′ (position 2997–3018), 3′-primer: 5′-GGACAGCTGGATACCAGAC-3′ (position 3377–3395) (length of amplified region 399 residues, U05596) [9]; bAE3: 5′-primer: 5′-ATCTGAGGCA-GAACCTGTGG-3′ (position 452–471), 3′-primer: 5′-TTTCACTAAGTGTCGCCGC-3′ (position 851–869) (length of amplified region 418 residues, U05596) [9]; cAE3: 5′-primer: 5′-TTTGAG-GATGGTGACCTGTG-3′ (position 260–279), 3′-primer: 5′-CTTGTCATCGTTGGGATGG-3′ (position 793–811) (length of amplified region 551 residues, U05597) [9].

2.3. Reverse transcription (RT)-PCR technique

RT-PCR was performed essentially as described [16]. Briefly, 2–4 μg of total RNA was used for RT with sequence-specific primers and SuperScript II reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA). The reaction was carried out in a 20 μl reaction containing 100 mM Tris–HCl pH 8.3, 2.5 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 50 mM KCl, 1 μM dNTPs, 10 μM of antisense primer, 1 μl of SuperScript II reverse transcriptase and incubated at 42°C for 1 h. The reaction was terminated by heating at 70°C for 15 min, then 1 μl of RNase H was added to the reaction and incubated for 20 min at 37°C.

Two μl of the RT reaction was diluted into a final volume of 50 μl of a PCR mix containing 100 mM Tris–HCl (pH 8.3), 2.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTPs, 1 μM of each antisense and sense primers, 0.5 μl of a mix of Taq DNA polymerase (Gibco BRL, Gaithersburg, MD, USA) and Taq Pfu (Stratagene, La Jolla, CA, USA) with the ratio of 16:1 (unit/unit). PCR reaction was carried out using a Microcycler programmable heating/cooling dry block (Perkin Elmer Corp., Norfolk, CT, USA) for 30–40 cycles of amplification (94°C, 30 s; 55°C, 1 min; 72°C, 30 s) followed by 10 min at 72°C. PCR products were separated by electrophoresis on a 1% agarose gel containing ethidium bromide (0.5 μg/ml). Bands of expected sizes were visualized under UV light utilizing Eagle eye II Still Video System (Stratagene, La Jolla, CA, USA).

2.4. In situ RT-PCR

This technique was performed as described by Nuovo [17] with minor modifications which have been standardized in our laboratory for the expression of AE isoforms in the intestinal tissues. Briefly, tissues fixed in formalin and stored in paraffin blocks were carefully selected to include specific regions of the human intestine. Slides with tissue sections ~5 μm thick were initially deparaffinized by three xylene washes, 2 min each, hydrated with graded ethanol and treated with pepsin (2 mg/ml+0.1 N HCl) for 15–30 min at 37°C in a moist chamber. Tissue sections were washed with DEPC water and then with 100% ethanol for 1 min each, and treated with RNase-free DNase at 37°C overnight in a moist chamber. RT reaction on the tissue section was performed utilizing gene-specific primers and RT reaction mixture (containing 100 mM Tris–HCl pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 1 mM dNTPs, 10 μM antisense primer, 2 U/μl of RNasin (Promega, Madison, WI, USA) and 5 U/μl of SuperScript II reverse transcriptase) in a total volume of 40 μl at 42°C in a moist chamber for 1 h. The tissue sections were then washed again with DEPC water and 100% ethanol. The cDNA is then amplified in situ by direct incorporation of the digoxigenin-dUTP (Boehringer-Mannheim, Indianapolis, IN, USA), in the amplified product, in 100 μl of a PCR reaction mix (containing 100 mM Tris–HCl pH 8.3, 50 mM KCl, 4.5 mM $MgCl_2$, 200 μM dNTPs, 10 μM of digoxigenin-dUTP, 1 μM of each primer, 8 U/100 μl Taq Pfu polymerase, 10% glycerol, 3 mg/ml bovine serum albumin) on the tissue sections and incorporating the reporter molecule, digoxigenin-dUTP (Boehringer-Mannheim, Indianapolis, IN, USA), directly in the amplified DNA. The PCR mixture is sealed on the tissue sections with a coverslip utilizing Gene-Frames (Marsh Biomedical, Rochester, NY, USA). The sealed slides with PCR mix on the tissue sections are immediately returned on a robocycler (Stratagene, La Jolla, CA, USA), heated to 96°C for 5 min, then the PCR reaction is carried out by 40 cycles of: 96°C for 1 min, 55°C for 2 min, 74°C for 50 s, followed by 7 min at 74°C. The slides are then

washed in phosphate-buffered saline (PBS) (pH 7.4) buffer and dehydrated with graded ethanol, then incubated with anti-digoxigenin antibody (Boehringer-Mannheim, Indianapolis, IN, USA) conjugated with alkaline phosphatase for 60 min at 37°C in a moist chamber then washed with PBS followed by staining with NBT/BCIP (Boehringer-Mannheim, Indianapolis, IN, USA) at 37°C for 10–15 min. Slides are then washed with PBS, dehydrated with graded ethanol and counterstained with BC-50 Red counterstain (Biomeda, Foster city, CA, USA). The tissue sections are mounted by situ mount (Biomeda, Foster city, CA, USA) and visualized under a microscope. Appropriate negative (omit RT step) and positive control (omit DNase treatment step) tissue slides are processed simultaneously to ensure the specificity of the PCR products synthesized. Adjacent tissue sections from the same tissue block are stained with H&E stain to identify specific cell types matched to the stained cells by the in situ RT-PCR methods.

2.5. Isolation of human intestinal apical and basolateral membrane vesicles

Human intestinal apical (brush-border) and basolateral membranes from the small intestine and colon were prepared as described [18–22], starting with a scraped mucosa from various regions of the intestine procured from organ donors provided by ROBI (Regional Organ Bank of Illinois, Chicago, IL, USA). The final pellet of the membrane vesicles was resuspended in PBS buffer containing protease inhibitors: 50 μM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μg/ml trypsin inhibitors, and 1 μg/ml chymostatin. The membranes were frozen for later analysis by immunoblotting. These investigations were approved by the Human Investigation Committee of the West Side VA Medical Center and the Institutional Review Board of the University of Illinois at Chicago.

2.6. Antibodies

The AE2 antibodies were raised against an 11 amino acid peptide representing amino acid residues 1230–1240 of the C-terminal end of human AE2 (VDEYNEMPMPV). For AE3 they were raised against a 12 amino acid peptide representing amino acids 1221–1232 of the C-terminal end of human bAE3, brain subtype of AE3 (GQDEYNELHMPV). Briefly, the peptides were commercially synthesized by Bio-Synthesis Inc., Lewisville, TX, USA, then conjugated to an adjuvant and injected into two rabbits for each antibody. Enzyme-linked immunosorbent assay testing was done on the immunized serum. The immune serum was passed over a column of activated beads which were covalently coupled to purified anti-immunoglobulin antibodies. The beads were washed and the antibodies were eluted by treating the column with a low pH buffer and then a high pH buffer (Bio-Synthesis Inc., Lewisville, TX, USA). These affinity-purified polyclonal antibodies were used as immunologic probes for immunoblotting studies.

2.7. Immunoblotting

150 μg each of purified brush-border and basolateral membranes were solubilized in Laemmli sample buffer (2% sodium dodecyl sulfate (SDS), 100 mM DTT, 60 mM Tris, pH 6.8, 0.01% bromophenol blue) and separated on 6% Tris–glycine SDS–polyacrylamide gel by the methods of Laemmli. Brush-border and basolateral membrane proteins from the same region and the same organ donor were separated on the same gel along with high range molecular weight protein standards (Amersham, Arlington Heights, IL, USA). The separated proteins were then electrotransferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) and visualized with Ponceau stain to ensure the efficiency of the transfer. Immunoblotting was performed by washing the nitrocellulose membranes three times, then blocking them for 1 h in Blotto solution containing 5% non-fat dry milk in PBS (150 mM NaCl, 10 mM Na-phosphate, 2 mM EDTA, 2% NP 40). The blots were then incubated with the primary antibodies diluted in the blocking solution for 1 h at room temperature, and washed extensively after that with the same solution. The blots were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (diluted 1:2000) in solution containing 1% non-fat dry milk in PBS. Blots were again washed extensively with the

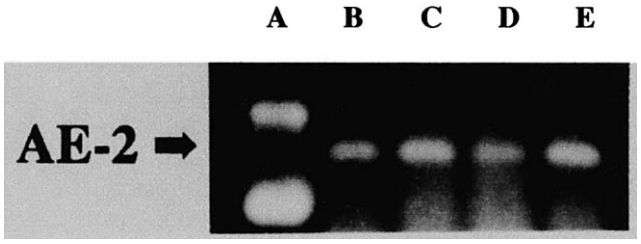

Fig. 1. RT-PCR analysis of AE2 message in the human intestine. Lane A: DNA ladder 123, lane B: ascending colon, lane C: transverse colon, lane D: sigmoid colon, lane E: descending colon.

same solution. The bands were visualized by enhanced chemiluminescence according to the manufacturer's instructions (Amersham, Arlington Heights, IL, USA).

3. Results

3.1. Detection of AE isoforms expressed in the human intestine

To identify the isoforms of AE gene family expressed in the human intestine, we performed RT-PCR on total RNA extracted from pinch biopsy samples obtained endoscopically from different regions of the human intestine. Gene-specific primers for AE1, AE2, bAE3 and cAE3 were designed from the amino-terminal regions of the exchangers which differ between the isoforms (see Section 2). As shown in Fig. 1, the AE2 transcript of an expected size of 181 bp is detected in the human ascending, transverse, sigmoid, and descending colon. AE2 mRNA was also detected in ileum, jejunum and esophagus (not shown). In controls, i.e. in the absence of the RT enzyme, the AE2 PCR products were not synthesized.

Utilizing gene-specific primers (see Section 2), the PCR product of an expected size of 520 bp for AE1 could not be detected in any of the regions of intestine tested. However, under the same conditions we were able to detect the same expected size band in the human kidney RNA (positive control) ensuring that the lack of detection of AE1 in the human intestine was not due to technical reasons.

RT-PCR with AE3 gene-specific primers showed (Fig. 2) that products with the expected size of 399 bp were detected in the jejunum, ileum, ascending, transverse, descending, sigmoid colon, and rectum. To elucidate whether bAE3 or cAE3 subtypes were expressed, another set of primers was designed from the variant N-terminal region of the two subtype isoforms, and RT-PCR with these primers was performed again on total RNA from various regions of the human intestine along with control total RNA for human brain and cardiac tissues. As shown in Fig. 3, the expected PCR products for bAE3 with the size of 418 bp were detected in all human intestinal regions and the brain (used as positive control), whereas cAE3 PCR product (expected size of 551 bp) was only detected with the cardiac RNA (positive control) but not in any region of the human intestine.

Representative PCR fragments (from the descending colon) with the expected size for AE2 and bAE3 were subcloned in PCRII vector utilizing TA cloning kit (In Vitrogen, San Diego, CA, USA) and sequenced to confirm the identity of the AE transcripts. The sequences were 100% identical to the respective published AE sequences [9,23] (not shown).

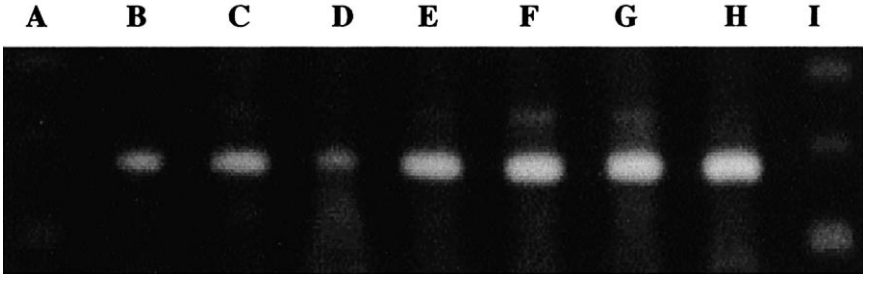

Fig. 2. RT-PCR analysis of AE3 in the human intestine. Lanes A and I represent DNA ladder 123, lanes B, C, D, E, F, G and H represent jejunum, ileum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum, respectively.

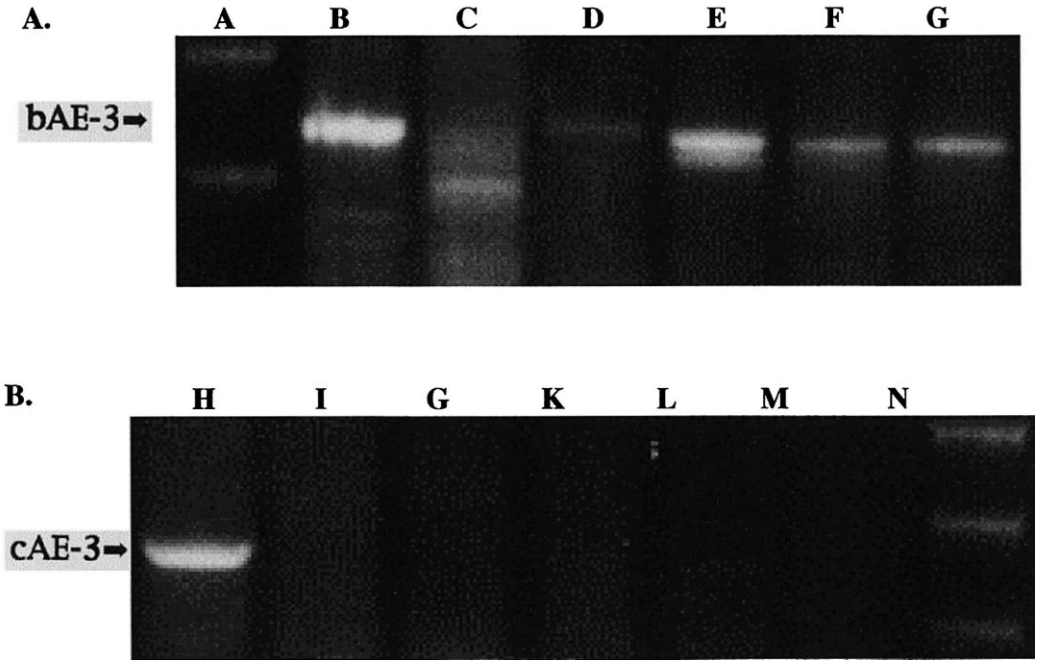

Fig. 3. RT-PCR analysis for bAE3 and cAE3 isoforms in the human colon. Lane B: brain control, lanes C, D, E, F, G represent bAE3 message (size 418 bp) in ascending, transverse, descending, sigmoid colon and rectum, respectively. Lane H represents cardiac control, lanes I–M represent the same colonic regions where no cAE3 message was detected (size 551 bp) compared to the control. Lanes A, N represent DNA ladder (123).

3.2. *Cellular and vertical axis distribution of AE2 transcripts in human intestine*

To further analyze the cellular and vertical axis distribution of AE2 mRNA in the colon, in situ RT-PCR was performed utilizing gene-specific PCR primers for AE2. Formalin-fixed paraffin-embedded archival tissue sections from the colon were treated as described in Section 2. Results are represented in Fig. 4 (left panel), which shows human colonic (de-

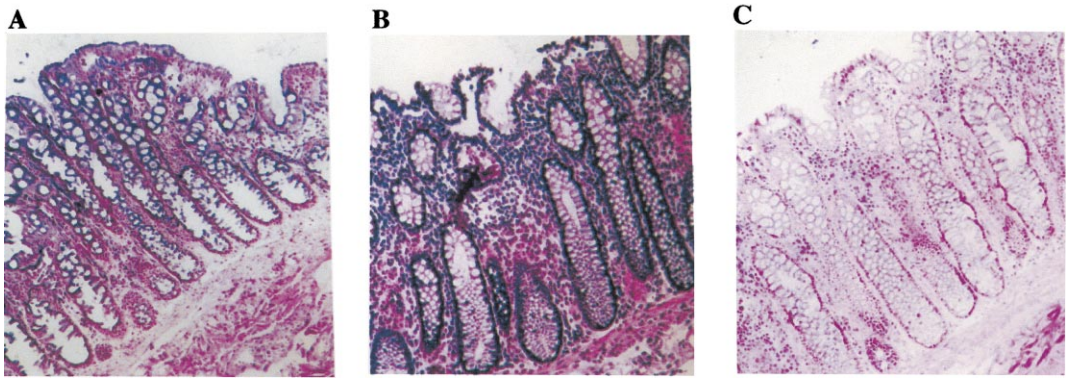

Fig. 4. In situ RT-PCR analysis of AE2 mRNA in descending human colon. A: The cytoplasmic blue signal represents AE2 mRNA which can be seen only in the epithelial cells in the crypt and the surface cells. B: Represents the positive control without DNase digestion where the signal is detected in all cell types and mostly in the nuclei. C: Represents the negative control where the RT enzyme was omitted, and no signal can be detected.

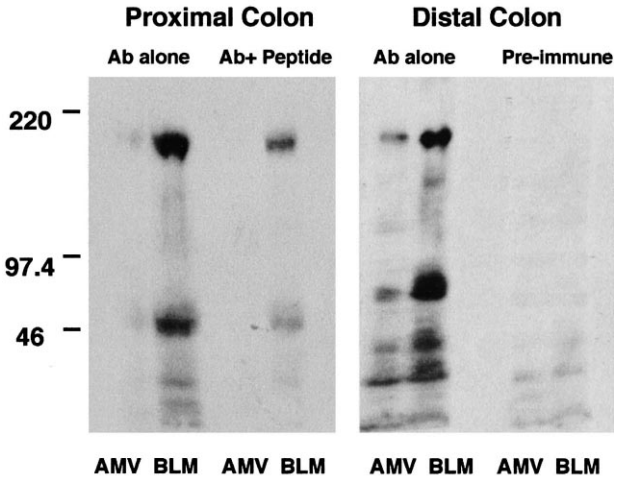

Fig. 5. Immunoblotting for AE2 in the colon. Bands with the expected size ~170 kDa predominantly in the basolateral (BLM) but not the apical (AMV) membranes in both proximal and distal colon are detected. The band signal is competed out significantly with the free peptide (left panel), and the preimmune sera showed no signal of expected size band (right panel).

scending colon) sections, and Fig. 4 (middle and right panels) which represent the positive and the negative controls, in which the DNase step and the RT enzyme were omitted, respectively. The blue cytoplasmic signal which represents AE2 mRNA is observed in all epithelial cell types including the crypt and the surface cells but not in the submucosal regions. The signal in the positive control is noticed in all cell types (epithelial and subepithelial cells) and mostly from the nuclei (representing signal from genomic DNA), whereas no signal was detected in the negative control indicating the specificity of the primers and the reliability of our results.

3.3. Membrane localization of AE2 and bAE3

To determine whether AE2 and bAE3 are apical or basolateral isoforms, we analyzed isolated purified apical and basolateral membranes from different regions of the human intestine by immunoblotting.

Antibodies, raised against peptides representing the C-terminal fragment of AE2 and bAE3 as described before (see Section 2), were used as immunologic probes. As shown in Fig. 5, the AE2 antibodies (1:5000 dilution, 1 h incubation with blot at 25°C) detected a band with the expected relative size of ~170 kDa predominantly in the basolateral membranes of both the proximal and distal colonic regions. As shown in the left panel, incubation of the AE2 antibody with the blot in the presence of free peptide (10 μg/ml) significantly competed out the AE2 band signal. Furthermore, as shown in the right panel, preimmune sera showed no signal of the expected size band, confirming the specificity of our

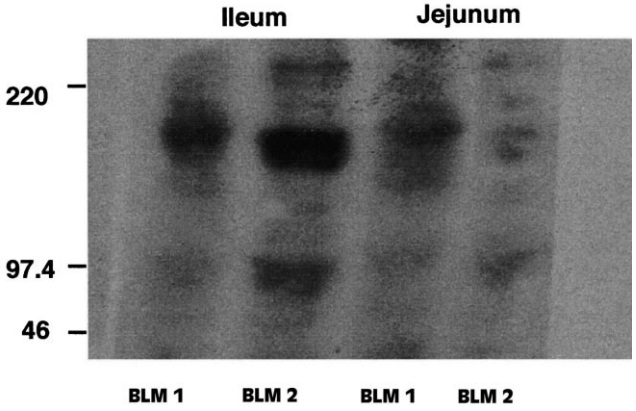

Fig. 6. Immunoblotting for AE2 in the small intestine. The expected size bands ~170 kDa in the BLM of the ileum and jejunum from two different organ donors 1 and 2, after overnight incubation with the antibody at 4°C.

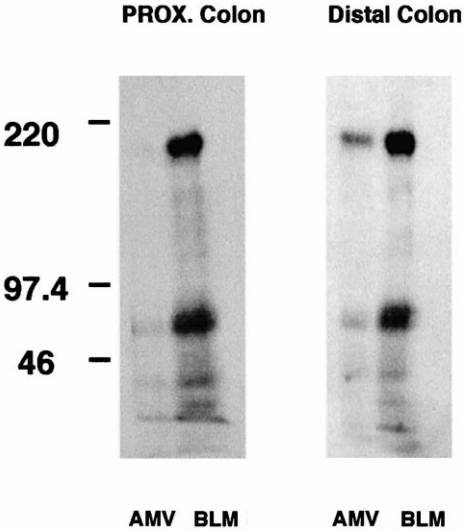

Fig. 7. Immunoblotting for bAE3 in the colon. The bands (size ~165 kDa) are shown predominantly in the basolateral but not the apical membrane, in both the proximal and distal colon.

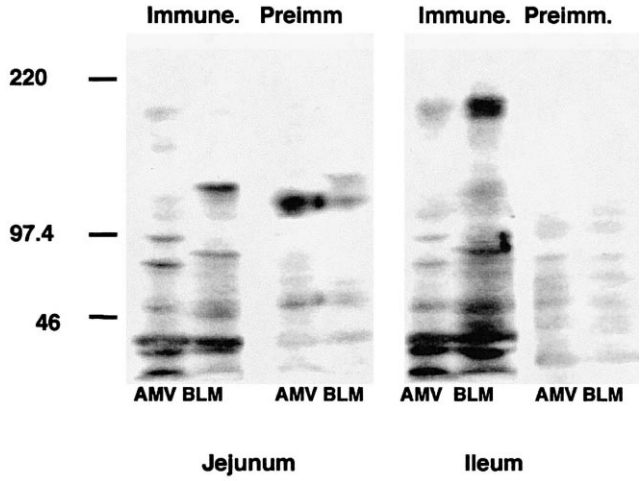

Fig. 8. Immunoblotting for bAE3 in the small intestine. The expected size (~165 kDa) is seen in the basolateral (BLM) but not in the apical membranes (AMV) in the ileum but no expected size bands are seen in the jejunum. No cross reactivity with the preimmune sera is observed.

antipeptide antibodies. Peptide competition and negative staining with the preimmune serum were the same in all the intestinal regions tested (not shown). A faint band of the expected size can also be detected in the human distal colonic apical membranes as well. In the ileum and jejunum, the basolateral band of AE2 could only be detected after overnight incubation of the 1:100 dilution of the primary antibodies, as shown in Fig. 6, which represents basolateral membranes from two separate organ donors. The apical membranes from the jejunum and ileum showed no cross reactivity with these antibodies (data not shown).

Similar to AE2, bAE3 antibodies (1:5000 dilution, 1 h incubation at 25°C) also detected the expected band of about ~165 kDa, predominantly only in the basolateral and not the apical membranes in both the proximal and distal colonic regions (Fig. 7). As shown in Fig. 8, bAE3 antibodies (1:1000 dilution, 1 h incubation at 25°C) detected the expected band of ~165 kDa only on the basolateral and not on the apical membranes in the ileum. In the jejunal regions, the expected size band was not detected. Incubation with the secondary antibodies alone did not show bands of the expected sizes. Bands with the approximately molecular weight of ~50–80 kDa were also detected when the primary antibodies were used, but not with the preimmune serum or the secondary antibodies alone. Most likely these bands represent degradation products of the native polypeptides.

4. Discussion

Utilizing purified apical membrane vesicles, we have previously demonstrated the presence of apical membrane Cl^-/HCO_3^- exchange activity in ileal, proximal and distal colonic regions of the human intestine [10,11,24]. We have also recently demonstrated the presence of kinetically distinct Cl^-/HCO_3^- exchange activity in the basolateral membranes of the human colon [12]. However, the proteins that mediate this transport remain largely unidentified. In the present study, we demonstrate for the first time the detection of the AE isoforms expressed in the human intestine and the cellular localization of their polypeptides in the epithelial cells. We have shown that AE2 and bAE3, not AE1 and cAE3, mRNAs were detected along the entire length of the human intestine. Furthermore, we showed that AE2 mRNA was expressed in all epithelial cell types, including surface cells along the entire vertical axis of the colonic crypt. Our localization studies demonstrated that AE2 and bAE3 polypeptides are localized to the basolateral but not the apical membranes of the epithelial cells.

Red cell band-3 AE1, the best characterized AE, is the prototype member of the AE gene family. Studies have shown that its predominant expression except for the erythrocytes is in the kidney [2]. Rajendran et al. have recently shown that AE1 is also expressed in the rat colon [25]. Our current studies, however, demonstrated that AE1 is not expressed in any region along the entire length of the human intestine. These disagreements in AE isoform expression could possibly be due to species differences.

The AE3 gene has been shown to encode two different AE3 subtypes (brain and cardiac) which are believed to be alternatively spliced transcripts. bAE3 (brain subtype) isoform has been cloned from the brain tissues [26], whereas cAE3 (cardiac subtype) and bAE3 have been cloned from the human heart tissues [9]. However, very little is known about the expression of AE3 in the intestinal epithelia. In

our current studies we have found that bAE3 but not cAE3 message was detected in the human intestine.

AE2 isoform is generally considered to be the epithelial isoform, however, studies from a number of laboratories have detected its mRNA in a variety of other non-epithelial tissues as well [2]. In earlier studies of Chow et al., the cDNA of AE2 was cloned from the enterocytes of the rabbit ileum, and their immunoblotting studies showed that AE2 was localized to brush-border membranes and might be responsible for mediating the chloride absorption by the enterocytes [7]. The full length cDNA of the human intestinal AE2 was obtained in our laboratory from human colonic T84 cells, and it showed 100% homology with the human kidney AE2 (data not shown). Consistent with these results, our current studies, utilizing the RT-PCR technique with gene-specific primers, showed that AE2 is expressed throughout the human intestine. The results of our current study in the human intestine regarding the surface–crypt axis distribution of AE2 mRNA indicated that the message for AE2 in the human colon was also restricted to the surface and crypt epithelial cells. However, no quantitative inferences could be drawn utilizing this technique.

Most of the localization studies of the AE2 isoform have shown that this polypeptide was restricted to the basolateral membranes of polarized epithelial cells including rat colon, mouse small intestine and kidney tubule cells [2,5,27,28]. However, Chow et al. have shown by immunoblotting that AE2 was localized to the apical membranes of the rabbit ileal enterocytes, using polyclonal antibodies generated against a fusion protein with the cytoplasmic fragment of the cloned AE2 [7]. In contrast, Rossman et al., by using antipeptide antibodies, have shown that AE2 was localized to the basolateral and not the apical membranes of the rabbit ileal enterocytes [8]. In the present study, the antibodies were raised against the same peptides which have previously been used [8,9] representing the C-terminal of AE2 and bAE3. Additionally, it has previously been shown [9] that these antibodies were highly specific as AE2 antipeptide antibodies did not detect bAE3 in transiently transfected CHOP cell and bAE3 antipeptide antibodies did not detect rat choroid plexus AE2. Utilizing these antibodies for immunoblotting, our results, in agreement with the previous AE2 localization studies [3–6], showed that AE2 is localized to the basolateral membranes of the human intestinal epithelial cells. Moreover, we also demonstrated here, for the first time, that bAE3 polypeptides were also localized to the basolateral but not the apical membranes of the human colon and ileum. In the jejunum, the bAE3 protein was not detected. The expression of AE2 and bAE3 polypeptides was significantly higher in the colon compared to the small intestine. Additionally, previous studies from our laboratories have shown a 6-fold higher V_{max} value for chloride uptake by the brush-border membrane Cl^-/HCO_3^- exchange in the ileum compared to the proximal and distal colon. The difference in the V_{max} values for the apical chloride uptake along with the differences in the relative abundance of the AE2 and bAE3 proteins in the distal versus proximal segments of the human intestine, may further indicate that the AE2 and bAE3 are probably not the isoforms which are responsible for apical membrane Cl^-/HCO_3^- exchange activity. In immunoblotting studies, faint bands with expected sizes ($\sim$165–170 kDa) for AE2 and bAE3 were also detected with the apical membranes of the distal colon as well. This faint expression can be explained by a possible cross contamination of the apical membranes with basolateral membranes, since the membranes from the proximal colon tend to demonstrate slightly higher fold purification of the apical membrane marker (cysteine-sensitive alkaline phosphatase assay) compared to their counterparts from the distal colon (unpublished results).

Our current results that AE2 and bAE3 are localized to the basolateral membranes raise the question about the identity of the protein that is responsible for Cl^-/HCO_3^- (OH^-) exchange process in the apical membrane of the human intestinal cells. In this regard, a series of recent studies have demonstrated that DRA (down regulated in adenoma) gene is mutated in congenital chloride diarrhea patients where the basic defect has been shown to be an impaired Cl^-/HCO_3^- (OH^-) exchange process in the human ileum and colon [29–33]. Although recent functional studies have shown that DRA is able to transport chloride [34,35], DRA was initially shown to be a sulfate/oxalate transporter, which shares high homology with a number of sulfate transporters but not

with any member of the AE gene family [36]. Moreover, previous studies, utilizing colonic purified apical membrane vesicles, have suggested that sulfate and chloride transport are mediated via distinct transporters [11]. Additional studies are required to clearly identify the apical membrane Cl^-/HCO_3^- (OH^-) exchanger and delineate whether it is DRA or a novel AE isoform.

In summary, our current results demonstrate that AE2 and bAE3, but not AE1 and cAE3 are expressed along the entire length of the human intestine. The AE2 mRNA was expressed throughout the surface–crypt axis. Furthermore, the polypeptide products for AE2 and bAE3 were found to be localized to the basolateral but not the apical membranes of the epithelial cells of the human intestine and their. These results suggest that human intestinal AE2 and bAE3 isoforms are differentially expressed and may play an important role in vital cellular functions, e.g. cell pH and volume regulation and maintenance of intracellular Cl^- concentrations. Further studies are needed to define the functions of the AE2 and bAE3 isoforms and their regulation in the human intestinal epithelial cells in normal conditions, and to identify the potential apical membrane isoform involved in luminal chloride absorption.

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