Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8

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ABSTRACT
Short-chain fatty acids (SCFA) are monocarboxylates produced by bacterial fermentation that play a crucial role in maintaining homeostasis in the large intestine. Two major transporters for SCFA, monocarboxylate transporter (MCT) and slc5a8 (or SMCT), exist in the digestive tract. The present histochemical study using in situ hybridization and immunohistochemistry revealed the distribution and subcellular localization of the MCT family in the digestive tract of mice, rats, and humans, comparing these with that of slc5a8. The expression of mucosal MCT1 in the mouse and rat was most intense in the cecum, followed by the colon, but low in the stomach and small intestine. Among other MCT subtypes, only MCT2 was detected in the parietal cell region of the gastric mucosa. Slc5a8 had predominant expression sites in the distal half of the large bowel and in the most terminal ileum. The mucosal MCT1 was localized in the basolateral membrane of enterocytes, while slc5a8 was restricted to the apical cell membrane, suggesting the involvement of slc5a8 in the uptake of luminal SCFA, and of MCT1 in the transport of SCFA towards blood circulation. The large intestine expressed both types of the transporter, but their distribution patterns differed along the longitudinal axis of the intestine and along the perpendicular axis of the mucosa.

Plant-derived dietary fiber and undigested carbohydrates are fermented by bacterial microflora in the large intestine and produce acetate, propionate, and butyrate, collectively called short-chain fatty acids (SCFA). These are monocarboxylates which contain less than five carbon atoms: acetate has two carbons, propionate has three, and butyrate has four. The SCFA in the bowel lumen have some effects on the intestinal wall, including the stimulation of colonic blood flow and of fluid and electrolyte uptake (30). Butyrate also functions as an energy source of epithelial cells in the large intestine and promotes differentiation of the cells (2, 21) while suppressing the proliferation of tumor cells by the induction of apoptosis (6). Thus, direct and indirect intraluminal supplementation of butyrate maintains and strengthens the epithelial integrity to suppress mucosal damage such as ulcerative colitis (15, 16, 19, 27). At least 60% of the SCFA uptake in the large intestine occurs by simple diffusion of the unionized form across the cell membrane; the remainder occurs by the active cellular uptake of ionized SCFA involving co-transport of inorganic protons, such as Na⁺, K⁺, and H⁺ (7).

As a transporter of SCFA, monocarboxylate transporter (MCT)-1 was first identified and localized in intestinal epithelial cells as well as the heart, kidney, and epididymis (9). MCT1 can transport lactate, pyruvate, and SCFA in a H⁺-dependent manner. The
original study by Garcia et al. (9) demonstrated immunohistochemically the selective localization of MCT1 at the basolateral membrane of enterocytes in the cecum of hamsters, suggesting a role for MCT1 in the transport of monocarboxylates from the epithelium to the blood. However, subsequent studies using immunohistochemistry and the immunoblotting of purified membrane fractions have reported the predominant localization of MCT1 in the luminal membrane of intestinal epithelial cells in humans (10, 26), pigs (26), rats (29), and in a colonic cell line (3). At present, discussion on the functional significance of MCT1 appears to be based on the apical localization of MCT1, as described in recent review articles (6, 13). We do not know the exact reasons for the discrepant findings on the subcellular localization of MCT1, but they may be due to species difference or different experimental conditions. In order to reveal the definite subcellular localization of MCT1 and other MCT subtypes, we need a systematic immunohistochemical analysis using different animal species as well as a confirmation of the cellular expression at an mRNA level.

More recently, a potent membrane transporter for SCFA, namely solute-linked carrier 5a8 (slc5a8), was identified as a Na⁺-coupled co-transporter for SCFA and lactate (5, 12, 20). In contrast to MCT, which functions as an H⁺-coupled electroneutral transporters, slc5a8 functions as an Na⁺-coupled electrogenic transporter. Affinities of slc5a8 for its major substrates were in the following order: butyrate > propionate > lactate >> acetate (20). Since the substrate specificity of slc5a8 is very similar to that of MCT1 (14), Ganapathy and coworkers proposed to term slc5a8 as a Sodium-coupled MonoCarboxylate Transporter (SMCT) against the H⁺-coupled MCT (13). The down-regulation of slc5a8 expression in a variety of cancers including colon cancer suggests that this transporter may have a tumor suppressive role (18, 23); this can explain partially the anti-tumorigenic effects of butyrate. In a previous study (28), we revealed the selective localization of slc5a8 on the brush border in the terminal ileum and large intestine of mice. Therefore, a comparative expression of slc5a8 and MCT, especially the topographical relationship of the two transporters, is important for a good understanding of a transporting system of SCFA produced by the luminal fermentation.

The present histochemical study at the protein and mRNA levels compared the expression patterns of MCT and slc5a8 in the digestive tract of mice, rats, and humans; it reveals their distinct subcellular localization to ascertain whether they exist apically or basolaterally. To date, fourteen MCT isoforms have been identified in mammals, each having a unique distribution and different affinity to monocarboxylates (14). Among them, we examined MCT1, MCT2, MCT3, MCT4, MCT5, and MCT8, whose substrates are known or whose expression in the gut has been suggested.

MATERIALS AND METHODS

Tissue samplings. Five adult male ddY mice (8-weeks old; Japan SLC, Shizuoka, Japan) and five Wistar rats (10-weeks old, Japan SLC) were used in each of the in situ hybridization analyses and immunohistochemical studies. For in situ hybridization, the animals were killed by bloodletting from the heart under deep anesthesia with pentobarbital sodium, and fresh tissues were collected from whole length of the gastrointestinal tract as well as from the brain, eyeball, and several visceral organs. The jejunum and ileum were obtained from the central region and terminal region of the small intestine, respectively. The distal colon corresponded to the segment 1 cm apart from the anus. These tissues were embedded in a freezing medium (OCT compound; Sakura FineTechnical Co. Ltd., Tokyo, Japan), and quickly frozen in liquid nitrogen. For immunohistochemistry, the animals were deeply anesthetized by an intraperitoneal injection of pentobarbital, and then perfused through the left ventricle of the heart with physiological saline and subsequently with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Tissue samples were collected from the gastrointestinal tract (from the stomach to the distal colon) and immersed in the same fixative for an additional 6 h. All experiments using animals were performed under protocols following the Guidelines for Animal Experimentation, Hokkaido University Graduate School of Medicine.

Human samples, collected from the terminal ileum, proximal colon, sigmoid colon, and rectum at surgery, were macroscopically normal and immersed in a 4% paraformaldehyde solution for more than 72 h. The sampling was performed with the informed consent of the patients.

In situ hybridization. Two non-overlapping antisense oligonucleotide probes (45 mer in length) were designed for each mRNA of mouse MCT1, MCT2, MCT3, MCT4, MCT5, MCT8, and slc5a8, and also rat MCT1. The sequence and database information are listed in Table 1. The probes were labeled with 33P-dATP using terminal deoxynucleotidyl transferase (Invitrogen, Carlsbad, CA). Fresh frozen
sections, 14-μm-thick, were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min, and then acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) for 10 min. Hybridization was performed at 42°C for 10 h with a hybridization buffer containing 10,000 cpm/μL 3²P-labeled oligonucleotide probe. Glass slides were rinsed at room temperature for 30 min in 2 × SSC (1 × SSC: 150 mM sodium chloride, 15 mM sodium citrate) containing 0.1% N-lauroylsarcosine sodium, then rinsed twice at 55°C for 40 min in 0.1 × SSC containing 0.1% N-lauroylsarcosine sodium, dehydrated through a graded series of ethanol, and air-dried. Sections were either exposed to BioMax MR film (Kodak, Rochester, NY) for 10 days or dipped in auroradiographic emulsion (NTB-2, Kodak) at 4°C for 8–12 weeks. The hybridized sections used for autoradiography were counterstained with hematoxylin after development.

In situ hybridization using the two non-overlapping antisense probes for each mRNA exhibited identical labeling in all the tissues examined. The specificity of the hybridization was also confirmed by the disappearance of the signals upon the addition of an excess of unlabeled antisense probe as a negative control.

Immunohistochemistry. The paraformaldehyde-fixed tissues were dipped in 30% sucrose solution overnight at 4°C, embedded in OCT compound, and quickly frozen in liquid nitrogen. Frozen sections, about 10 μm in thickness, were mounted on poly-L-lysine-coated glass slides and stained by the avidin-biotin complex (ABC) method. They were pretreated both with 0.3% Triton X-100-containing PBS (pH 7.2) to enhance the penetration of antibodies and with 0.03% H₂O₂ in methanol to block endogenous peroxidase activities. After preincubation with normal goat serum, the sections were incubated overnight with chicken anti-MCT1 antibody (AB1286, Chemicon International, Temecula, CA; diluted 2,000 fold), rabbit anti-MCT1 antibody (Biogenesis, Poole, UK; diluted 1,600 fold), or a rabbit anti-human SLC5A8 serum (RY1617; diluted 6,000 fold) (28). They were then incubated with biotinylated goat anti-chicken IgY (Santa Cruz Biotechnology, Santa Cruz, CA) or goat anti-rabbit immunoglobulins (Nichirei, Tokyo, Japan) followed by incubation with the avidin-peroxidase complex (Vestastain ABC kit; Vector, Burlimgame, CA), each for 1 h. The antigen-antibody reaction was visualized by incubation in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3’-diaminobenzidine and 0.001% H₂O₂.

Double immunofluorescence staining. Frozen sections from fixed samples were incubated with the rabbit anti-SLC5A8 serum (1: 600 in dilution) overnight, followed by incubation with Cy3-labeled donkey anti-rabbit IgG (1: 200; Jackson ImmunoResearch, West Grove, PA). After rinsing in PBS, same sections were incubated with the chicken anti-MCT1 antibody (1: 400 in dilution) overnight, followed by incubation with FITC-labeled goat anti-chicken IgY (1: 200; Santa Cruz), and were observed under a confocal laser scanning microscope (Fluoview;
Silver-intensified immunogold method for electron microscopy. Frozen sections, 15 μm in thickness, were prepared from the paraformaldehyde-fixed tissues of the mouse cecum and processed for the silver-intensified immunogold method. The sections on glass slides were incubated with the rabbit anti-MCT1 antibody diluted 1 : 800 overnight and subsequently reacted with goat anti-rabbit IgG covalently linked to 1-nm gold particles (1 : 200; BBI International, Golden Gate, UK). Following silver enhancement using HQ silver (Nanoprobes, Stony Brook, NY), the sections were osmicated, dehydrated, and directly embedded in Epon (Nissin EM, Tokyo, Japan). Ultrathin sections were prepared and stained with an aqueous solution of uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi, Tokyo, Japan).

RESULTS

mRNA expression of MCT family throughout the digestive tract of mice

The messenger RNA expression of six MCT subtypes in the digestive tract of mice was examined by in situ hybridization technique (Fig. 1). X-ray images of hybridized sections for MCT1 mRNA showed its broad expression from the stomach to the distal colon (Fig. 1a). However, the expression was much more intense in the large intestine than in the stomach and small intestine. The most intense signals were detected in the cecum, in agreement with the early report by Garcia et al. (9), and the signals in the colon considerably decreased in intensity. Among other MCT subtypes, transcripts of only MCT2 were detected in the gastric corpus (acid-secreting region) (Fig. 1b). No significant signals for MCT3, MCT4, MCT5 and MCT8 were visible on X-ray films throughout the digestive tract (data for MCT5 not shown); in situ hybridization analysis for these negative MCT isotypes detected intense and specific signals of each subtype in other organs used as positive controls, such as the retinal pigment epithelium (MCT3), placenta (MCT4), and kidney (MCT5 and MCT8). For the rat, mRNA expression of MCT1 only was analyzed with the same method, and the expression pattern was essentially identical to that in the mouse. The large intestine of rats intensely expressed MCT1 mRNA along the entire length, with the most intense signals in the cecum (Fig. 2). In addition, weak to moderately intense signals were detectable in the terminal ileum compared with faint signals in the more proximal regions.

For comparison, mRNA expression of slc5a8 was observed with the same technique using adjacent sections from the digestive tract of mice (Fig. 1f). As reported previously (28), transcripts of slc5a8 were found throughout the large intestine, with the most intense expression in the distal colon, followed by the cecum. Noteworthily, the expression levels in the proximal half of colon-rectum were lower compared with those in the distal half. Another site showing an intense expression of slc5a8 mRNA was the terminal ileum, where the expression was restricted to the distal 10 cm segment of the ileum (28).

Light microscopically, gastric pits of the gastric corpus and pyloric antrum weakly expressed MCT1 mRNA. In the small intestine, the weak signals were localized in the crypt region, with no significant signals in the villous region (data not shown). A dense aggregation of silver grains showing intense expression for MCT1 was found in crypts of the cecum, especially in the middle regions of crypts (Fig. 3a). The proximal and distal colon possessed less intense signals in the upper region of crypts (Fig. 3b, c). In contrast, the expression of slc5a8 mRNA was very intense in the distal colon, in which the signals gathered in the middle region of crypts (Fig. 3d), as reported previously (28). Signals for MCT2 mRNA were restricted to the parietal cell region in the acid-secreting area of the stomach (Fig. 3e, f).

Immunohistochemistry of MCT1 in the intestine of mice, rats and humans

When we compared stainability with the MCT1 antiserum between paraffin sections and frozen sections in a preliminary staining, better results were always obtained in frozen sections. Furthermore, when we stained tissue sections of the intestine with two MCT1 antisera raised in a chicken and rabbit, we could obtain identical staining results with a lower background with use of the chicken antiserum. Therefore, we used frozen sections and the chicken antiserum in the following stainings at a light microscopic level. The distribution and intensity of MCT1 immunoreactivity coincided with the expression pattern of MCT1 mRNA throughout the digestive tract of mice. The staining results of MCT1 in the rat were essentially identical to those in the mouse. In both animals, intense immunoreactivity was found in the cecum and localized in the basolateral membrane of the surface (covering) epithelium and crypts (Fig. 4a). The surface epithelium
Fig. 1  X-ray film images showing mRNA expressions for MCT1 (a), MCT2 (b), MCT3 (c), MCT4 (d), MCT8 (e), and slc5a8 (f) in the stomach and intestine of a mouse. Serial sections from the stomach (S), antro-duodenum (A-D), terminal ileum (I), cecum (Ce), proximal colon (pC) and distal colon (dC) were arranged on each glass slide and hybridized. The most intense expression of MCT1 is seen in the cecum (a). The significant expression of MCT2 is restricted to the stomach (b). No expression is detectable for the other MCT subtypes. Signals for slc5a8 are distributed from the terminal ileum to the distal colon, with a more intense expression in the terminal ileum and distal colon (f).

Fig. 2  X-ray film analysis for MCT1 mRNA expression in the digestive tract of a rat. The expression levels are high in the cecum (Ce), the proximal colon (pC), and distal colon (dC). A faint expression is found in the stomach (S), antro-duodenum (A-D) and jejunum (J), but a moderately intense expression is seen in the ileum (I).

... contained a more intense immunoreactivity, while the bottom of crypts was less intense in immunoreaction. In the proximal and distal colon, the immunoreactivity generally decreased in intensity and gathered in the surface epithelium and the upper region of crypts (Fig. 4b, c). Lower parts of colonic...
Fig. 3  Light microscopic observation of tissue sections hybridized for MCT1 and MCT2 mRNAs in the large intestine and stomach of mice. Silver grains showing mRNA expression of MCT1 aggregate at the middle portion of crypts in the cecum (a). The proximal (b) and distal colon (c) contain less intense signals at the upper portion of crypts. slc5a8 mRNA is intensely expressed around the middle regions of crypts in the distal colon (d). MCT2 mRNA expression is found in the parietal cell region of the stomach (e) (f: dark field image). Bar = 50 µm
Fig. 4  Immunohistochemistry for MCT1 in the digestive tract of mice. The most intense immunoreactivity for MCT1 is seen in the surface epithelium and crypts of cecum, where the basolateral membrane is selectively immunolabeled (a). The immunoreactivity in the proximal colon (b) and distal colon (c) decreases in intensity and predominates in the surface epithelium and the upper region of crypts. The slc5a8 immunoreactivity in the distal colon is restricted to the luminal side of crypts (d). MCT1 immunoreactivity in the pyloric antrum is localized in the gastric pits (e). In the ileum (f), the basolateral immunolabeling for MCT1, though not intense, is seen only in crypts (C), but not in villi (V). Bar = 20 µm
crypts which were occupied by goblet cells (mucus-secreting cells) often lacked significant immunoreactivity. The MCT1 immunoreactivities in the mucosa of the stomach (Fig. 4e) and small intestine (Fig. 4f) were much weaker than those in the large intestine and were restricted to the gastric pits or intestinal crypts (without a Paneth cell region), where the basolateral cell membrane was also immunolabeled. The villus epithelium in the small intestine was completely free from the immunoreactivity. In the stomach and small intestine, the muscle layers rather than the pit and crypt epithelia displayed a more distinct immunoreactivity for MCT1.

We stained human samples obtained from the terminal ileum, proximal colon (Fig. 5a), sigmoid colon (Fig. 5b), and rectum (Fig. 5c) using the chicken antiserum against MCT1. Here again, the MCT1 immunoreactivity in the proximal colon was found in the basolateral cell membrane of the surface epithelium and upper regions of crypts. In the sigmoid colon and rectum, only the surface epithelial cells displayed a moderately intense immunoreactivity for MCT1 with the basolateral labeling; crypt cells were largely negative in reaction. No immunoreactivity was found in the terminal ileum. This staining pattern in humans was essentially identical to those in the mouse and rat.

**Double staining of MCT1 and slc5a8 in the mouse large intestine**

Immunostaining for slc5a8 in the digestive tract of mice confirmed our previous findings (28): the slc5a8 immunoreactivity was localized selectively in the luminal surface of crypts in the large intestine (Fig. 4d). For concomitant demonstration of the two transporters, double immunostaining was carried out in the large intestine of mice. In the cecum (Fig. 6a), the slc5a8 immunoreactivity was restricted to the brush border at the middle part of crypts, while the basolateral labeling of epithelial cells with the MCT1 antibody was observed extensively in both the surface epithelium and crypts. Crypt cells showed a dual expression of the two transporters, but the surface epithelium contained only MCT1 immunoreactivity. The slc5a8 immunoreactivity in the proximal colon was faint or under a detectable level, while consistent basolateral labeling with the MCT1 antibody was visible in the surface epithelium and upper region of crypts (data not shown). In the distal colon (Fig. 6b), the slc5a8 immunoreactivity was very intense on the luminal side of crypts and appeared as a solid, lineal staining, while the basolateral MCT1 immunoreactivity predominated from the surface epithelium to the upper region of crypts. Thus, coexistence of the two transporters in the large intestine was found in a minor population of enterocytes.

**Immunoelectron microscopy for MCT1**

The subcellular localization of MCT1 in the cecum of mice was investigated by the pre-embedding silver-intensified immunogold method using the antiserum raised in a rabbit. Gold particles showing the existence of MCT1 gathered in the basolateral membrane of enterocytes but not on the apical, microvillar region (Fig. 7). The immunoreactivity on the lateral cell membrane was absent at the junctional complexes including tight junctions, and tended to increase in intensity towards the basal lamina (Fig. 7a). Aggregation of the gold particles was found along the basal cytoplasmic membrane, being more remarkable at developed microfoldings (Fig. 7b). Goblet cells, enteroendocrine cells, and intraepithelial lymphocytes lacked the immunoreactivity.

**DISCUSSION**

**Expression of MCT1 throughout the gastrointestinal tract**

MCT1, the first isoform of the MCT family, may play a central role in the metabolism of SCFA and other monocarboxylates in the digestive tract, but even findings on its expression pattern along the longitudinal axis of the intestine are controversial. Abundant expressions of MCT1 mRNA and protein in the cecum of hamsters were reported in the original studies by Garcia et al. (8, 9); the expression levels were very low in the stomach and small intestine (duodenum). Northern blot analyses in humans and pigs also demonstrated a greater abundance of the MCT1 transcript in the colon than in the small intestine (25, 26). A Western blot analysis of membrane fractions obtained from the human intestine has shown that the expression of MCT1 increased along the length of the intestine, with a maximum expression in the distal colon, followed by the proximal colon and ileum, but was very low in the jejunum (10). However, an immunostaining for MCT1 in the rat documented the consistent existence of MCT1 from the stomach to the large intestine, with greater intensity in proximal regions of the duodenum-ileum axis (29). The present immunostaining in the mouse and rat confirmed the rich existence of MCT1 in the large intestine, where the immunoreactivity was most intense in the cecum, followed by the proximal colon and distal colon.
The specificity of our immunostaining for MCT1 was supported by the mRNA expression revealed by in situ hybridization technique. Our in situ detection of MCT1 at both mRNA and protein levels displayed weak signals in the small intestine compared with those in the cecum, and both signals in the small intestine were restricted to the crypts occupied by proliferating and immature cells. The lack of significant signals for MCT1 in the villus epithelium in the small intestine of the mouse and rat is in sharp contrast to the previous immunohistochemical staining by Tamai et al. (29). The abundant expression of MCT1 in the large intestine is reasonable since bacterial fermentation occurs predominantly in the proximal regions of the large intestine (30), especially in the cecum in rodents (7). Interestingly, recent Western blot and RT-PCR analyses of the bovine digestive tract hardly detected any significant expression of MCT1 in the glandular stomach (abomasum) and small intestine; they instead revealed its abundant expression in the forestomach as well as large intestine (17). The forestomach in ruminants is known to be the most predominant site for SCFA production, while very low levels of SCFA exist in the glandular stomach and small intestine of these animals (22). Although our immunohistochemical analysis of the human large intestine is not sufficient due to difficulty in the sampling of fresh materials, the MCT1 immunoreactivity in the human proximal colon was superior in intensity to those in the terminal ileum and distal colon. Thus, the expression patterns of MCT1 along the long axis of the intestine reflect the active production of SCFA.

Fig. 5 Immunostaining for MCT1 in the large intestine of humans. MCT1 immunoreactivity in the proximal colon is found in the surface epithelium and upper regions of crypts (a). In the sigmoid colon (b) and rectum (c), it is limited to the surface epithelium between crypts. Bar = 20 µm.

Fig. 6 Double immunofluorescence staining for MCT1 (green) and slc5a8 (red) in the cecum (a) and distal colon (b) of a mouse. More intense immunoreactivity for MCT1 is distributed in the surface epithelium and upper regions of crypts, while that for slc5a8 is located at the brush border of enterocytes in the crypts. Bar = 50 µm.
in the three mammalian species.

Expression patterns of other MCT members and slc5a8 in comparison with MCT1
Reportedly, MCT2 in the digestive tract of hamsters is expressed mainly in the stomach, where parietal cells are the cellular source of production, and the expression levels of MCT2 are much lower in the small (duodenum) and large intestine (cecum) (8). The intense expression of MCT2 in the parietal cell area was confirmed by the present in situ hybridization study at the mRNA level. A Western blot analysis of the human small and large intestine (10) described the intense expression of MCT1, MCT4, and MCT5 in the distal colon, with less intense expressions of all three subtypes in the proximal colon and ileum. In the immunoblotting by Gill et al. (10), MCT3 was present at a relatively low level compared with other isoforms, but significant immunoreactive bands for MCT3 were detected in the human small intestine and colon. A Northern blot analysis of human small and large intestine demonstrated an intense expression of MCT8 (XPCT) comparable to the expression of MCT1 (25). However, the present study failed to obtain any visible signals for MCT3, MCT4, MCT5, or MCT8 throughout the digestive tract of the mouse. Our survey in the mouse indicates that MCT1 is a predominant subtype expressed in the gastrointestinal tract, except for MCT2 in the glandular stomach.

A Northern blot analysis for mouse slc5a8 mRNA demonstrated its abundant expression in the large intestine, small intestine, and kidney (12). The present study and our previous study (28) confirmed the predominant expression of slc5a8 in the distal colon and terminal ileum. Interestingly, the slc5a8 expression in the colon-rectum of mice was drastically upregulated in the distal half. Since this region makes feces into a solid form, this finding raises the possibility that slc5a8 is involved in the absorption of water. It is still unknown whether slc5a8 expressed in the terminal ileum is responsible for the transport of SCFA which is locally produced at the end of the small intestine or for the transport of dietary nicotinic acid (vitamin B3), which exists as a monocarboxylate anion in vivo (11). Nevertheless, it is concluded that the large bowel in mammals expresses two types of monocarboxylate transporters, MCT1 and slc5a8.

Subcellular localization of MCT1 and slc5a8
There were opposite findings regarding the subcellular localization of MCT1 in the intestinal epithelial cells: the basolateral membrane versus the apical

![Fig. 7](image_url)
Cellular expression of MCT in the digestive tract

The morphological observation further confirmed the distinct basolateral localization of MCT1 throughout the intestine in the hamster. In accordance with the morphological findings, H+-linked monocarboxylate transport activity has been characterized physiologically in the basolateral membrane of both intestinal epithelial cells and renal tubules (4, 24). However, subsequent immunostainings of tissue sections and the immunoblotting of purified membrane fractions from the intestine of rats, pigs, and humans indicated the predominant existence of MCT1 in the brush-border membrane rather than basolateral ones (10, 26, 29). A similar apical staining for MCT1 was noted in a cell line of human colonic tumor cells (Caco-2 cells) (3). The maturation-dependent localization of MCT1 was documented in the immunostaining of the rat small intestine (29). Namely, immature epithelial cells at the crypts showed its basolateral localization, while matured villous epithelial cells displayed the immunoreactivity predominantly at the apical site of cells. However, the present immunostaining selectively labeled the basolateral membrane of enterocytes in all regions in the three animal species examined. Our observation further confirmed the subcellular localization of MCT1 for the first time at an electron microscopic level. Thus, consistent immunolabeling in the basolateral membrane in this study supports the original finding by Garcia et al. (9) beyond any species difference. In contrast, the slc5a8 immunoreactivity was concentrated in the brush border of enterocytes in mice (this study) and rats and humans (our unpublished data). The morphological findings simply indicate that slc5a8 but not MCT regulates the entry of SCFA from the lumen into enterocytes.

Coexpression or differential expression of MCT1 and slc5a8?

Taking the functional relationship of basolateral MCT and apical slc5a8 into consideration, it is worth noting that there are two kinds of gaps in the expression sites between MCT1 and slc5a8 at least in the digestive tract of mice. First, the regional distribution of the two transporters is different along the longitudinal axis of the intestine. The most intense signals for MCT1 were found in the cecum, while those for slc5a8 appeared in the distal colon close to the anus. Secondly, the most intense expression sites along the perpendicular axis of the mucosa differ between the two transporters. Namely, the MCT1 immunoreactivity is more intense at the surface (covering) epithelium, while the slc5a8 immunoactivity was localized only at the crypts, although it is true that some crypt cells co-expressed both transporters, as seen in the cecal mucosa of mice with comparatively short crypts. Thus, the exact expression sites of slc5a8 and MCT1 in the gastrointestinal tract do not coincide, indicating that these transporters exist differentially.

The driving force for the entry of butyrate from the lumen into epithelial cells via the MCT is much less intense than via slc5a8 (13). Since the MCT1-mediated transport is believed to be bidirectional (9, 29), the basolateral localization of MCT1 should be linked to the transport of SCFA absorbed by enterocytes to the bloodstream. Generally, the bacterial fermentation predominates in the proximal portion of large intestine, and SCFA products move to the distal regions by the fecal stream, resulting in the decrease in concentrations of SCFA along the large bowel. This decline correlates very well with the intensity gradient of MCT1 along the large bowel. Another major substrate of the basolateral MCT1, as described by Garcia et al. (9), may be lactate. Since the intestinal epithelium converts large amounts of glucose to lactate after a carbohydrate-rich meal, it must expel the lactate to avoid intracellular acidosis (1). These data totally support the idea that MCT1 functions mainly in the transport of monocarboxylates within enterocytes towards the blood circulation or an internal milieu.

In conclusion, our immunohistochemical findings indicate that the MCT1-mediated transport of monocarboxylates across the basolateral membrane occurs mainly at the surface epithelium in the large intestine, and may decrease in potency towards the anus. Epithelial cells in the distal colon, especially crypt cells, may require the apical slc5a8 for an efficient uptake of luminal SCFA because of a decrease in concentrations of SCFA and of the absorption of water.

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