

Downregulation in aquaporin 4 and aquaporin 8 expression of the colon associated with the induction of allergic diarrhea in a mouse model of food allergy

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Abstract

Food allergies have become increasingly prevalent during the past few decades. Diarrhea is one of the most frequent intestinal symptoms caused by food allergens and is characterized by imbalanced ion exchange and water transfer; however, the underlying mechanism of allergic diarrhea remains unclear. Water transfer across the intestinal epithelial membrane seems to occur via aquaporins (AQPs). However, the molecular mechanism of water transfer and the pathophysiological roles of aquaporins in the intestine have not been fully established. The present studies have focused on the alterations of AQPs in a mouse model of allergic diarrhea in which BALB/c mice developed diarrhea following repeated challenges of orally administered ovalbumin. Quantitative real-time PCR analysis and immunohistochemical technique were used for expression of mRNA and protein of AQPs, respectively. AQP4 and AQP8 mRNA levels were significantly decreased in the proximal colon of allergic mice compared to controls; likewise, expression of AQP4 and AQP8 proteins was reduced in the proximal colon of the allergic mice. These results suggest that allergic diarrhea is associated with a downregulation in AQP4 and AQP8 expression.

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Introduction

Since discovery of the aquaporin 1 (AQP1) water channel protein (Preston and Agre, 1991), the distribution and expression of a family of AQPs in various tissues have been reported (King and Agre, 1996; Ma and Verkman, 1999; Takata et al., 2004; Verkman, 2002). AQPs constitute a family of small integral membrane proteins that are selectively permeable to water, and driven by osmotic gradients (King and Agre, 1996; Ma and Verkman, 1999; Takata et al., 2004; Verkman, 2002). At present, 13 members of AQP subtypes have been cloned from mammals (Itoh et al., 2005; Matsuzaki et al., 2004; Morishita et al., 2005; Verkman and Mitra, 2000). The gastrointestinal

(GI) tract, particularly colon is an organ system that is responsible for an enormous amount of water transport and the transepithelial water permeability of the proximal colon is higher than that of the distal colon in the mouse (Wang et al., 2000). It is estimated that daily water absorption in the human colon, is about 1.5 L. The absorption of water is one of the key functions of the intestine, which adjusts the osmotic pressure in the whole body. The regulation of transepithelial fluid transport in the gastrointestinal tract is based on ion transport and also on water transport by AQP-type water channels (Kunzelmann and Mall, 2002). Various subtypes of AQP are localized in the intestine (AQP1, 3, 4, 5, 7, 8, 9), and play an important role in a number of physiological and pathological processes. It is widely thought that AQPs are involved in diseases that are characterized by alterations in fluid transport. It has been reported that a defect in the expression and/or function of AQPs underlies renal diabetes insipidus (Deen et al., 1994), brain edema (King and Agre, 1996; Saadoun et al., 2002) and dry eye (Ohashi et al., 2003).

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Diarrhea is a common symptom of patients with inflammatory bowel disease, and a reduction in expression of AQP4 appears to be correlated with increased disease activity in patients with ulcerative colitis and Crohn's (Hardin et al., 2004). The GI tract is capable of secreting large amounts of water, and transepithelial hypersecretion of fluid is the basis of secretory diarrhea; however, defects in water absorption in the colon are also important factors in the pathogenesis of diarrhea. Studies of AQP4 deficient mice have demonstrated that AQP4 is involved in fluid absorption mainly in the proximal colon (Wang et al., 2000). AQP8 has been proposed to be a potentially important water transporter in the GI tract (Laforenza et al., 2005; Yang et al., 2005). AQP8 transcripts are expressed in the mouse GI tract, including small intestine and colon, and subsequent immunolocalization studies have localized AQP8 protein expression in the proximal colon (Yang et al., 2005).

Food allergies are basically an adverse immunological response to proteins in food, and are common, affecting up to 6% of young children and up to 4% of adults (Sicherer and Sampson, 2006). One animal model of food allergy consists of sensitizing BALB/c mice to ovalbumin by intraperitoneal injections and then later oral administration of the protein (Kweon et al., 2000). Furthermore, no studies have reported on the changes in the tissue levels of AQP expression in the allergic diarrhea. To investigate the pathophysiological role of AQPs in experimental oral allergen-induced diarrhea, first of all we examined the changes in the expression of AQP4 and AQP8 transcripts and proteins and the distribution of AQP4 and AQP8 proteins in the proximal colon of a BALB/c mouse diarrheal model of food allergy.

Materials and methods

Induction of intestinal anaphylaxis

Male BALB/c mice (8–12 weeks old) were sensitized twice, 2 weeks apart, with 50 µg of ovalbumin (OVA; fraction V) in

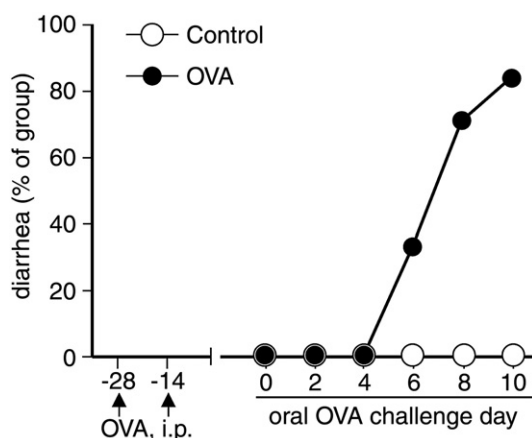


Fig. 1. Oral OVA administration evokes acute allergic diarrhea in BALB/c mice previously sensitized to OVA. Occurrence of diarrhea within 1 h after OVA challenge was recorded. Quantal data show the percent of the control group (○) and the OVA-challenged group (●) that exhibited acute diarrhea. $n=14$ mice per group.

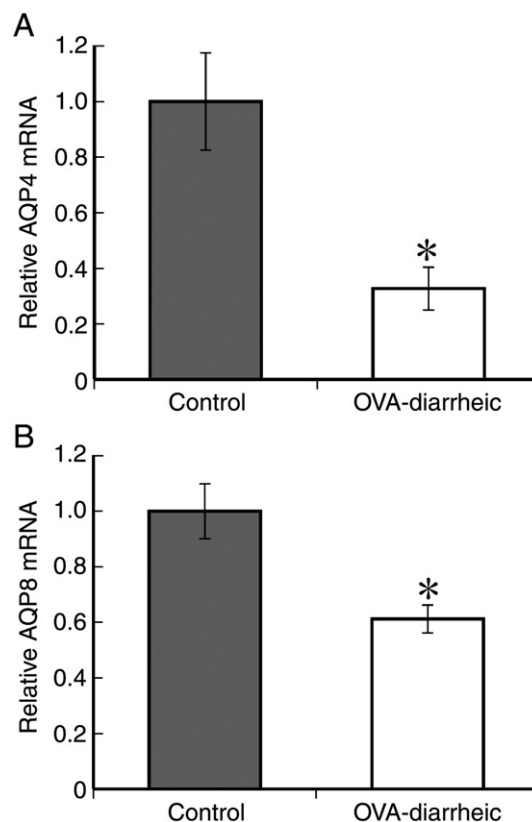


Fig. 2. The mRNAs for AQP4 and AQP8 are downregulated in the proximal colon of mice sensitized to OVA and exhibiting acute diarrhea following oral administration of OVA. Real-time PCR for AQP4 mRNA (A) and AQP8 mRNA (B) of control mice ($n=7$) and OVA-diarrheic mice (OVA-sensitized, oral OVA; $n=5$). Individual samples were normalized to GAPDH mRNA levels and data are expressed as a ratio of relative AQP mRNA in OVA-diarrheic:control groups. * = $P<0.05$.

the presence of 1.3 mg of aluminum hydroxide gel (Sigma) as an adjuvant by intraperitoneal injection. Two weeks after the systemic priming, mice were repeatedly given 50 mg of OVA dissolved in 0.3 mL of water with intragastric feeding needles three times per week. Diarrhea was assessed by visually monitoring mice for up to 1 h following intragastric challenge. Mice that responded to oral OVA with profuse liquid stool were recorded as diarrhea-positive animals. One hour after the last oral administration, mice were sacrificed and antigen-induced allergic reactions were analyzed. The proximal colon was removed and used for quantitative measurements of mRNA and immunohistochemistry.

Expression of aquaporins mRNA

The 2-cm of proximal colon was flushed with ice-cold saline and frozen at -80°C in liquid nitrogen. Total RNA was extracted from the proximal colon by Sepasol Super (Nacalai, Tokyo, Japan) according to the manufacturer's instruction. Reverse transcription PCR was performed using the Exscript RT reagent kit (Takara, Tokyo, Japan) and random primers, followed by real-time PCR. Real-time PCR amplification of AQP4, AQP8 and GAPDH was performed using the SYBR Premix Ex Taq (Takara). The following primer pairs were used:

AQP4, forward: 5'-CTTTCTGGAAGGCAGTCTCAG-3'; reverse: 5'-CAGTCACAGCGGGATTGATGT-3'; AQP8, forward: 5'-TGTGTAGTATGGACCTACCTGAG-3'; reverse: 5'-ACCGATAGACATCCGATGAAGAT-3'; GAPDH forward: 5'-TGACCACAGTCCATGCCATC-3'; reverse: 5'-GACGGACACATTGGGGGTAG-3'. Real-time PCR was carried out using Mx3000p (Stratagene, CA, USA). PCR reaction conditions were 10 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 20 s at

63 °C. Target mRNA was normalized to the GAPDH mRNA as internal control in each sample. Results were expressed as relative ratio to the normal group average.

Immunohistochemistry

Mice were sacrificed and the proximal colons removed, the lumens washed with saline, and fixed by immersion in

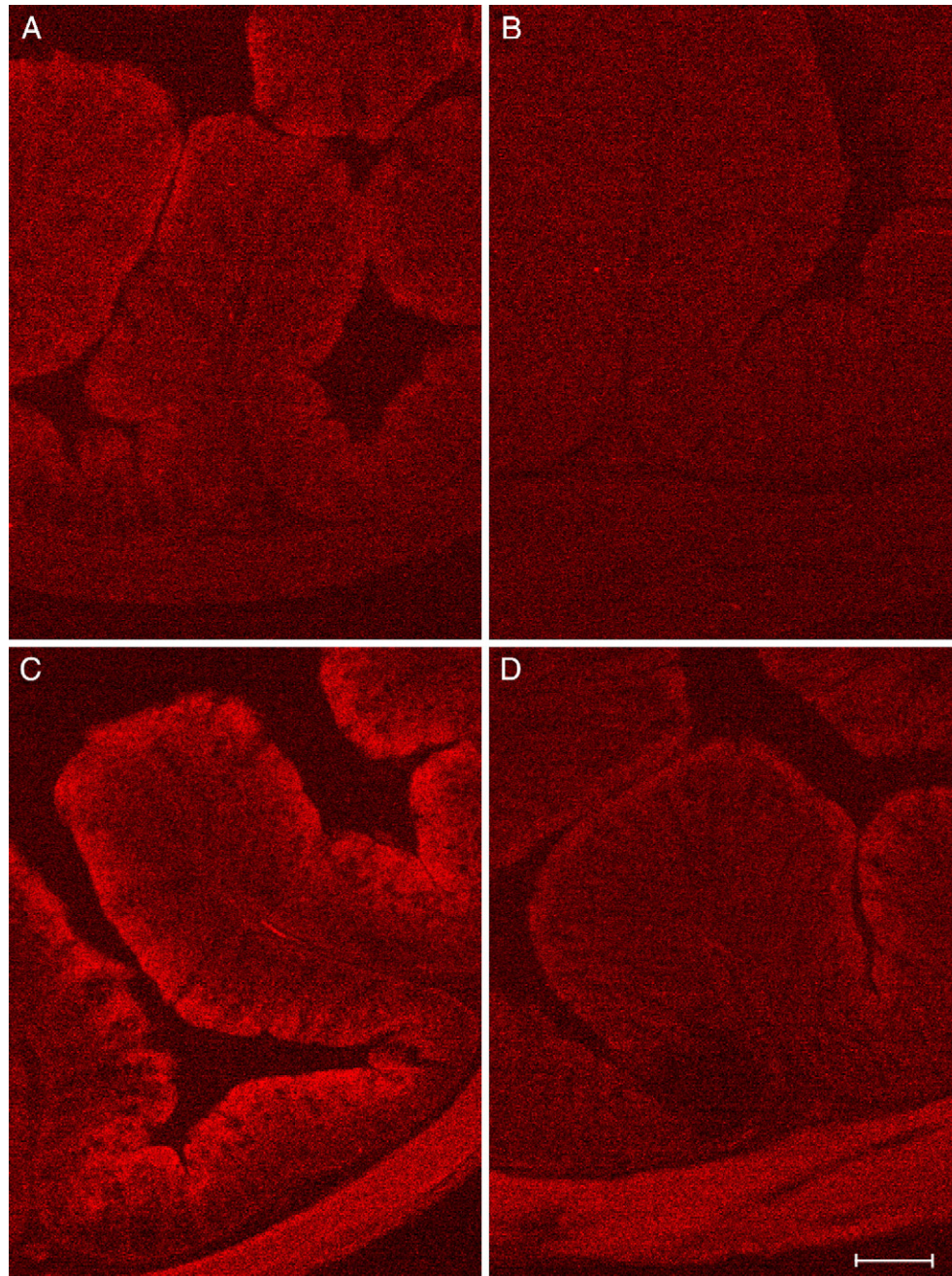


Fig. 3. Immunoreactivity (IR) for AQP4 and AQP8 is reduced in the proximal colon of OVA-sensitized mice that exhibit diarrhea following oral OVA challenge. AQP4-IR (A) and AQP8-IR (C) are distributed throughout the colonic mucosa in control mice. Following the onset of acute diarrhea after repeated oral OVA challenge, AQP4-IR is absent (B) and AQP8-IR is greatly reduced (D). Representative photomicrographs showing the changes in AQP4 and AQP8 immunolocalization in cryosections of colonic tissues from mice with allergic diarrhea. The sections from control mice ($n=6$) and oral OVA challenge mice ($n=6$) were immunostained with anti-AQP4 or anti-AQP8. Bar=100 μ m.

4% paraformaldehyde (w/v) in 0.1 M sodium phosphate buffer (PB, pH 7.3) at 4 °C for 12–18 h. The tissues were briefly cleared in dimethylsulphoxide followed by washes in 0.01 M phosphate-buffered saline (PBS, pH 7.3), and cryo-protected by infiltration with 30% sucrose in 0.01 M PBS and embedded in OCT-compound and frozen sections (50 µm) cut at –20 °C using a cryostat microtome. Sections were permeabilized for 12–18 h in 0.01 M PBS containing 0.3% Triton X-100, incubated for 30 min to normal donkey serum (1:10; Chemicon International, Temecula, USA) to block nonspecific binding of antisera, and then washed in 0.01 M PBS. Sections were exposed for 12–18 h to anti-AQP4 serum (rabbit polyclonal, 1:100; Chemicon) or anti-AQP8 serum (rabbit polyclonal, 1:100; Chemicon), washed with PBS, and incubated for 2 h with Cy3-conjugated donkey anti-rabbit IgG (1:200; Jackson Laboratories, West Grove, Pa.). Finally, the sections were washed in 0.01 M PBS, and coverslips were mounted using sodium carbonate-buffered glycerol (pH 8.6). All incubations were carried out at room temperature. The immunostained sections were examined using a fluorescence microscope (IX71 system, Olympus, Tokyo, Japan) with a filter set U-MWIG3 (Olympus) and photographed by using an Olympus Digital camera (DP70, Olympus). The brightness and contrast of the images were modified with Adobe Photoshop Elements 2.0 (Adobe Systems, San Jose, USA).

Statistics analyses

Data are expressed as the mean $\pm$ S.E. Statistical comparisons were made with Student's unpaired *t*-test and significant differences defined as $P < 0.01$.

Results

Intestinal allergic symptom induced by oral OVA challenges

Exposure of the primed mice to repeated oral OVA challenges developed severe diarrhea 20–60 min after the fourth OVA challenge. The intestinal allergic symptoms were induced in 80–90% of mice after six OVA challenges, whereas, repeated oral OVA administration without systemic priming did not induce any significant clinical sign of intestinal anaphylaxis (Fig. 1).

Analysis expression of AQP mRNA

We determined the expressions of AQP4 and AQP8 mRNA by real-time PCR in the non-treated normal mice and in mice with OVA-induced allergic diarrhea (Fig. 2). The expression of AQP4 mRNA in the proximal colons of mice with allergic diarrhea was 33% of the level of expression in tissues from control mice (normal mice: 1.0 ± 0.14 ; OVA-sensitized and challenged mice: 0.33 ± 0.08 , $P < 0.01$). Likewise, the expression of AQP8 mRNA was significantly decreased in colons of the diarrhea mice (normal mice: 1.0 ± 0.10 ; diarrhea mice: 0.61 ± 0.05 , $P < 0.01$).

Immunohistochemistry

Typical photomicrographs of each group have been shown in Fig. 3. Similar results on immunolocalization of AQP4 and AQP8 have been noted in every mouse of each group ($n=6$). AQP4 immunoreactivity (IR) was localized to mucosal epithelia in the proximal colon of the non-treated normal mouse (Fig. 3A). The AQP4-IR was observed in columnar epithelial cells, in the mucosal surface as well as in upper and middle crypt regions (Fig. 3A). AQP4-IR was greatly reduced in the proximal colon of the mice with the allergic diarrhea following repeated oral OVA challenges (Fig. 3B). Likewise, AQP8-IR was observed in the surface and upper crypt epithelium of the proximal colons of normal mice (Fig. 3C), but was reduced after exposure of the primed mice to repeated oral OVA challenges (Fig. 3D).

Discussion

To investigate whether the development of allergic diarrhea is associated with an altered expression of AQPs in the colon, we examined the changes in the expression of AQP4 and AQP8 transcripts and proteins and the distribution of AQP4 and AQP8 proteins in the proximal colon of a BALB/c mouse with allergic diarrhea. In the present study, we found that the expression of both the mRNAs encoding AQP4 and AQP8 as well as the proteins was decreased in the proximal colons of mice exhibiting severe diarrhea following an allergic response to oral ovalbumin. These data make it possible to suggest that these reductions resulted in the dysfunction on the water absorption in the proximal colon and then caused diarrhea to wash out the food allergen. The reduction in both AQP4 and AQP8 following antigenic challenge in sensitized animals results in enteropooling of fluid, and could constitute a protective mechanism designed to wash the food allergen out of the GI tract.

The two AQP subtypes, AQP4 and AQP8, examined in this study responded to the induction of food allergy. At least 13 subtypes of AQPs (AQP0–AQP12) have been identified in mammals (Itoh et al., 2005; Matsuzaki et al., 2004; Morishita et al., 2005; Verkman and Mitra, 2000). At least seven AQP subtypes (AQP1, 3, 4, 5, 7, 8, and 9) have been reported to be expressed in the GI tract and play an important role in a number of physiological and pathological processes (Matsuzaki et al., 2004; Ma and Verkman, 1999). In particular, the colon is a major site of AQP1, 3, 4 and 8 expression (Matsuzaki et al., 2004; Ma and Verkman, 1999) and there are a few pieces of evidence for physiological roles and functions of AQP1, 3, 4 and 8 in the colon. The water content of defecated stool is higher in AQP4 knockout mouse (Wang et al., 2000), and inhibition of AQP8 expression by small interfering RNA decreases osmotic water permeability (Laforenza et al., 2005). On the other hand, along the colon, AQP3 is expressed abundantly in the distal colon and rectum and AQP3 is absent in the cecum and proximal colon (Matsuzaki et al., 2004). Furthermore, AQP1 null mouse exhibits only defective dietary fat processing in the digestive system. In addition, both AQP1 null mouse and AQP3

null mouse do not appear to have a deficiency in colonic fluid transport (Ma et al., 2000, 2001). There is little information about the function of other AQP subtypes in the large intestine. Therefore, we have focused particularly on AQP4 and AQP8 and have not studied AQP1 and AQP3.

There are some reports on the physiological role of AQP in water absorption by the colon. Yang et al. have shown only minor phenotypic differences between wildtype and AQP8 null mice on intestinal fluid transport, suggesting that compensatory changes in the expression of other water or solute transporters in the AQP8 null mice might account for their unremarkable phenotype (Yang et al., 2005). In contrast, AQP4 null mice have been found to excrete feces that have elevated water content (Wang et al., 2000). The transepithelial water permeability of the proximal colon of wildtype mice is higher than that of the distal colon of wildtype mice, and is also higher than that of the proximal colon of AQP4 null mice. AQP4 deletion does not affect water permeability in distal colon, resulting in the increase of the water content of defecated stool. These data imply that AQP4 has a role in transcellular water movement across surface colonocytes (Wang et al., 2000). Furthermore, inhibition of AQP8 expression by small interfering RNA significantly decreased water absorption in the rat proximal colon (Laforenza et al., 2005). Thus AQP4 and AQP8 may play an important role in the colonic water absorption, particularly in the proximal colon and it seems that decreased AQP expression might lead to an insufficiency of water absorption in this region of the GI tract.

Changes in intestinal tissue levels of various AQPs have been reported in animal models and human diseases (Hardin et al., 2004). The expression of AQP4 and AQP8 mRNA is significantly decreased immediately after exposure to dextran sodium sulphate (DSS) in a C57BL/6 mouse model of colitis. Protein expression followed a similar pattern to that observed for AQPs mRNA. The rapid decrease in AQP4 and AQP8 expression is associated with an alteration in colonic water transport, leading to a shift in fluid flux from an absorptive state to a secretory state.

Furthermore, colonic fluid transport returns to an absorptive state with an increase in AQP4 and AQP 8 expression following cessation of DSS, indicating a possible functional correlation between fluid transport and AQP expression in the colon. These results from the mouse DSS colitis model correlate with data from clinical investigations of human inflammatory bowel disease. Patients with active ulcerative colitis, Crohn's colitis or infectious colitis who develop severe diarrhea have similar reductions in AQP8 expression. These results are consistent with our findings of the altered expression of AQP4 and AQP8 in mice with severe diarrhea following an experimentally induce food allergy.

Our findings have implications for the understanding of diarrhea caused by the intestinal anaphylaxis.

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