INFLAMMATORY BOWEL DISEASE

# Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis

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**Background/Aims:** Recent evidence has implicated the involvement of aquaporins (AQPs) in cellular functions that are unrelated to transepithelial water transport. Although AQPs are expressed in the gastrointestinal tract, their importance has so far been unclear. AQP3 is a water/glycerol transporter expressed at the basolateral membrane of colonic epithelial cells. The aim of this study was to investigate the involvement of AQP3 in enterocyte proliferation using mouse models of inflammatory bowel disease.
**Methods:** Expression and function of AQP3 in mouse colonic epithelium were established. Colitis was induced in wild-type and AQP3 null mice by oral dextran sulphate administration or intracolonic acetic acid administration. Outcome measures included clinical disease severity, survival, pathology and cellular responses. Some mice were administered glycerol to test whether disease progression could be altered.
**Results:** AQP3 null mice given dextran sulphate developed severe colitis after 3 days, with colonic haemorrhage, marked epithelial cell loss and death. Wild-type mice, which had comparable initial colonic damage as assessed by cell apoptosis, developed remarkably less severe colitis, surviving to >8 days. Cell proliferation was greatly reduced in AQP3 null mice. Oral glycerol administration significantly improved survival and reduced the severity of colitis in AQP3 null mice. Survival was also reduced in AQP3 null mice in the acetic acid model.
**Conclusions:** The results implicate a novel role for AQP3 in enterocyte proliferation that is probably related to its glycerol-transporting function. AQP3 is thus a potential target for therapy of intestinal diseases associated with enterocyte destruction.

The aquaporins (AQPs) are a family of water channels expressed in many epithelial, endothelial and other cell types. They facilitate transepithelial water transport in kidney tubules for urine concentration, and in glandular, choroidal and ciliary epithelia for fluid secretion.[1 2] AQPs in non-epithelial tissues in the central nervous system and eye are also involved in the regulation of tissue hydration. Recently, analysis of transgenic mice lacking specific AQPs has revealed new cellular roles for AQPs that are unrelated to transcellular water transport.[3] We found impaired angiogenesis in AQP1-deficient mice as a consequence of reduced endothelial cell migration,[4] which may be caused by slowed water movement into lamellipodia at the leading edge of migrating cells. A subset of AQPs (AQPs 3, 7 and 9), called 'aquaglyceroporins', transport water as well as glycerol, and possibly other small solutes. Mice lacking AQP3 have dry skin and delayed epidermal healing, with reduced glycerol content in epidermis and stratum corneum,[5 6] caused by impaired glycerol transport from the dermis through the normally AQP3-expressing basal keratinocytes. Recent studies provided evidence for a new role for AQP3 in cell proliferation. Mice lacking AQP3 were found to have significantly impaired epidermal proliferation in a wound healing model,[7] and impaired corneal epithelial cell proliferation after epithelial injury.[8] Mice lacking AQP7 manifest age-dependent adipocyte hypertrophy and glycerol accumulation,[9] which we proposed is caused by reduced glycerol exit from the normally AQP7-expressing adipocytes.

AQPs are expressed strongly in gastrointestinal organs including the stomach (parietal cells), liver (hepatocytes and cholangiocytes), pancreas (acinar epithelia), gallbladder (epithelium), small intestine (lacteals, enterocytes) and colon (colonocytes).[10 11] Consequently, roles for AQPs in the secretion of bile and pancreatic fluid have been postulated, as well as in intestinal fluid absorption and secretion. However, phenotype analysis of AQP1, AQP4 and AQP8 knockout mice has revealed little or no consequence of AQP deletion on major gastrointestinal fluid-transporting functions.[12–16] The absence of overt gastrointestinal phenotypes in AQP-deficient mice is surprising in view of the renal, central nervous system, ocular, glandular, cutaneous and other phenotypes found in these mice, particularly since the magnitude of gastrointestinal fluid transport is second only to that in kidney.

The epithelium lining the intestine maintains its architecture by a balance between the continuous processes of epithelial cell generation from clonal stem cells[17] at the base of intestinal crypts, and death of cells near the luminal surface. Recent evidence has suggested dysregulation of these processes in inflammatory bowel diseases (IBDs) such as ulcerative colitis, and has emphasised the importance of cell proliferation in disease progression.[18 19] There is evidence for a crucial role for Toll-like receptor (TLR) signalling and commensal bacteria in the initiation and transduction of the inflammatory and tissue repair responses.[20 21] TLR-4 null mice and related MyD88 null mice show significantly more severe disease progression in murine models of colitis as a result of impaired epithelial cell proliferation.[20]

Here, we present evidence for a new AQP function in the gastrointestinal tract. We found remarkably greater colonic pathology and mortality in mice lacking AQP3 than in wild-type mice in experimental models of colitis, with impaired epithelial cell proliferation at the base of colonic crypts in the null mice. The motivations for this study included the strong expression of AQP3 in colonic epithelial cells that undergo rapid turnover,[17] and, as mentioned above, the impairment of

**Abbreviations:** AQP, aquaporin; BrdU, bromodeoxyuridine; DAI, disease activity index; IBD, inflammatory bowel disease; PPAR, peroxisome proliferator-activated receptor; TLR, Toll-like receptor

epidermal and corneal cell proliferation in AQP3 deficiency.[7 8] We used the dextran sulphate and intracolonic acetic acid models of colitis[21–24] to induce epithelial damage and restitution in the colon. The primary model used was the dextran sulphate model, a well-described oral model of colitis. In order to verify the findings using a direct damage, non-oral model, we also used intracolonic instillation of acetic acid.[25 26] Enterocyte turnover is strongly stimulated in these models, which we predicted could expose defects in enterocyte proliferation and/or migration in AQP3 deficiency. Consequences of the findings here include the potential involvement of AQP3 in epithelial cell proliferation in a variety of intestinal inflammatory diseases, and the possibility of new therapies based on pharmacological modulation of AQP3 expression.

## MATERIALS AND METHODS

### Mice

AQP3 null mice in a CD1 genetic background were generated by targeted gene disruption as described.[25] Six- to ten-week-old male weight-matched wild-type and AQP3 null mice were used. For some control studies, AQP1 null mice in a CD1 genetic background were used.[28] The mice were maintained in air-filtered cages and fed normal mouse chow in the UCSF Animal Care facility. All procedures were approved by the UCSF Committee on Animal Research.

### Dextran sulphate model of colitis

Dextran sulphate sodium (molecular weight 36 000–50 000 Da; MP Biomedicals, Solon, OH, USA) was given ad libitum in the drinking water. Wild-type mice were given 6% dextran sulphate to induce acute colitis after 4 days. Since AQP3 null mice have increased daily water intake (see the Results section), in order to achieve the same dextran sulphate load[24] AQP3 null mice were given 2% dextran sulphate. Colitis induction in AQP1 mice was carried out as for AQP3 mice. Fluid consumption, body weight, stool consistency and colonic bleeding were monitored, and a disease activity index (DAI) was calculated as described.[29 30]

### Acetic acid model of colitis

Prior to acetic acid administration, mice were deprived of food for 24 h (but given 5% sucrose in water). Mice were then anaesthetised using 125 mg/kg 2,2,2-tribromoethanol (Avertin; Sigma-Aldrich, St Louis, MO, USA). As modified from published protocols,[25 26] a PE-50 catheter was inserted 3–4 cm into the lumen of the colon via the anus for infusion of 0.1 ml of 3% acetic acid in saline. After 1 min, the acetic acid was washed out with three lavages of saline, 0.3 ml each. Mice were assessed daily for body weight and survival for 10 days. Colon histology was obtained in some mice on day 3.

### Histology, immunocytochemistry and immunoblot analysis

Mice were killed and colons excised, fixed in 10% neutral buffered formalin, and embedded in paraffin. For immunohistochemistry, tissue sections (5 μm) were cut, deparaffinised, and stained with a rabbit polyclonal AQP3 antibody (Chemicon, Temecula, CA, USA) and Texas Red mouse anti-rabbit IgG (Vector Labs, Burlingame, CA, USA). Wide-field fluorescence images were taken using a Leica DM4000B epifluorescence microscope and Spot RT CCD camera; confocal images were taken on a Leitz microscope with a Nipkow wheel confocal attachment. Immunoblot analysis was done on homogenates of full-thickness colon. For histology, tissue sections (5 μm) were stained with H&E. Colon length was measured after excision. Crypt length and density were measured from at least two sections per mouse colon. Colonic epithelial damage was scored as follows: 0 = intact crypt; 1 = mild crypt loss (10–33%); 2 = moderate crypt loss (33–66%); 3 = severe crypt loss (66–100%); 4 = complete loss of crypt and surface epithelium intact. Infiltration with inflammatory cells was also scored (0 = normal, 1 = mild, 2 = modest, 3 = severe, 4 = very severe).

### Glycerol transport

To measure transepithelial glycerol permeability, closed colonic loops were created as described.[16 31] Loops were filled with phosphate-buffered saline containing 10 mM glycerol and 250 mM raffinose (to maintain loop volume over the course of the experiment). Based on preliminary experiments showing an ~2 h half-time for glycerol disappearance from loops, fluid was sampled using a syringe from loops at 0 and 2 h for colorimetric assay of glycerol concentration (R-Biopharm AG, Germany).

### Bromodeoxyuridine and apoptosis measurements

For measurement of cell proliferation, mice were injected intraperitoneally with 1 mg of bromodeoxyuridine (BrdU; Sigma, St Louis, MO, USA) at 3 days after initiation of dextran sulphate administration. Intestines were excised 2 h post-injection, fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections (5 μm) were cut, deparaffinised, stained with rat anti-BrdU antibody (Chemicon, Temecula, CA, USA) and peroxidase/diaminobenzidine (DAB; Vector Labs), and counterstained with haematoxylin. Apoptosis staining was done on fixed paraffin sections at 2 days using a DNA fragmentation assay kit (APO-BrdU-IHC, Biovision Inc., Mountain View, CA, USA). The extent of apoptosis was quantified from the staining intensity by image analysis (Image J, Wayne Rasband, NIH) of 3–5 areas per section (two sections per mouse).

### Glycerol replacement experiments

In some studies, wild-type and AQP3 null mice were supplemented with oral glycerol (2% (w/v) in drinking water, Fluka Chemie GmBH, Germany). Dextran sulphate was administered as described above, and assessment of DAI and histology were done.

### Data analysis

All values are expressed as mean ± SEM for data on different mice. Differences between groups were analysed using one-way analysis of variance (ANOVA) with Tukey–Kramer post hoc test or Student t test where appropriate.

## RESULTS

### AQP3 expression and function in murine colon

Previous studies have shown AQP3 expression in human and rat colon.[32 33] Immunostaining of mouse colon showed AQP3 localisation in the basolateral membrane of colonic epithelial cells, mainly at the base of colonic crypts (fig 1A). Immunoblot analysis confirmed the antibody specificity, with AQP3 expression seen in colon homogenates from wild-type but not AQP3 null mice (fig 1B). To confirm functional AQP3 expression in colon, glycerol transport from lumen to blood was measured in closed colonic loops. Figure 1C shows an ~2-fold reduced rate of glycerol disappearance from colonic loops of AQP3 null mice, indicating reduced transepithelial glycerol permeability. The actual fold difference in enterocyte basolateral membrane glycerol permeability may be much greater because of unstirred layer effects and serial permeability barriers. Histology of the colonic mucosa in wild-type and AQP3 null mice were indistinguishable, without significant differences in crypt length or density (fig 1D).

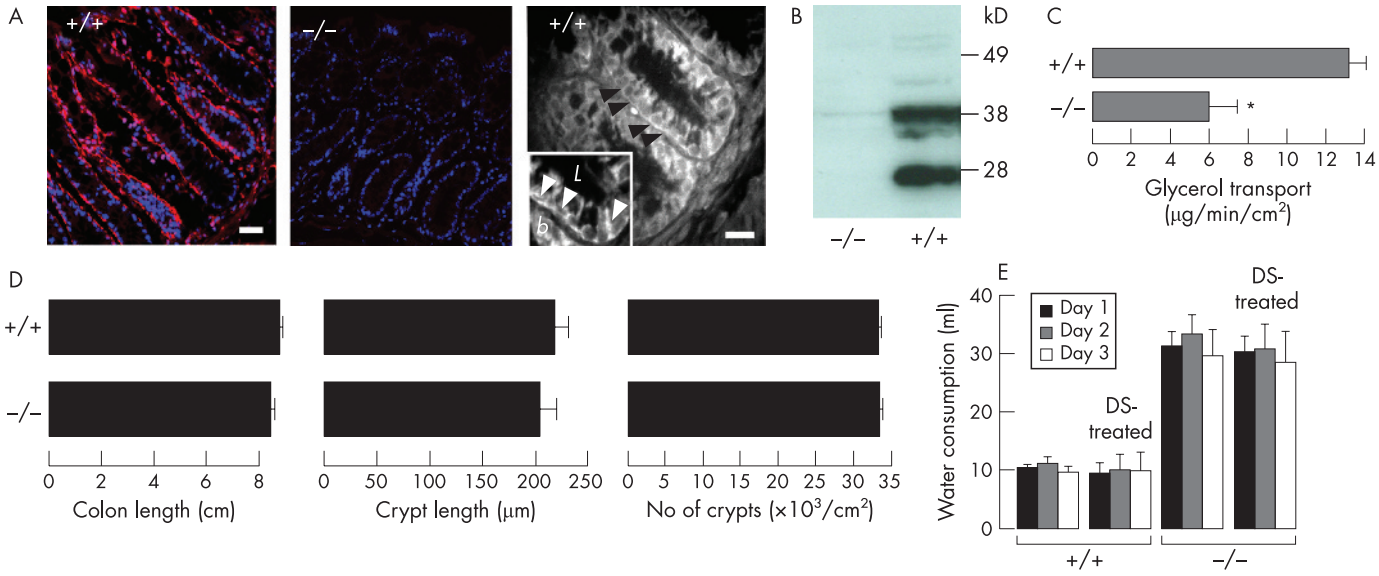


**Figure 1** Expression and function of aquaporin 3 (AQP3) in mouse colon. (A) AQP3 immunofluorescence in the proximal colon of a wild-type mouse (wide-field left, confocal right) and an AQP3 null mouse (middle). Nuclei stained blue with 4′,6-diamidino-2-phenylindole (DAPI). Arrows indicate basolateral membrane staining. Scale bars = 20 μm. The inset shows a higher magnification view (b, basolateral; l, lumen). (B) Immunoblot of whole colon homogenate. (C) Transepithelial glycerol transport measured in closed colonic loops in vivo (three loops per mouse) (SEM, n = 4 mice. $p<0.05$). (D) Colon length (left), crypt length (middle) and crypt density (right) (SEM, n = 5 mice). (E) Daily water consumption (SEM, n = 16 mice). DS, dextran sulphate.

## Reduced survival with severe colonic pathology in dextran sulphate-treated AQP3 null mice

Based on the postulated roles of AQP3 in epidermal and corneal cell proliferation, we tested whether AQP3 has a role in colonic epithelial cell regeneration/restitution after inflammatory damage. A well-established model of acute colitis produced by oral dextran sulphate was used to induce colonic inflammation and epithelial cell damage.[22] Pilot studies on wild-type mice established the dextran sulphate load required in our mouse strain to produce colitis reliably within 7 days. Previous studies have shown that AQP3 null mice are polyuric because of reduced water permeability in the kidney collecting duct,[27] with consequently greater daily fluid consumption than wild-type mice.[34] In order to match dextran sulphate load in the wild-type and AQP3 null experimental groups, the amount of dextran sulphate was normalised by daily water intake (fig 1E).

Figure 2A shows remarkably reduced survival of AQP3 null mice after dextran sulphate-induced colitis, with no mice surviving to 6 days. Figure 2B summarises the progression of body weight and DAI following dextran sulphate administration, revealing significantly more severe colitis in AQP3 null than wild-type mice, with an earlier appearance of clinical signs of colitis. At 3 days, the colons of AQP3 null mice were grossly haemorrhagic with decreased colon length (fig 2C).

Control experiments were done to rule out the possibility that systemic factors related to polyuria in AQP3 null mice could account for their worse survival. Dextran sulphate experiments were done on mice lacking AQP1, which have similar polyuria to AQP3 null mice.[28] AQP1 is not expressed in colonic epithelium. Figure 2A shows that the survival of AQP1 null mice was similar to that of wild-type mice, and notably better than that of AQP3 null mice, suggesting that the greater disease progression in AQP3 null mice results from greater colonic pathology.

Sections of colon from wild-type and AQP3 null mice were compared to assess structural and epithelial damage after dextran sulphate administration. Figure 3A shows considerably more epithelial damage in AQP3 null mice than in wild-type mice at 5 days, with near complete loss of the epithelium. Because dextran sulphate-induced colonic epithelial damage can be patchy, histological scoring of many sections was done (fig 3B), showing significantly increased mucosal damage in AQP3 null mice. In contrast, markers of inflammation, such as infiltration of leucocytes and neutrophils into the mucosa, were similar in wild-type and AQP3 null mice (fig 3B). Interestingly, by immunostaining, colonic AQP3 expression was elevated in wild-type mice at 3 days after dextran sulphate administration compared with untreated wild-type mice (fig 3C), providing further evidence for involvement of AQP3 in the colonic cellular response to injury. Figure 3D shows similar numbers of apoptotic epithelial cells in wild-type and AQP3 null mice at 2 days after dextran sulphate administration, suggesting similar cellular insult.

## Reduced survival in acetic acid-treated AQP3 null mice

An intracolonic infusion acetic acid model was developed as an alternative model of colitis that does not require oral administration. After testing a series of models, including intracolonic trinitrobenzenesulphonic acid (TNBS), we adapted the well-described acetic acid model used previously to produce acute colitis in mice.[25 26] Following pilot studies to determine acetic acid dose and volume, administration details and washout procedures, conditions were established in which survival of wild-type mice was 30–40% at 10 days after treatment (fig 4A). Under identical treatment conditions there was significantly reduced survival of AQP3 null mice at 3–10 days after treatment. Acetic acid treatment produced marked epithelial and goblet cell loss, with distortion of crypt architecture and marked infiltration of inflammatory cells in the lamina propria (fig 4B). Prominent mucosal congestion and haemorrhage was seen at the macroscopic level (fig 4C). These results support the conclusion that AQP3 deficiency is associated with a greater severity of colitis.

## Impaired enterocyte proliferation in AQP3 deficiency

Proliferation of epithelial cells after mucosal damage is an important determinant of disease progression in colitis,[35] with studies showing marked enterocyte proliferation in dextran sulphate-induced colitis.[36] BrdU pulse–chase experiments were done to compare colonic epithelial cell proliferation in wild-type

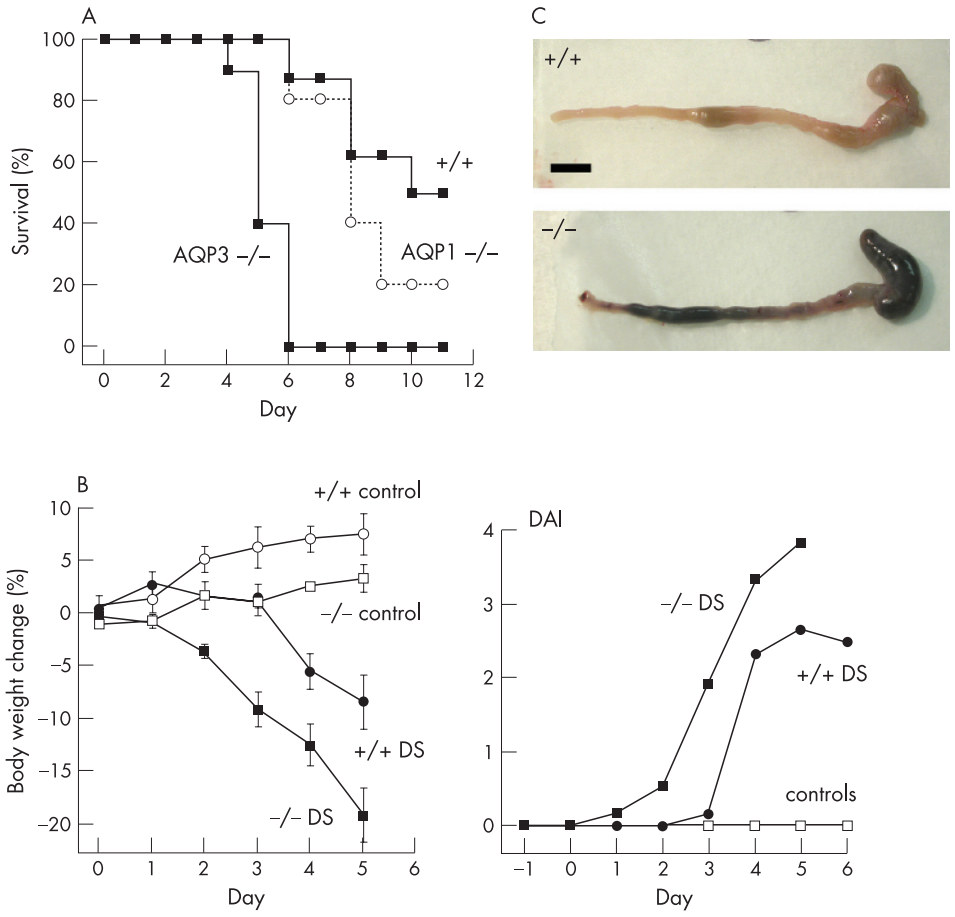


**Figure 2** Aquaporin 3 (AQP3) deficiency worsens the severity of dextran sulphate (DS)-induced colitis. (A) Survival after administration of DS. AQP1 −/−, aquaporin 1 null. (B) Left: body weight (SEM, 4 mice for control groups, 8 mice for DS-treated groups). Right: disease activity index (DAI) after DS treatment (4 mice for control groups, 8 mice for DS-treated groups). (C) Diseased bowel.

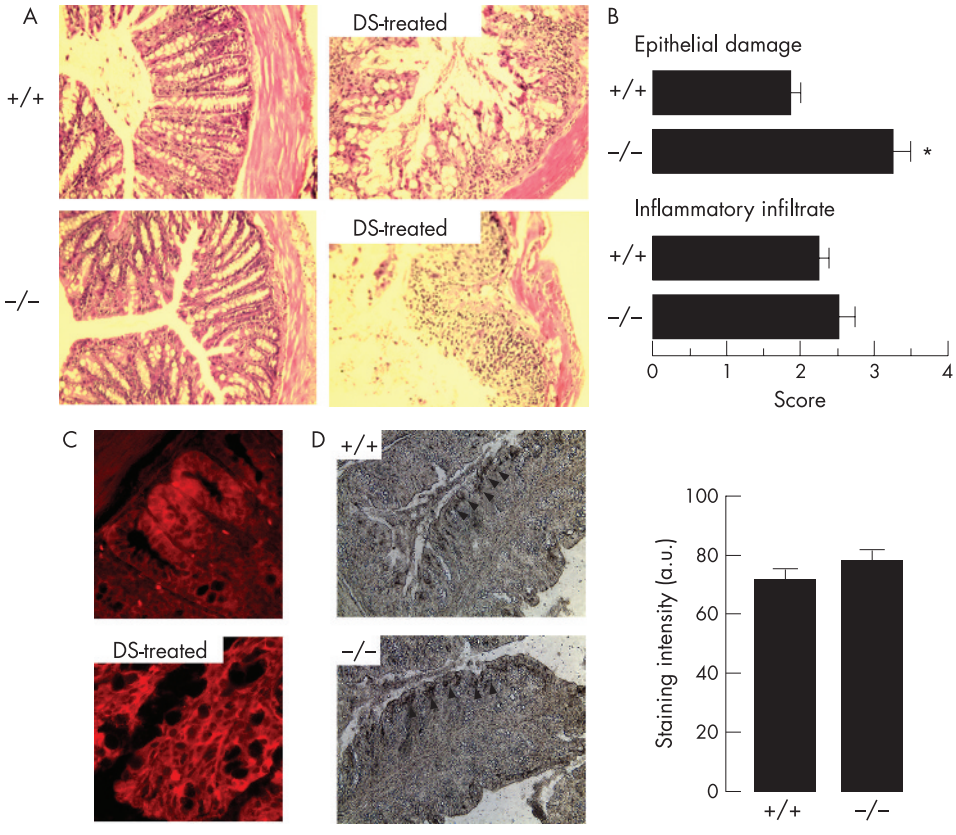


**Figure 3** Analysis of colonic structural responses to dextran sulphate (DS)-induced colitis. (A) H&E-stained sections of colon from control and DS-treated mice at 5 days. (B) Histological scores of epithelial damage and inflammatory cell infiltrate (5 mice per group, $p<0.005$). (C) Increased AQP3 immunostaining in wild-type mice at 3 days vs before DS treatment (representative of sections from four wild-type and AQP3 null mice). (D) Micrographs (left) showing apoptosis 2 days after DS treatment. Arrows indicate apoptotic cells. Right: staining intensity (SEM, n = 3 mice).

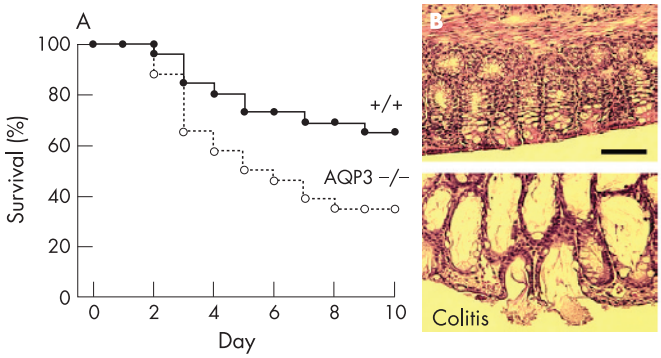


**Figure 4** Reduced survival of aquaporin 3 (AQP3) null mice in an acetic acid model of colitis. Acetic acid (3%) applied by intracolonic administration as described in the Materials and methods section. (A) Mouse survival after acetic acid treatment (26 mice per group, p = 0.02, log-rank test). (B) H&E-stained sections of ascending colon from a wild-type control (top) and an acetic acid-treated (bottom) mouse at 3 days. Scale bar = 100 μm. (C) Colonic mucosa of control and acetic acid-treated mice.

and AQP3 null mice in the dextan sulphate model. BrdU-labelled sections of colonic mucosa (fig 5A) showed the expected increase in cell proliferation in wild-type mice at 3 days after dextran sulphate treatment, with 12±2 BrdU-labelled cells per crypt (fig 5B). Cell proliferation in AQP3 null mice was also increased, though significantly less than in wild-type mice, with 6.6±0.6 BrdU-labelled cells per crypt (fig 5B)

### Oral glycerol administration reduces the severity of colitis in AQP3 null mice

To test whether AQP3-facilitated glycerol transport may be involved in enterocyte proliferation and responsible for the colitis phenotype difference in wild-type vs AQP3 null mice, we administered glycerol to dextran sulphate-treated mice to determine whether the course of the colitis could be modified. A similar glycerol replacement strategy was used to demonstrate that the glycerol-transporting function of AQP3 was responsible for the difference in skin phenotype in wild-type vs AQP3 null mice.[6 7] Figure 6A and B shows significantly greater survival and reduced severity of colitis in the glycerol-treated AQP3 null mice. Histological examination at 5 days (fig 6C) showed evidence of reduced crypt/epithelial damage compared with that seen in untreated AQP3 null mice (fig 4A).

## DISCUSSION

These studies revealed an interesting gastrointestinal phenotype in AQP3 null mice with potential clinical relevance and broad implications to tissues outside of the colon. In a dextran sulphate model of colitis, mice lacking AQP3 had remarkably more severe colonic pathology and reduced survival than control mice. Wild-type mice showed basal crypt cell hyperproliferation in response to colonic epithelial cell injury, whereas the AQP3 null mice had reduced epithelial cell proliferation, probably accounting for their more rapid worsening of clinical signs and more severe colonic pathology. Differences in outcome and cell proliferation in the AQP3 null mice could not be accounted for by differences in initial epithelial cell damage, as both groups of mice had the same dextran sulphate exposure and showed a comparable apoptotic cell response. Reduced survival of AQP3 null mice was also demonstrated in an acetic acid model of colitis, which involved intracolonic infusion rather than oral administration. Our results provide the first evidence for involvement of AQP3 in intestinal cell proliferation, which adds to a growing list of aquaporin functions that are unrelated to their classical role in transcellular water transport.[3]

Previous phenotype studies of mice lacking aquaglyceroporins provide clues about possible mechanisms for AQP3 involvement in cell proliferation. In skin, AQP3 deficiency reduces stratum corneum hydration and elasticity, and delays epidermal wound healing and restoration of barrier function after stratum corneum removal.[5 6] Mechanistic analysis indicated reduced glycerol content in the epidermis and stratum corneum in AQP3 null mice as a consequence of reduced glycerol transport through basal epidermal cells. Reduced glycerol content impairs skin hydration because of its humectant (water-retaining) properties, and delays healing because of its precursor role in epidermal biosynthesis. Topical or oral glycerol supplementation corrected the skin abnormalities in AQP3 null mice.[6] More direct evidence for AQP3 involvement in epidermal cell proliferation was found recently,[7] where AQP3 deficiency was associated with impaired cellular ATP generation and p38 mitogen-activated protein (MAP) kinase signalling, and absent tumorigenesis (unpublished results). Whether a similar mechanism exists in colon was not determined here, since, unlike epidermis, suitable primary enterocyte cell culture models do not exist. Existing transformed colonic cell lines such as T84 and HT29 are not suitable for proliferation studies, as they are derived from colon carcinomas.

Deficiency of AQP7, another aquaglyceroporin that transports both water and glycerol, produced progressive adipocyte hypertrophy with intracellular accumulation of glycerol and

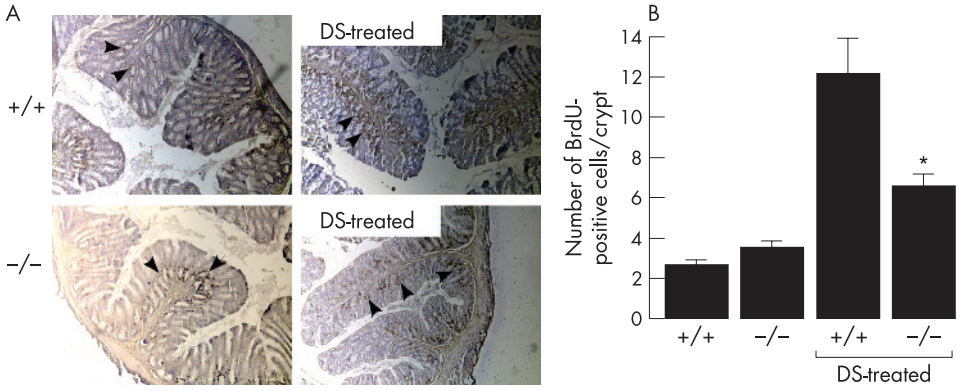


**Figure 5** Enterocyte hypoproliferation in colitis in aquaporin 3 (AQP3) null mice. Bromodeoxyuridine (BrdU) pulse–chase experiments were carried out as described in the Materials and methods section at 3 days after dextran sulphate (DS) administration. Sections are representative of four or more obtained per mouse (n = 5 mice). (A) BrdU-stained cells in control and DS-treated colons. (B) Number of BrdU-positive cells per crypt (SEM, n = 5 mice).

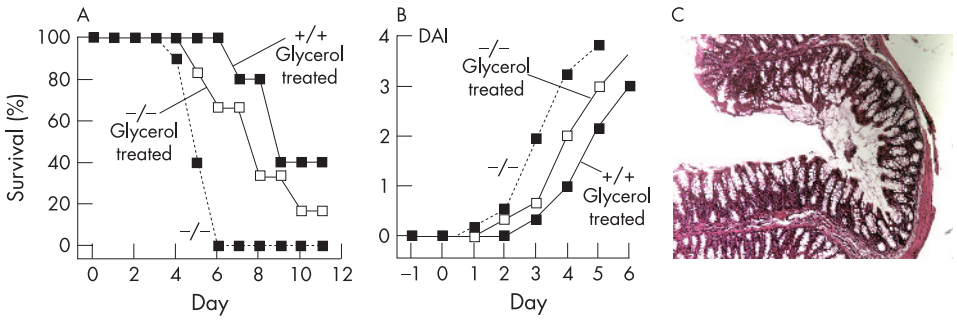


**Figure 6** Oral glycerol administration reduces the severity of dextran sulphate (DS)-induced colitis in aquaporin 3 (AQP3) null mice. (A) Survival curve for wild-type and AQP3 null mice after DS administration with oral glycerol treatment (6 mice per group). For comparison, survival is shown for AQP3 null/DS-treated mice without glycerol (dashed line). (B) Disease activity index (DAI) after glycerol treatment (SEM, n = 6 mice). (C) H&E-stained sections of colon from a glycerol-treated AQP3 null mouse at 5 days after DS administration.

triglycerides.[9] Mechanistic analysis suggested that reduced adipocyte permeability in AQP7 deficiency results in progressive glycerol accumulation and a consequent shift in adipocyte metabolism favouring triglyceride biosynthesis. Taken together with the data here showing partial correction of the AQP3 defect by glycerol administration, these studies suggest an important physiological role for aquaglyceroporin-mediated glycerol transport in cellular metabolism and biosynthesis.

Based on the previous work, the impaired cell proliferation in AQP3 deficiency reported here might be related to reduced AQP3-facilitated glycerol transport and consequent altered cell metabolism. AQP involvement in cell proliferation may thus be a general paradigm in tissues with rapid cell turnover. In addition to, or as an alternative to altered cell metabolism, AQP3 involvement in cell proliferation may be via interactions with other associated proteins. Recent studies have begun to link aquaglyceroporins with activation of peroxisome proliferator-activated receptors (PPARs) and also to regulation of proteins involved with glycerol metabolism.[35] Activation of PPARs has also been shown to be involved in colitis, with evidence for reduced disease progression with PPAR agonist treatment.[36 37] As described in the Introduction, recent studies have highlighted the central role of cell proliferation in response to a wide range of intestinal stimuli, including TLR activation and parasitic infection.[18 20 38]

Though the primary defect producing severe colitis in AQP3 deficiency appears to be impaired crypt cell proliferation, there may also be impairment of cell migration as a consequence of reduced colonocyte water permeability. Re-epithelialisation of the colon involves proliferation of basal crypt epithelial cells, as well as epithelial cell migration from the bottom of the crypts towards the colonic surface. Evidence from vascular endothelial and AQP-transfected cells has suggested a role for AQP-mediated water transport in cell migration, which could have a number of physiologically important consequences such as in endothelial cell migration in tumour angiogenesis.[4] Recent studies have shown impaired corneal re-epithelialisation in response to injury in AQP3-deficient mice, with impairment of both cell migration and proliferation.[8] Thus, impairment of AQP3-facilitated migration of new colonocytes might also contribute to the severe colitis in AQP3 deficiency, though we believe that this is less likely in the colon than in cornea because AQP3 is a substantially less efficient water transporter than the water-selective AQPs,[39] and colonocytes also express at least two water-selective AQPs, AQP4 and AQP8.[14 16 40] Previous studies have demonstrated AQP4-dependent transepithelial water permeation in colon, though little effect of AQP4 or AQP8 deletion on vital colon functions was found, including faecal dehydration, fluid absorption and fluid secretion.[14 16]

In summary, our results support a novel function of AQP3 in enterocyte proliferation, and provide the first evidence for a major gastrointestinal phenotype associated with AQP deficiency. Further work is needed to establish the precise metabolic link between AQP3-facilitated enterocyte glycerol transport and proliferation, and to investigate the possibility of modulation of AQP3 expression in therapy for diseases, such as Crohn's disease, that are associated with enterocyte hyper- or hypoproliferation.

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# EDITOR'S QUIZ: GI SNAPSHOT

## Answer

*From question on page 1511*

The clinical and endoscopic findings together with a re-evaluation of dietary recall, showing an absence of dietary vitamin C sources, suggested a diagnosis of scurvy. An ascorbic acid assay yielded no detectable amounts (<0.12 mg/dl, normal range 0.2–1.9 mg/dl). The patient was treated for 2 weeks with a daily oral dose of 200 mg vitamin C and a balanced diet including daily consumption of fruit. In the following week a prompt resolution of all symptoms was observed and a normal vitamin C level (1.1 mg/dl) was achieved after 5 days of therapy.

Clinical manifestation of scurvy reflects the role that vitamin C plays in collagen synthesis. Ascorbate is a cofactor for prolyl and lysyl hydroxylases in the biosynthesis of collagen. The characteristic tissue and capillary fragility of scurvy has been attributed to a defect in the praline hydroxylation step of collagen biosynthesis.[1 2] Nowadays, scurvy reports are rarer and less common in children, except in underdeveloped countries and in some ''at risk'' categories, including persons with neurological or behavioural disorders, anorexia and intestinal malabsorption.[3] It is important for the gastroenterologist to include scurvy in the differential diagnosis of patients presenting with purpuric lesions (also at gastric mucosa levels), joint pain and bleeding gums, because appropriate diagnosis and treatment is life saving, even when patients present in the most advanced stages.

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