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Loss of the Tight Junction Protein ZO-1 in Dextran Sulfate Sodium Induced Colitis

Lisa S. Poritz, M.D.,[1] Kristian I. Garver, Ph.D., Cecelia Green, M.S., Leo Fitzpatrick, Ph.D., Francesca Ruggiero, M.D., and Walter A. Koltun, M.D.

Department of Surgery and Department of Pathology, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania

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Background. **Inflammatory bowel disease (IBD) is associated with increased intestinal permeability and decreased expression of tight junction (TJ) proteins in the inflamed mucosa. Whether this alteration in TJ expression is a prerequisite for the development of intestinal inflammation or a secondary result of that inflammation is unknown. This study looked at the expression of the TJ protein ZO-1 and the corresponding permeability changes in dextran sulfate sodium (DSS) induced colitis in a mouse model.**

Materials and methods. **BALB/c mice were fed 3% DSS or water for 1, 3, 5, or 7 days. The animals were weighed, stool was checked for blood, and the colon length measured. Segments of the colon were used for histology, immunohistochemistry for ZO-1, or Western blot for TJ proteins. Colonic permeability was measured using Evan's Blue dye.**

Results. **DSS treated animals had heme positive stools, colitis by histology, significant weight loss, and colon shortening. There was an absence of ZO-1 by Western blot in the 7-day DSS treated animals, double the amount of claudin-1 and normal cytokeratin. The loss of ZO-1 started after 1 d of DSS treatment and was followed by a significant increase in permeability to Evan's blue by day 3.**

Conclusions. **The loss of ZO-1 and increased permeability preceded the development of significant intestinal inflammation suggesting that in DSS colitis alterations in the TJ complex occur before the intestinal inflammation and not as a consequence of it. These changes in the TJ complex may facilitate the development of the inflammatory infiltrate seen in colitis.**

Key Words: **tight junction proteins; ZO-1; inflammatory bowel disease; ulcerative colitis; intestinal permeability; intestinal inflammation; dextran sulfate sodium colitis.**

[1] To whom correspondence and reprint requests should be addressed at Section of Colon and Rectal Surgery, H137, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033. E-mail: lporitz@psu.edu.

INTRODUCTION

Inflammatory bowel disease (IBD), which consists of Crohn's disease (CD) and ulcerative colitis (UC), is an immune-mediated illness characterized by chronic intestinal inflammation with or without other systemic manifestations. The etiology of IBD is unknown and there is no known medical cure. One theory of pathogenesis suggests that there is a compromise in the intestinal permeability barrier exposing the underlying immuno-regulatory mechanisms to normally excluded agents that results in an aberrant and self-perpetuating inflammatory process. Consistent with this theory is the finding of increased intestinal permeability in both patients with CD and at-risk relatives who remain asymptomatic [1].

The tight junction complex (TJ) is a cluster of proteins that forms a physiologically active barrier at the level of the intestinal epithelial cell that can change its permeability based on the cellular environment. The organization of the TJ is similar to other intercellular junctions and consists of transmembrane proteins that mediate adhesive function linked to the underlying plaque proteins that in turn associate with the cytoskeleton [2]. Three of the key proteins of the TJ are ZO-1, occludin, and the more recently identified family of claudins. ZO-1, one of the plaque proteins, was the first TJ protein characterized. It is a 225 kda membrane bound protein that localizes to the TJ. It binds the transmembrane proteins occludin and the claudins linking them to cytoskeletal actin [3, 4]. Contraction of cytoskeletal actin is thought to have a role in the regulation of paracellular permeability and ZO-1 may be the direct link between actin and the transmembrane proteins.

The expression of TJ proteins has been shown to be decreased in the intestinal inflammation of IBD [5–7]. This corresponds to an increase in intestinal permeability and decrease in transepithelial resistance

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(TER) [8, 9]. Increased intestinal permeability in IBD patients correlates with disease activity and has been demonstrated to be a predictor of relapse, after both medical and surgical remission [10–13]. Whether this alteration in tight junction expression and increase in permeability is a prerequisite for the development of intestinal inflammation or a secondary result of that inflammation is unknown. The goal of this present study was to measure expression of the TJ protein ZO-1 and the corresponding permeability changes associated with dextran sulfate sodium (DSS) induced colitis in a mouse model.

MATERIALS AND METHODS

Mice

Female BALB/c mice, 8 to 10 weeks (17–25 g) were purchased from Jackson Laboratory (Bar Harbor, ME) and handled in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Induction of Colitis

Colitis was induced in the mice by replacing their drinking water with 3% (wt/vol) DSS in tap water. Control mice received tap water. Animals were allowed water or 3% DSS and food ad lib. See Table 1 for animal groups. Weight change and fecal blood assessment was made in all animals. The animals were then subdivided into smaller groups and after sacrifice the colon was used for Western blot, histology, and immunofluorescence or Evan's blue permeability studies. Change in colon length was calculated only in the animals used for histology and Western blot. Animals were sacrificed by exsanguination under sodium pentobarbital anesthesia (20 mg/kg i.p.).

Clinical Evaluation

Weight Loss

Animals were weighed before DSS treatment and on the day of sacrifice. Percent weight loss was calculated as: [(post-DSS weight − pre-DSS weight)/pre-DSS weight]*100.

Fecal Blood

On the day of sacrifice the fecal material was evaluated for blood. If there was visible blood in or on the fecal material it was scored as gross blood. If no blood was visible the fecal material was Hemoccult (Beckman Coulter, Fullerton, CA) tested and recorded as positive or negative.

TABLE 1

Mouse Groups

Mouse group	Treatment	Days of treatment	*N*
Control	Water	7	20
DSS 1	3% DSS	1	17
DSS 3	3% DSS	3	17
DSS 5	3% DSS	5	15
DSS 7	3% DSS	7	18

Colon Length

At the time of sacrifice, in all animals except those to be used for permeability assays, the colon was transected in the distal rectum and just distal to the cecum. The colon was measured and the colon length was corrected for the mouse's pre-treatment weight and calculated as: colon length (cm)/pre-treatment weight (g). The colon was then opened longitudinally and washed twice in phosphate-buffered saline (PBS) to remove fecal matter. It was then either processed for histology and immunofluorescence or Western blot (see below).

Histology

Sections of proximal and distal colon (1 section from each mouse) were embedded in paraffin and stained for morphology with hematoxylin and eosin. The histology scoring system for DSS colitis was used to quantify the histological changes and assess the severity of inflammation in the colon. The sections were scored by an investigator blinded to treatment group (L.F.). The sections were graded 0 to 3 for inflammation and depth of inflammation with 3 being most severe, and 0 to 4 for crypt damage and regeneration. The score was then multiplied by percent of the section involved: 1 for 1 to 25%; 2 for 26 to 50%, 3 for 51 to 75%, and 4 for 76 to 100% [14].

Immunofluorescence and Confocal Microscopy

Frozen sections (8 μm) of distal colon (1 section of each from each mouse) from control animals and animals treated with 7 days of DSS were fixed in 2% paraformaldehyde for 10 min. They were then rinsed in PBS twice for 5 min and then incubated with 200 ul of blocking buffer (10% donkey serum in PBS with 0.1% triton) in a humidified chamber for 1 h at 23°C. They were then incubated with the primary antibody, polyclonal rabbit anti ZO-1 (Zymed Laboratories, San Francisco, CA) overnight at 4°C. After washing 3 times for 20 min in 0.1% triton in PBS they were incubated with the secondary antibody Cy 3-conjugated donkey anti-rabbit (Jackson ImmunoResearch Labs, West Grove, PA) and Hoechst (Sigma, St. Louis, MO) nuclear counterstain at 1:1000 in a humidified chamber for 1 h at 23°C. They were washed again with 0.1% triton in PBS three times for 20 min and mounted with aquapolymount and a coverslip.

Digital images of the colon were captured with the Leica TCS SP2 AOBS confocal microscope. The images were created as a z-stack and three dimensional images were created by merging the z-scans and are shown as an average projection. All images were treated equivalently.

Western Blot

The mucosa was scraped from the entire opened and washed colon with a microscope slide. The mucosa was then immediately immersed in urea extraction buffer [6 M Urea, 0.1% Triton X-100, 10 mM Tris (pH 8.0), 1 mM DTT, 5 mM MgCl2, 5 mM EGTA, 150 mM NaCl, 0.2 mM PMSF] with anti-proteases to prevent protein degradation. The samples were sonicated to release the membrane bound proteins. Protein concentration was measured with BCA protein assay Kit (Pierce, Rockford, IL) according to the manufacturers instructions. Samples (25 ug/lane) were run on a 4 to 15% gradient gel (Bio-Rad Laboratories, Hercules, CA) at a constant voltage of 100 volts for 1 h against high molecular weight standards. The proteins were then transferred to nitrocellulose for one hour at 100 V or 20 volts overnight at 4°C. The blots were blocked in 5% nonfat milk for 1 to 2 h to prevent non-specific binding. They were then incubated with the primary antibody, monoclonal mouse anti-human ZO-1 (Zymed) for 2 h. The blots were then washed and incubated with the secondary antibody, HRP conjugated rabbit anti-mouse (Zymed). Immunoreactive bands were detected using a chemiluminescence kit according to the manufacturer's directions (Perkin-Elmer Life Sciences, Boston, MA). The chemiluminescent signals were detected using autoradiographic film. Blots were then stripped and reprobed