The role of the complement and contact systems in the dextran sulfate sodium-induced colitis model: the effect of C1 inhibitor in inflammatory bowel disease

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Lu F, Fernandes SM, Davis AE 3rd. The role of the complement and contact systems in the dextran sulfate sodium-induced colitis model: the effect of C1 inhibitor in inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 298: G878–G883, 2010. First published March 25, 2010; doi:10.1152/ajpgi.00400.2009.—The complement and contact systems may be involved in the pathophysiological process of inflammatory bowel disease (IBD). C1 inhibitor (C1INH) is the most important inhibitor of both the complement and contact systems. We evaluated the role of these systems and the effect of both active and inactive forms of C1INH (iC1INH) in dextran sulfate sodium (DSS)-induced colitis mouse model. Three percent DSS was used in drinking water to induce colitis in complement C3-deficient (C3−/−) mice, bradykinin type 2 receptor deficient (BkR−/−) mice, and C57BL/6 mice. After ten days DSS exposure, C3−/− mice exhibited markedly less weight loss than wild-type (WT) mice (12 ± 3.3% vs. 30 ± 1.2%, P < 0.05) and developed a milder disease-activity index (DAI), histological score, colon shortening, and myeloperoxidase (MPO) elevation (P < 0.05, respectively). The BkR−/− mice were not protected from the disease. Seven-day treatment with either native C1INH or iC1INH reduced the severity of the disease in WT mice, as indicated by decreased weight loss (15 ± 1.8%, 14 ± 2.1% vs. 30 ± 1.2%, P < 0.05, respectively), DAI, intestinal tissue damage, and MPO elevation compared with untreated WT DSS control mice (P < 0.05, respectively). These findings suggest that complement plays a role in the development of DSS-induced colitis and that blockade of the complement system might be useful for the acute phase of IBD treatment. C1INH, however, leads to an amelioration of DSS-induced colitis via a mechanism that does not involve the inhibition of complement or contact system activation but does result in significant suppression of leukocyte infiltration.

C3; bradykinin; colon; leukocyte infiltration

INFLAMMATORY BOWEL DISEASE (IBD) is a chronic disorder of the bowel that encompasses ulcerative colitis (UC) and Crohn’s disease (CD). Although the etiology and the precise pathogenic mechanism of IBD are not known, numerous inflammatory mediators have been implicated in its pathogenesis. These include cytokines, eicosanoids, reactive oxygen species, nitric oxide, and complement system activation products (22, 29). Complement activation products, C3a, C5a and the terminal membrane attack complex, are potent inflammatory mediators that are associated with pathology in various disease states, such as systemic lupus erythematosus and autoimmune arthritis, ischemia-reperfusion injury, sepsis, transplantation rejection, burn, Alzheimer’s disease, multiple sclerosis, and Guillain-Barre syndrome. A role for the complement system in the pathogenesis of IBD has been suggested in some studies. C3 and C4 gene expression has been found increased in intestinal tissues from patients with CD (23). C3b and other activation fragments have been detected on intestinal cells in lesions of chronic IBD in humans (15, 40). Pathologically, polymorphonuclear cell infiltration characteristics is also noted (21).

Contact system activation, leading to the formation of bradykinin, also may be involved in the pathogenesis of IBD (36). The kallikrein-kinin system consists of circulating kininogens, the proteolytic enzymes factor XII and prekallikrein, kinins, and two G protein-coupled receptors termed kinin B1 and bradykinin B2 receptors, which mediate the biological effects of kinins (1, 24). Both B1 and B2 receptors have been immunolocalized in the epithelial cells of affected human intestinal tissue (38). A kinin B1 antagonist attenuates the inflammatory changes in the experimental colitis model (17). However, it is not known whether the B2 receptor might also play a role.

C1 inhibitor (C1INH), a member of the serpin family, is the most important inhibitor of both the complement and contact systems, which are involved in the mediation of inflammation. However, there is limited evidence that inhibition of complement and/or contact system activation results in suppression of the development of IBD. The observations by Potter and Devani (12, 31) showed a higher level of functional C1INH in CD patients. In contrast, significantly lower functional C1INH concentration has been detected in patients with active UC than in normal controls or patients with inactive disease, which agree with the finding of Oshitani et al. (28, 37), who found a decrease in the level of functional plasma C1INH in active CD patients. This diminution of C1INH in IBD may be a result of inhibitor consumption during both complement and contact system activation.

There is some evidence that inhibition of the complement system results in a protective effect in several colitis models. An antibody to complement receptor 3 ameliorated the intestinal inflammation in trinitrobenzene sulfonic acid (TNBS)-colitis and T cell transfer colitis (25). The complement activation inhibitor K-76 produced symptomatic improvement of UC (20). A strong protective effect of a C5a receptor antagonist has been demonstrated in the TNBS-induced rat colitis model and was confirmed recently in the dextran sulfate sodium (DSS)-colitis mouse model (19, 42). Conversely, a study by Deguchi (11) showed that DSS-induced colitis is aggravated in C5a-deficient mice, which suggested that the complement system might play a protective role in the development of this experimental colitis. This apparent contradiction prompted us to perform a study to further evaluate the roles of the complement and contact systems in the pathogenesis of IBD and...
whether C1INH might be therapeutically useful in IBD. C1INH has been shown to be beneficial in a variety of animal models of inflammatory disease, including sepsis, ischemia-reperfusion injury, hyperacute transplant rejection, and burn injury (10). In the present study, we investigated the development of DSS-induced colitis in complement C3-deficient (C3\(^{-/-}\)) mice, bradykinin type 2 receptor-deficient (Bk2R\(^{-/-}\)) mice, and wild-type (WT) mice, and in WT mice treated with both C1INH and inactivated C1INH (iC1INH).

**MATERIALS AND METHODS**

*Animals.* C57BL/6 mice and Bk2R\(^{-/-}\) mice were purchased from Jackson Laboratory (Bar Harbor, ME). C3\(^{-/-}\) mice were obtained from Michael Carroll, PhD, Immune Disease Institute, Boston, MA. Experiments were performed with mice at 8–12 wk of age and an average weight of 25 g at the beginning of the experiments. All experiments were performed in compliance with relevant laws and institutional guidelines and were approved by the Immune Disease Institute Animal Care and Use Committee.

*Reagents.* C1INH (Berinert) was generously provided by CSL Behring (King of Prussia, PA). iC1INH was prepared by limited digestion of native C1INH with trypsin agarose (no. T4019; Sigma, St. Louis, MO) (2). Briefly, C1INH was incubated with trypsin agarose (1U:8 μl) at room temperature for 1 h, then subjected to centrifugation (5000 g) at 4°C. The supernatants were collected and used immediately. The function of C1INH and iC1INH was characterized by analyzing complex formation with Cls. C1INH and iC1INH were incubated separately with Cls (1:1 molar ratio) at 37°C for 60 min and then subjected to SDS-PAGE and stained with Coomassie blue. Native C1INH reacts with Cls to form a 200-kDa complex. However, reactive-center-cleaved C1INH (iC1INH) loses the ability to complex with Cls and is therefore regarded as inactive. In addition, iC1INH migrates on SDS-PAGE with a slightly smaller apparent molecular weight than intact active C1INH (~105 kDa).

*Model of colitis.* Colon inflammation was induced by oral administration of 3% DSS (molecular weight: ~36–40 kDa; MP Biomedicals, Aurora, OH) in the drinking water. Sham control mice had drinking water only. The mice were observed three times a day, and their body weight was recorded daily. The colons were collected after 10 days for histological and biochemical analysis.

Five groups of mice were exposed to DSS to induce colitis, namely C57BL/6 controls, a C1INH-treated C57BL/6 group, an iC1INH-treated C57BL/6 group, a C3\(^{-/-}\) group, and a Bk2R\(^{-/-}\) group. C1INH- or iC1INH-treated WT mice were dosed from the third day of DSS exposure and then every 8 h for 7 days. C1INH or iC1INH were administered at 5 mg/kg body wt in 75 μl buffer by intravenous injection; the same volume of normal saline was used in the WT control mice (n = 12 in the WT control group and n = 8 in all other groups).

*Evaluation of colitis.* In all animals, body weight, stool hemoccult positivity, or gross bleeding, and stool consistency were determined daily. Disease activity index (DAI) was determined by combining scores of 1) weight loss 2) stool consistency, and 3) bleeding. Each score was determined as follows, change in weight (0: <1%, 1: 1–5%, 2: 5–15%, 4: >15%), stool consistency (0: normal, 2: loose stools, 4: diarrhea), and stool blood (0: negative, 2: positive, 4: gross bleeding) as previously described (7). Body weight loss was calculated as the percent difference between the original body weight and actual body weight on any particular day. Evaluation of DAI scores of the mice was conducted by a researcher who was blinded to the treatment.

*Colon length and histological scoring.* After mice were anesthetized with high-dose ketamine/xylazine with carbon dioxide asphyxia, their colons were immediately resected between the ileocecual junction and the proximal rectum, close to its passage under the pelvis ternum. To measure the length, the colon was placed on a nonabsorbent surface and measured with a ruler, taking care not to stretch the tissue. For the histology, ~1.5–2 cm of colon was fixed in 10% buffered formalin, embedded in paraffin block, cut into 5-μm sections, and stained with hematoxylin and eosin. The remainder of the colon was weighed and snap frozen at ~80°C for other analyses. Stained sections of colons were scored by an independent pathologist in a blinded fashion, using the standard histological scoring system described by Cooper et al. (7). Three serial sections of five to six different sites were examined, and the most affected part was scored. A score of 0 reflects normal epithelium, without blunting, normal crypt appearance, low mononuclear cell infiltration, and very few or absent neutrophil infiltration; a score of 1 indicates loss of single epithelial cells, mild blunting of epithelium, single inflammatory cell infiltration of crypts, slight mononuclear cells, and few neutrophil infiltrate; a score of 2 signifies loss of multiple epithelial cells (in patches), obvious flattening of epithelia, cryptitis, and a moderate increase in neutrophils and mononuclear cells; a score of 3 indicates frank epithelial ulceration with crypt abscesses and a marked increase in neutrophil infiltration.

*Myeloperoxidase activity.* Myeloperoxidase (MPO) activity, an index of leukocyte recruitment, was measured in colon tissue homogenates by a modification of the method of Grisham (14). Briefly, colon homogenate was sonicated in 1% hexadecyltrimethylammonium bromide buffer for 10 min and subjected to centrifugation at 12,000 revolution/min at 4°C for 20 min. The supernatants were collected and reacted with O-dianisidine dihydrochloride (0.167 mg/ml) and H2O2 (0.005%) in potassium phosphate buffer at 25°C. The change in absorbance was measured spectrophotometrically at 450 nm. MPO activity was expressed as units per 100 mg of wet tissue.

*Statistical analysis.* Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad, La Jolla, CA), with significance defined as P < 0.05. DAI and histological score are expressed as median and interquartile range and analyzed by using Kruskal-Wallis test followed by Dunn’s post hoc test. All other parametric data are expressed as means ± SE, and a one-way ANOVA analysis of variance with Bonferroni’s multiple-comparison tests was used for data analysis.

**RESULTS**

*Body weight change.* All mice lost weight after 10-day exposure to 3% DSS. WT control mice started to lose weight from day 5 (5% loss) and continued until reaching a 30 ± 1.2% reduction from their original weight at day 10. Bk2R\(^{-/-}\) mice lost 7% weight at the second day and progressed to 29 ± 2.0% reduction at day 10. Body weight change in response to DSS was significantly milder in C3\(^{-/-}\), C1INH-, and iC1INH-treated WT mice compared with WT DSS control mice, with 12 ± 3.3%, 15 ± 1.8%, and 14 ± 2.1% reductions at day 10, respectively (Fig. 1).

*Disease activity.* The DAI, defined as described in MATERIALS AND METHODS, was used to analyze the roles of the complement and contact systems and the therapeutic benefit of C1INH. In WT control and Bk2R\(^{-/-}\) mice, rectal bleeding and severe diarrhea appeared from day 5 and continued until day 10. Compared with WT control mice, the development of DSS-colitis was eliminated in C3\(^{-/-}\) mice, and both C1INH and iC1INH treatment reversed the course of the disease induced by DSS in the WT mice (Table 1).

*Colon shortening.* At day 10, WT mice exposed to DSS exhibited significant colon shortening compared with sham (water only) controls. This effect was eliminated in C3\(^{-/-}\) mice and by C1INH or iC1INH treatment but was not attenuated in Bk2R\(^{-/-}\) mice (Fig. 2).

*Histological scores.* Microscopically, colon sections from WT control mice and Bk2R\(^{-/-}\) mice showed more severe...
pathology after 10-day DSS exposure, including disruption of the epithelium, ulcer formation, mucosal edema, and cellular infiltration. By comparison, sections of colon from C3−/− mice, C1INH-, and iC1INH-treated WT mice showed much milder signs of damage, indicated by less edema, less neutrophil infiltration, less mucosa erosion, and ulcer formation (Fig. 3). Histological scores were significantly reduced in C3−/− mice and in C1INH- and iC1INH-treated WT mice compared with untreated WT colitis mice (Fig. 4).

Colon MPO levels. MPO activity, an index of neutrophil infiltration, was measured in colon tissue homogenates. As indicated by elevated MPO levels, DSS ingestion led to increased neutrophil infiltration into the intestinal tissue. After 10-day DSS exposure, colonic MPO levels were similarly elevated in WT and Bk2R−/− mice compared with sham control mice that were not exposed to DSS (11.28 ± 1.18, 9.95 ± 0.83 vs. 5.41 ± 0.71, P < 0.05, respectively). In WT colitis mice treated with C1INH and iC1INH, or in C3−/− mice, the colonic MPO levels were significantly reduced compared with WT colitis controls (5.85 ± 0.38, 6.61 ± 0.66, 5.50 ± 0.56 vs. 11.28 ± 1.18, P < 0.05, respectively) (Fig. 5).

DISCUSSION

DSS-induced colitis in mice has been exploited as a model of superficial intestinal inflammation. In this model, colonic mucosal inflammation, ulceration, weight loss, and bloody diarrhea develop upon addition of the drug to drinking water. It is a reliable model that has been widely used to investigate the pathogenesis of IBD (7, 13, 19, 27). Although the exact etiologies remain uncertain, results from research in animal models, human genetics, basic science, and clinical trials have provided important new insights into the pathogenesis of chronic, immune-mediated, intestinal inflammation. Activation of immune cells is thought to play a major role in IBD; once the cell is activated, proinflammatory cytokines and chemokines are strikingly elevated, and expression of adhesion molecules and costimulatory molecules is also increased (34). Blockade of their production is one of the tools recently introduced in therapy (33). The complement system involves a cascading series of proteins that act as early defense components of the immune system. The complement anaphylatoxin C5a is a potent chemotactic agent that helps to recruit and activate immune and inflammatory cells. Although primarily protective, uncontrolled complement activation leads to tissue injury in several inflammatory diseases. A role for complement in tissue destruction in IBD has been suggested by the identi-

Table 1. DAI in mice exposed to DSS

<table>
<thead>
<tr>
<th></th>
<th>WT DSS Control</th>
<th>Bk2R−/−</th>
<th>C1INH-Treated</th>
<th>iC1INH-Treated</th>
<th>C3−/−</th>
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<tbody>
<tr>
<td>Day 5</td>
<td>1.0</td>
<td>1.9–3.0</td>
<td>1.3</td>
<td>1.0–2.0</td>
<td>0.2*</td>
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<tr>
<td>Day 6</td>
<td>2.2</td>
<td>1.5–3.9</td>
<td>1.7</td>
<td>1.3–3.5</td>
<td>0.7†</td>
</tr>
<tr>
<td>Day 7</td>
<td>3.7</td>
<td>2.3–4.0</td>
<td>2.2</td>
<td>1.5–4.0</td>
<td>1.2‡</td>
</tr>
<tr>
<td>Day 8</td>
<td>4.0</td>
<td>4.0–4.0</td>
<td>3.3</td>
<td>2.0–4.0</td>
<td>1.2‡</td>
</tr>
<tr>
<td>Day 9</td>
<td>4.0</td>
<td>4.0–4.0</td>
<td>4.0</td>
<td>3.0–4.0</td>
<td>1.2‡</td>
</tr>
<tr>
<td>Day 10</td>
<td>4.0</td>
<td>4.0–4.0</td>
<td>4.0</td>
<td>3.5–4.0</td>
<td>1.3‡</td>
</tr>
</tbody>
</table>

Values are presented as medians and interquartile ranges (IQR). n = 12 in wild-type (WT) control group and 8 in the other group. There is no significance between the groups from days 1–4; data are not shown here. *, †, ‡P < 0.05 compared with WT dextran sulfate sodium (DSS) control and bradykinin type 2 receptor-deficient mice (Bk2R−/−) group. DAI, disease-activity index; C1INH, C1 inhibitor; iC1INH, reactive center-cleaved inactive C1INH.
fication of increased deposition of the active terminal complement complex in intestinal tissue from IBD patients (15). However, the effect on attenuation of the development of IBD by blocking the activation of complement is still in question (11, 19, 42). In this study, we found that C3−/− mice showed less tissue damage and disease severity compared with the WT mice, which is consistent with the study by Woodruff and Johswich et al. (19, 42), indicating that complement-depleted animals were less susceptible to DSS-colitis and that inhibition of complement activation might be an effective strategy for treating IBD in animal models.

Fig. 3. Selective photographs of colon sections from colitis mice stained with hematoxylin and eosin (magnification, ×100). A: normal colon with no lesions. The mucosa is of uniform thickness, and the crypts are straight. There is no cellular infiltration, edema, or exudate. B: score of 1, colon with mild lesions. There are mucosal erosion and small superficial ulcers scattered along the length of the colon, with slight crypt loss and mononuclear cell infiltration. C: score of 2, colon with moderate lesions. Intestines have extensive erosion and ulceration, with moderate crypt loss and neutrophil infiltration. Only a small area of fairly normal crypts is seen at right. D: score of 3, colon with very severe ulceration. Much of the mucosa is thin with loss of crypts and markedly increased infiltration of neutrophils and acute inflammatory exudate.

Fig. 4. Colon histological score after 10-day exposure to DSS. Histological scores of DSS-affected colon were significantly reduced in C3−/− mice, C1INH−, and iC1INH-treated mice compared with untreated WT DSS control and Bk2R−/− mice. Value are expressed as medians and interquartile ranges (n = 12 in WT DSS control group and n = 8 in other groups); P < 0.05 C3−/−, C1INH−, and iC1INH-treated WT mice vs. WT DSS control and Bk2R−/− mice.

Fig. 5. Colon myeloperoxidase (MPO) levels after 10-day exposure to DSS. The colonic MPO level in untreated WT DSS control and Bk2R−/− mice were significantly increased compared with sham control mice. In WT mice treated with C1INH and iC1INH, or C3−/− mice, the colonic MPO levels were significantly reduced. The data represent the means ± SE (n = 12 in WT DSS control group and n = 8 in other groups). P < 0.05, WT DSS control and Bk2R−/− mice vs. WT sham control; P < 0.05 C3−/−, C1INH−, and iC1INH-treated WT mice vs. WT DSS control and Bk2R−/− mice.
C1INH is a heavily glycosylated plasma protein that belongs to the serpin superfamily of serine proteinase inhibitors. It inhibits activation of the classical and lectin pathways by inactivation of the early proteases involved in activation, C1r, C1s, and mannin-binding lectin serine protease-2 (30, 32, 35, 41, 43). In addition, via a mechanism that does not involve protease inhibition, it also may play a role in regulation of alternative pathway activation (18). The beneficial effect of C1INH in inflammatory disease has been assumed to result primarily from its ability to suppress complement-mediated damage. The present study demonstrated that treatment with C1INH resulted in improvement of histopathology scores and in other signs of intestinal damage and inflammation, perhaps providing additional evidence suggesting a central role for the complement system in IBD. However, iC1INH, which is cleaved at its reactive center and, therefore, has no protease inhibitory activity, also exerts effective protection against DSS colitis in C57BL/6 mice. Therefore, C1INH must have suppressed inflammation via some mechanism other than protease inhibition.

Regulation of the contact system via inactivation of coagulation factor XIIa and plasma kallikrein is another important action of C1INH (9). Bradykinin stimulates sensory nerve endings, produces pain, enhances eicosanoid and nitric oxide production by both macrophages and endothelial cells, increases capillary permeability, and contributes to diarrhea by stimulating intestinal electrolyte secretion (3, 8). In addition, bradykinin can stimulate macrophage secretion of interleukin-1 stimulating intestinal electrolyte secretion (3, 8). In addition, via a mechanism that does not involve protease inhibition, it also may play a role in regulation of complement activation but clearly results in marked suppression of neutrophil infiltration. Although IBD is a chronic disease that might not be sufficiently simulated by an acute model, our data suggest that C1INH might be considered as a therapeutic option at least in an acute phase of IBD. Future studies using purified mouse C1INH in chronic colitis models are warranted to further evaluate the potential efficacy of C1INH for human IBD treatment.

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DISCLOSURES
The authors have no financial conflict of interest.

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