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*J Immunol* 2012; 188:6309-6318; Prepublished online 7 May 2012;
doi: 10.4049/jimmunol.1200553
http://www.jimmunol.org/content/188/12/6309

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Complement-dependent injury and protection in a murine model of acute dextran sulfate sodium-induced colitis

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Complement plays a key role in the pathophysiology of many inflammatory diseases, and in this study, we investigated the role of complement in the pathogenesis of inflammatory bowel disease. Compared to wild-type mice, mice deficient in C3 or factor B were protected from acute dextran sulfate sodium (DSS)-induced colitis. C1q/mannose-binding lectin (MBL) double-deficient mice, however, exhibited more severe colitis than wild-type mice. When mice were allowed to recover after DSS treatment, all C1q/MBL−/− mice died by day 2 of recovery period, and, surprisingly, all C3−/− and factor B−/− mice died by day 5. Serum endotoxin levels were significantly increased in complement-deficient mice prior to death, particularly in C1q/MBL−/− mice, and antibiotic treatment prevented the lethal effect of DSS in all complement-deficient mice. In contrast to complement deficiency, targeted complement inhibition with either complement receptor 2 (CR2)-Crry (blocks all pathways at C3 activation) or CR2-factor H (blocks alternative pathway) was highly protective at treating established acute colitis. Endotoxin levels remained low in complement-inhibited mice, and complement inhibition also reduced inflammatory cytokines, leukocyte infiltration, and tissue injury while improving wound repair and mucosal healing. CR2-factor H provided more effective protection than CR2-Crry.

Thus, complement has both pathogenic and protective roles in acute DSS-induced colitis, and whereas the alternative pathway appears to play a key role in tissue inflammation and injury, the classical/lectin pathway provides important protection in terms of host defense and wound repair. Targeted inhibition of the alternative pathway may represent a therapeutic modality for treating acute phases of inflammatory bowel disease. The Journal of Immunology, 2012, 188: 6309–6318.

The etiology of inflammatory bowel disease (IBD) is multifactorial, and contributing factors thought to be involved include genetic predisposition, environmental factors, gut microbiota, and dysregulated inflammatory mediators. The complement system is a key mediator of inflammation, and given the fact that complement plays a central role in the pathophysiology of many inflammatory and autoimmune diseases, there is surprisingly little known about the role of complement in the pathogenesis of IBD. Complement can be activated by one of three different pathways: the classical, lectin, or alternative pathways. Activation by any pathway leads to the generation of complement activation products that can promote inflammation and cause tissue injury by direct and indirect mechanisms (e.g., direct cell lysis, modulation of cytokine expression, and immune cell recruitment). Complement activation products also play important roles in shaping adaptive immune responses. However, although complement plays a role in the pathophysiology of many disease conditions when inappropriately or excessively activated, complement has important physiological roles in host defense, immune modulation, immune homeostasis (e.g., clearance of apoptotic cells and immune complexes), and tissue repair and regeneration.

In early studies using clinical specimens, complement activation was shown to be associated with Crohn’s disease and ulcerative colitis (1–6). The expression of cell-surface complement-inhibitory proteins has also been shown to be altered in gut epithelium of patients with IBD (7–9). More recent studies in rodent models of colitis have made a stronger case for a pathogenic role of complement, at least in experimental IBD. Mice deficient in the complement inhibitor decay-accelerating factor have decreased protection from complement activity and increased susceptibility to dextran sulfate sodium (DSS)-induced colitis (10). Deficiency of C3, the central component of the complement cascade, has also been reported to be protective against DSS-induced colitis (11). A role for the complement activation product C5a in acute trinitrobenzene sulfonic acid-induced colitis has been implicated by the protective effect of a C5a receptor (C5aR) antagonist (12) and anti-C5a Ab (13). C5aR deficiency is also protective against acute DSS-induced colitis, although interestingly, C5aR deficiency aggravated disease in a chronic model (13). Finally, in an apparent contradiction to the above reports, C5-deficient mice were shown to have increased susceptibility to acute DSS-induced colitis (14).

Complement can be activated via three different pathways, namely the classical, lectin, and alternative pathways. Classical pathway activation is traditionally thought of as being Ab dependent and is initiated when the recognition molecule C1q binds to immune complexes. However, Clq can also bind directly to bacterial surfaces, as well as apoptotic and injured cells. The lectin pathway recognition molecule mannose-binding lectin (MBL) recognizes conserved carbohydrate structures and molecular patterns present on many pathogens and, like Clq, also recognizes...
apoptotic and injured cells. In addition to their complement-activating function, C1q and MBL also have direct opsonic functions. Both classical and lectin pathway activation proceeds through the classical pathway C3 convertase. The alternative pathway is activated by spontaneous hydrolysis of C3 to a cleavage product (C3b analog) that binds factor B (B), leading to formation of the alternative pathway C3 convertase. Alternative pathway activation can proceed on any surface that lacks appropriate control molecules, and the alternative pathway also provides an amplification loop for the classical and lectin pathways. All pathways converge at C3 activation with the subsequent cleavage of C5. During this process, the anaphylatoxins C3a and C5a are generated, and C5 cleavage initiates formation of the membrane attack complex (MAC). The role played by different complement activation products (opsonins, C3a/C5a peptides, and the MAC) in mediating various disease processes has been extensively studied, although in many cases how the different activation pathways contribute to disease is less well understood. Nevertheless, it has become clear that in most disease processes, the alternative pathway plays a key role in driving inflammation and propagating injury (15).

In this study, we investigate the role of complement and the contributions of alternative versus classical/lectin pathway activation in acute DSS-induced intestinal inflammation. For these studies, we used mice deficient in C3 (blocked in all pathways), deficient in B (blocked in the alternative pathway), or deficient in both C1q and MBL (blocked in the classical and lectin pathways).

We also use a therapeutic paradigm to investigate the role of complement and alternative pathway activation in a more clinically relevant setting by treating mice with the fusion proteins complement receptor 2 (CR2)-factor (inhibits all complement pathways at C3 activation step) (16) or CR2-factor H (fH) (specifically inhibits the alternative complement pathway) (17). These inhibitors were developed in our laboratory, and they target to sites of complement activation via the CR2 moiety that binds to deposited C3 activation products (18). Of relevance to the current study, we have shown previously that the targeting of a complement inhibitor by CR2 not only markedly enhances its inhibitory and anti-inflammatory efficacy, but also that, unlike systemic inhibition with an untargeted inhibitor, does not impair host defense against experimental polymicrobial sepsis (16).

**Materials and Methods**

**Preparation and purification of CR2-fH and CR2-Crry**

The recombinant proteins CR2-fH and CR2-Crry were produced and purified as previously described (16, 17). Briefly, the proteins were expressed in Chinese hamster ovary cells that were stably transfected with plasmids encoding either mouse CR2-fH or CR2-Crry. The proteins were purified from culture supernatants by anti-mouse CR2 (mAb 7G6) affinity chromatography.

**Mice**

The wild-type (wt) C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C3−/− and B−/− mice on the C57BL/6 background were obtained from an in-house breeding colony. A breeding pair of double-deficient C1q−/− MBL−/− C3−/− mice on the C57BL/6 background (referred to as C1q/MBL−/−) were kindly provided by Dr. Kazue Takahashi (Massachusetts General Hospital for Children, Boston, MA) and bred in house. All animals were used between 8 and 10 wk old. Animals were maintained under standard laboratory conditions, and all animal procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee, in accordance with the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

**Induction of DSS colitis and complement inhibitor treatment**

Acute colitis was induced by oral administration of DSS (MP Biomedical, Solon, OH) at 5% (w/v) in drinking water ad libitum for 5 d. In the acute therapeutic model, following 5 d treatment with 5% DSS, the mice were allowed to rest for 7 d while receiving normal drinking water. Control mice received regular drinking water. Differences in DSS susceptibility did not correlate with differences in the consumption of DSS-supplemented water, and both wt and complement-deficient mice consumed an average of 3.5 ml DSS-supplemented water per mouse per day. Mice were checked each day for morbidity, and their weight was recorded. Upon sacrifice (at day 5, 7, or 12, depending on study), the colon was dissected and colon length measured. Induction of colitis was determined by weight loss and length of colon. The wt mice with established acute DSS-induced colitis were treated with either CR2-H or CR2-Crry in a therapeutic protocol. Mice received a 0.25 mg i.p. injection of either inhibitor every 48 h beginning upon replacement of DSS with normal drinking water on day 5. Control groups received a saline (vehicle) injection. Mice were monitored for 7 d following cessation of DSS treatment (total 12 d). The dose and frequency were based on prior pharmacokinetic and efficacy experiments (16, 17).

**Histology and immunohistochemistry**

For histological analysis, formalin-fixed sections of colon from control and DSS-treated animals were stained with H&E. Sections were assessed in a blinded fashion for evidence of epithelial damage and inflammatory cell infiltration using a modified (cumulative) score scale based on a previously described scoring system (19). Three parameters were determined, namely: 1) severity of inflammation (0, none; 1, slight; 2, moderate; and 3, severe); 2) depth of injury (0, none; 1, mucosal; 2, mucosal and submucosal; and 3, transmural); and 3) crypt damage (0, none; 1, basal one-third damaged; 2, basal two-thirds damaged; 3, only surface epithelium intact; and 4, complete loss of crypt and epithelium). The maximum possible score was 10. Immunohistochemical analysis was used to detect deposit C3d (anti-C3d mAb; DakoCytomation, Capiteria, CA), neutrophils (anti-GR1 mAb; BD Biosciences, San Diego, CA), and macrophages (anti-F4/80 mAb; BD Biosciences). Cell proliferation was assessed by immunohistochemical analysis of Ki-67 (DakoCytomation). All analyses were performed on paraffin-processed sections with sections receiving microwave Ag retrieval in citrate buffer. All Abs were visualized with a standard avidin-biotin detection system (Vector Laboratories, Burlingame, CA). Neutrophils and macrophage numbers were quantified in five random high-power fields of each colon section and quantified by two independent investigators. Cell proliferation in colon epithelium was assessed in colon sections by immunohistochemical detection of Ki-67 as described (20). Assessments were made after 5 d DSS treatment and 2 d rest (normal drinking water), and data are expressed as percent Ki-67-positive cells per crypt.

**Myeloperoxidase activity**

The activity of the enzyme myeloperoxidase (MPO) was used to further assess neutrophil infiltration as previously described (21). Briefly, colons were homogenized in lysis buffer plus protease inhibitors (Roche, Indianapolis, IN), and the extracted protein was measured for MPO levels using a mouse MPO ELISA kit according to the manufacturer’s instructions (Hyclut, Plymouth Meeting, PA).

**Cytokine assays**

Levels of TNF-α, IFN-γ, IL-10, IL-12 p70, and IL-17 were measured in homogenized colon samples. Colonos were homogenized, and protein was extracted from homogenized tissue as previously described (21). Cytokine levels were measured in extracted samples using ELISA kits according to the manufacturer’s instructions (BD Biosciences).

**Endotoxin measurements and antibiotic treatment**

Endotoxin levels were detected in serum after 5 d DSS treatment and 2 d rest (normal drinking water) using the endosafe-PTS handheld spectrophotometer according to the manufacturer’s instructions (Charles River Laboratories, Wilmington, MA). Contamination control was the supplied Reagent Water in tubes used for serum collection and sample dilution. For antibiotic treatment, mice received drinking water supplemented with broad-spectrum antibiotics (ciprofloxacin 200 mg/l and metronidazole 500 mg/l) for 7 d after 5 d of DSS treatment.

**Intestinal permeability**

Intestinal permeability was assessed following luminal enteral administration of FITC-dextran 4000 (Sigma-Aldrich, St. Louis, MO). Mice were gavaged with FITC-Dextran (40 mg/100 g body weight) 4 h before sacrifice. Whole blood was obtained by cardiac puncture at the time of death, and
FTC-dextran measurements were performed in triplicate by fluorescence. Dilutions of FTC-dextran in PBS were used as a standard curve, and adsorption of 100 µl serum or standard was measured in a plate reader at 488 nm.

Statistical analysis

All data are presented as mean ± SD. All data were subjected to statistical analysis using Prism Software version 4 (GraphPad, San Diego, CA). Parametrical analysis was done using one-way ANOVA with Bonferroni’s comparison between groups. Nonparametrical analysis between groups (histology scoring) was done using the Mann–Whitney U test. A p value <0.05 was considered significant.

Results

Complement activation in DSS-induced colitis

To validate the model for these studies, we first confirmed that complement was activated in the colon of C57BL/6 mice following treatment with 5% DSS. Five days after DSS treatment, marked deposition of the complement activation product C3d was detected on the colon mucosa of DSS-treated, but not untreated, mice (Fig. 1).

The role of complement in clinical disease activity and colon injury after acute DSS treatment

To determine how different complement activation pathways are involved in the development of acute DSS-induced colitis, we investigated the effect of different complement deficiencies on clinical disease activity, colon injury, and inflammation on day 5 after 5% DSS treatment. We used mice deficient in C1q/MBL (blocked in classical and lectin pathways of activation), fB (blocked in the alternative pathway of activation), and C3 (blocked in all pathways at C3 activation step).

Following DSS treatment, both fB−/− and C3−/− mice had a significantly improved outcome compared with wt mice in terms of weight loss and colon length (Table I). The outcome in fB−/− and C3−/− mice was also improved in terms of stool consistency and the absence of fecal blood. In contrast, C1q/MBL−/− mice had a worse outcome compared with wt mice following DSS treatment, with significantly increased weight loss and significantly shorter colons (Table I).

Acute DSS-induced colitis is histopathologically characterized by crypt loss, epithelial ulceration, and infiltration of inflammatory cells into the bowel wall. The most severely affected segment of the bowel is the distal colon, and multiple sections of the distal colon from DSS treated wt and complement-deficient mice were stained with H&E for histological assessment of injury and inflammation. Compared to wt and C1q/MBL−/− mice, there was reduced DSS-induced focal loss of crypts and reduced colonic infiltration of inflammatory cells in fB−/− and C3−/− mice (Fig. 2). Histological scores were significantly improved in fB−/− and C3−/− mice and significantly worse in C1q/MBL−/− mice following DSS treatment (Fig. 2).

The role of complement in modulating the inflammatory response after acute DSS treatment

Prolongatory mediators play a central role in the pathogenesis of IBD, and we investigated how different complement activation pathways impact inflammatory cell infiltration and the production of various pro- and anti-inflammatory cytokines in the mucosa.

The complement-activation products C3a and C5a recruit immune cells to the sites of inflammation, and influx of macrophages and neutrophils is known to play an important role in epithelial injury associated with the DSS model. Once these cells enter the tissue, they produce reactive oxygen metabolites and release proinflammatory cytokines. As a measure of neutrophil accumulation, MPO levels were measured in homogenized colon samples. Following 5 d of DSS treatment, MPO levels were significantly reduced in colon samples from fB−/− and C3−/− mice, but significantly increased in C1q/MBL−/− mice compared with DSS-treated wt mice (Fig. 3A). Immunohistochemical analysis of colon sections for neutrophils (GR1) and macrophages (F4/80) concurred with the MPO data with regard to relative profiles of infiltrating leukocytes in the different types of mice (Fig. 3B).

As previously documented, we found that acute DSS-induced colitis in wt C57BL/6 mice was associated with a characteristic Th1/Th17 response, marked by increased colonic levels of IFN-γ and IL-17, as well as the inflammatory cytokines TNF-α and IL-12. Compared to wt mice, the level of all four inflammatory cytokines was significantly reduced in fB−/− and C3−/− mice after DSS treatment (Fig. 4). In contrast, and consistent with the above data, C1q/MBL deficiency resulted in higher levels of the inflammatory cytokines compared with wt mice (except for IL-17). Furthermore, levels of IL-10, a protective cytokine in this model, were significantly increased in fB−/− and C3−/− mice compared with wt and C1q/MBL−/− mice.

Taken together, the above data indicate that the alternative pathway of complement activation plays a key role in the pathogenesis of acute DSS-induced colitis, whereas the classical and/or lectin pathway appears to serve an important protective role.

A therapeutic paradigm: the effect of targeted complement inhibition on acute DSS-induced colitis

To investigate the above findings in a more clinically relevant setting, we performed a therapeutic study using two types of targeted complement inhibitor to treat acute established colitis. Acute colitis persists for up to several weeks in C57BL/6 mice after cessation of DSS treatment, and we treated mice with either CR2-IIH (alternative pathway inhibitor) or CR2-Cry (inhibits all pathways at C3 activation step) every 48 h during a 7-d rest period following 5 d of DSS treatment. We also included groups of

FIGURE 1. Complement activation in acute DSS-induced colitis. Immunofluorescence staining for C3d (indicated by brown staining) on colon sections from untreated mice (Sham Control) and from mice treated with DSS for 5 d. Representative image, n = 3.
complement-deficient mice (not treated with complement inhibitors) in this 12-d study.

Unexpectedly, all complement-deficient mice died during the 7-d rest period after DSS treatment, and average survival of C1q/MBL\(^{-/-}\) mice was significantly shorter than fB\(^{-/-}\) (\(p, 0.01\)) and C3\(^{-/-}\) mice (\(p, 0.05\)) (Fig. 5). In contrast, all wt mice treated with either CR2-fH or CR2-Crry survived through day 7 after DSS treatment, at which time the mice were sacrificed. Compared to PBS-treated mice, CR2-Crry– and CR2-fH–treated mice had a significantly improved outcome in terms of weight loss and colon length (Table II). Both complement inhibitors also improved outcome in terms of stool consistency and the absence of fecal blood (Table II).

Furthermore, compared with control PBS treatment, CR2-fH and CR2-Crry treatment significantly reduced histological colon injury score (Fig. 6A), neutrophil infiltration (MPO; Fig. 6B), and the level of various inflammatory cytokines in colon homogenates (Fig. 7). Levels of the protective cytokine, IL-10, were also significantly increased in CR2-fH–treated mice (Fig. 7). Except for IL-10 levels, there were not significant differences between the effect of CR2-fH and CR2-Crry on other parameters of injury and inflammation, although there was a strong trend toward improved outcome with CR2-fH treatment compared with CR2-Crry treatment for some measurements (see figure legends for \(p\) values).

A protective role for complement in DSS-induced colitis

The acute 5-d DSS model is a useful model to study the contribution of innate immune mechanisms to IBD, and data presented above indicate an important role for the alternative pathway of complement in DSS-induced colitis.
complement in DSS-induced inflammation and injury. However, the above data obtained from the 12-d study with complement-deficient mice indicate that over a longer period, complement also has a critical protective role after the induction of colitis. With regard to why complement inhibition results in an opposite effect compared with complement deficiency, we have previously shown that targeted complement inhibition is protective against intestine ischemia and reperfusion injury, but unlike systemic complement inhibition (16) or deficiency (22), it does not increase susceptibility to experimental polymicrobial sepsis (16). We therefore investigated whether the apparent dual role of complement in the pathogenesis of DSS-induced colitis may be due to a balance between the inflammatory and the host-defensive functions of complement.

We first determined serum endotoxin levels after 5 d of DSS treatment and 2 d of rest (day 7). Endotoxin levels were significantly higher in all complement-deficient DSS-treated mice compared with wt DSS-treated mice, and, in correlation with survival data, endotoxin levels were significantly higher in C1q/MBL−/− mice compared with fB−/− and C3−/− mice. In contrast, endotoxin levels in mice treated with either CR2-fH or CR2-Crry were not different to levels in wt mice (Fig. 8). We next administered broad-spectrum antibiotics to mice during the 7-d rest period after DSS treatment. Antibiotic treatment rescued all complement-deficient mice, with 100% survival on day 12 with antibiotic treatment versus 0% survival without antibiotic treatment (Fig. 9A). Furthermore, antibiotic treatment of complement-deficient mice resulted in significant weight gains compared wt untreated mice, as well as increased colon lengths and reduced colon injury and inflammation (Fig. 9B–D). Of note, antibiotic treatment alone of wt mice did not reduce the severity of DSS-induced colitis.

In addition to the known role of complement in host-defense mechanisms, there is a growing appreciation for the role of complement regenerative and repair mechanisms, particularly in terms of liver regeneration and neuroprotective/regenerative mechanisms. And because mucosal healing and epithelial regeneration have emerged as important treatment goals for patients
with IBD, we additionally investigated how complement may be modulating mucosal healing and wound repair in the intestine by measuring epithelial cell proliferation and intestinal permeability after 5 d DSS treatment and 2 d rest (day 7). Cell proliferation was measured by immunohistochemical detection of Ki-67-positive cells, and on day 7, there was a significant decrease in epithelial cell proliferation in fb- and C3-deficient mice and in surviving C1q/MBL-deficient mice, but not in inhibitor-treated mice, compared with wt controls (Fig. 10A). We also determined how complement deficiency influenced intestinal permeability during the recovery period after DSS treatment by measuring plasma fluorescence after a single oral administration of FITC-dextran on day 7 (5 d DSS treatment and 2 d rest). Compared to wt DSS-treated mice, both C3−/− and fb−/− DSS-treated mice exhibited a significant increase in mean fluorescent plasma FITC-dextran concentrations, indicating increased intestinal permeability to macromolecules and impaired mucosal healing compared with wt mice. In addition, there was no evidence of increased intestinal permeability in wt DSS-treated mice that received either CR2-fH or CR2-Crry (Fig. 10B).

Discussion

Administration of DSS in drinking water induces a reversible form of colitis in mice. The model is characterized by acute tissue inflammation in the colon, and the adaptive immune system is not required for development of this self-limiting colitis. In this study, we focused on the role of complement in the innate immune response during the acute phase of DSS-induced colitis and show that the complement system plays an important role in the balance of the immune system in the gut.

The alternative pathway has been shown to play a key role in mediating inflammation and injury in multiple models of disease, including models of intestinal inflammation such as intestinal ischemia/reperfusion injury and hemorrhage (17, 23, 24). As such, the protective effect of C3 or fb deficiency after 5 d of DSS treatment was perhaps not surprising, and our data indicate that

\[ \text{TNF-alpha} \]

\[ \text{IFN-gamma} \]

\[ \text{IL-12} \]

\[ \text{IL-17} \]

\[ \text{IL-10} \]
the alternative pathway of complement plays a key role in acute DSS-induced colitis. We did not expect, however, that C1q/MBL deficiency would exacerbate inflammation and injury following 5 d of DSS treatment. This is because in most models of inflammation in which complement activation pathways have been studied, an intact classical and/or lectin pathway, in addition to the alternative pathway, has been shown to also be important for expression of inflammation and injury. It has been generally inferred from these studies that complement activation is initiated by the classical and/or lectin pathway and that alternative pathway amplification is necessary for driving inflammation. In contrast to the studies in other models of inflammation, however, we show in this study that C1q and/or MBL, recognition molecules that initiate the classical and lectin pathways, respectively, have a protective function in severe acute DSS-induced colitis. Serum endotoxin measurements, together with the protective effect of antibiotic treatment in C1q/MBL−/− mice in the acute DSS model, suggest that C1q and/or MBL control inflammation by controlling infection when bacteria cross the damaged intestinal epithelium. Thus, complement activation via the classical pathway C3 convertase, or direct bacterial opsonization with C1q and/or MBL, appear to be important mechanisms for limiting the inflammatory effects of microbes crossing the disrupted epithelium. The protective effect of both C3 and fB deficiency on acute DSS-induced colitis (5-d model) indicates a less important role for the alternative pathway in host defense during the early stages of acute intestinal inflammation, although it is important for promoting tissue injury.

Perhaps more surprising than the effect of C1q/MBL deficiency on exacerbating acute DSS-induced colitis in the 5-d model was the death of all complement-deficient mice during the rest period after cessation of DSS treatment in the 12-d model (5 d of DSS treatment followed by 7 d of rest). Analysis of serum isolated shortly before death of DSS-treated mice revealed elevated levels of endotoxin in C3−/− and fB−/− mice compared with wt mice. In addition, antibiotics rescued C3−/− and fB−/− mice, as well as C1q/MBL−/− mice, from the lethal effects of DSS treatment in the 12-d model. We interpret these data as indicating that although intestinal inflammation is initially reduced in C3−/− and fB−/− mice after DSS administration, ongoing mucosal damage leads to access of increasing numbers of commensal organisms to the circulation and that under these circumstances, a fully functional complement system is necessary to control effects of epithelial translocation of microbes and the associated intestinal inflammation. Thus, in acute DSS-induced colitis, there appears to be a balance between complement-dependent tissue injury and complement-dependent host defense, with different relative contributions of the complement pathways to these processes.

Complement may also have a protective role in colitis via epithelial repair mechanisms. Complement is known to play a role in certain regenerative and repair mechanisms, and complete epi-
or the MAC to both pathogenic and protective mechanisms will require further study.

C3 deficiency has been shown previously to be protective against acute DSS-induced colitis (11). In this previous study, mice were given DSS in their drinking water for 10 d, after which C3<sup>−/−</sup> mice displayed reduced inflammation and injury compared with wt mice. This appears to conflict with our data because in the current study, all C3<sup>−/−</sup> mice died by day 10, 5 d after cessation of DSS treatment. This apparent discrepancy is likely related to differences in severity of disease because we used a model of more severe colitis (5% DSS in drinking water versus 3.5%). Also of interest, the same report described amelioration of DSS-induced colitis by C1 inhibitor. Because C1 inhibitor blocks both the classical and lectin pathways, this again would appear to be contradictory to the current data that show exacerbated disease in C1g/MBL<sup>−/−</sup> mice. However, it was further shown that C1 inhibitor protected against colitis via a mechanism that does not involve inhibition of complement (11).

Most previous studies in animal models have indicated that complement inhibition may have therapeutic potential for the treatment of acute IBD. However, the current data obtained using complement-deficient mice suggest that complement inhibition should be approached with caution, not least because it plays an important role in controlling infection. We nevertheless investigated a complement inhibitory strategy for the treatment of established acute colitis. We characterized two targeted complement inhibitors, CR2-Crry and CR2-fH. The CR2 moiety of these fusion proteins targets the complement inhibitor to sites of complement activation and C3d deposition. CR2-Crry inhibits all complement pathways at the C3 activation step, and relevant to the current study, we have shown previously that CR2-Crry effectively ameliorates intestinal inflammation and injury after ischemia and reperfusion, without affecting susceptibility to polymicrobial sepsis (16). In contrast, untargeted Crry results in systemic complement inhibition, and, whereas it also ameliorates intestinal ischemia reperfusion injury (albeit much less effectively than CR2-Crry), it also significantly increases host susceptibility to acute polymicrobial sepsis. In short, the CR2-mediated targeting of Crry improves bioavailability, significantly enhances efficacy, does not systemically inhibit complement, and maintains host resistance to infection. Our data using complement-deficient mice indicated a key role for the alternative pathway in mucosal inflammation and injury after DSS administration, and we therefore also characterized the more recently developed inhibitor CR2-fH, a targeted inhibitor specific for the alternative pathway. This inhibitor will leave the classical and lectin pathways intact, pathways that the current data indicate are particularly important for controlling bacterial infection in the early stages of acute intestinal inflammation. We found that both CR2-Crry and CR2-fH effectively reduced clinical symptoms, mucosal inflammation, and injury in a therapeutic protocol that treated established acute colitis. By all measures, CR2-fH was more effective than CR2-Crry at ameliorating disease, although differences were not always significant. We assume that this is due to specifically targeting the pathway that is central for driving tissue inflammation and injury in this model. In this respect, although CR2 targeting reduces the systemic effects of complement inhibition, CR2-Crry will also inhibit C3 activation via the classical and lectin pathways, which appear to have a protective role. Of note, a human counterpart of CR2-fH (TT30) is currently in phase 1 clinical trials for patients with nocturnal paroxysmal hemoglobinuria (http://clinicaltrials.gov/ct2/results?term=tt30).

With regard to potential therapeutic application, it is significant that targeted inhibition of complement does not appear to have
a significant effect on host-defensive mechanisms. In this context, host immune response to intestinal microbiota, as well as to infections, are implicated in the onset and relapse of IBD. Also the disease itself (and current treatments) can predispose patients to some infections, and IBD patients have an increased risk of death caused by infection. Furthermore, an important immunologic feature in IBD is not a lack of tolerance to autoantigens per se, but a disturbed tolerance to luminal Ags. These Ags are mostly derived from the intestinal microflora, which could be altered by treatments affecting host immunity to infection, as well as by antibiotic treatment. Although tolerance generally refers to an adaptive immune response, pathologic activation of innate immune mechanisms may favor a break of tolerance.

The exacerbated disease seen in C1q/MBL−/− mice appears due at least in part, to commensal microorganisms gaining access to the systemic circulation as a result of impaired host defense, leading to a breakdown of tolerance. Of note, there is clinical and experimental evidence to support the role of MBL in limiting pathogen-induced mucosal damage. Although not without controversy, there are reports that MBL deficiency or low MBL levels are associated with IBD, at least in certain populations (26–28). It has also been shown that in a DSS-induced colitis model, MBL deficiency results in exacerbated colitis in response to normally mild gut pathogens (29). The classical pathway is also an important one for controlling bacteria infections, and C1q plays an important role in IgG-independent opsonization of bacteria. The classical pathway has also been shown to be required for effective antimicrobial defense against experimental polymicrobial sepsis (30), and although the alternative pathway also plays a role in host response against experimental sepsis, there appear to be distinct and different contributions of the classical and alternative pathways (31). Another consideration is that C1q and MBL are also involved in the opsonization and phagocytic uptake of apoptotic and injured cells. Apoptosis is a feature of injury in IBD, and macrophage uptake of apoptotic cells generally results in production of anti-inflamatory cytokines. Failure to clear apoptotic cells can lead to secondary necrosis and inflammation, and uptake of necrotic cells can induce macrophages to release cytokine danger signals. The role of complement in apoptotic cell clearance and wound healing in the gut is a current area of investigation in our laboratory.

In conclusion, we present evidence for a dual role of complement in the pathogenesis of acute DSS-induced colitis, with a key role for the alternative pathway in driving colon inflammation and tissue damage. The focus of the current study was acute colitis, and our data suggest that targeted complement inhibitors, particularly of the alternative pathway, may be of therapeutic benefit in acute phases of IBD. Nevertheless, DSS-induced colitis is only one of several models of IBD, and further studies are also needed to address the role of complement in chronic disease to better understand the potential benefits of complement inhibition for treatment of this condition.

Acknowledgments

We thank Emily Pauling for histology work and expert technical assistance.

Disclosures

S.T. received royalty payments for licensed patents of CR2-targeted complement inhibitors. The other authors have no financial conflicts of interest.

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