Neutralization of complement component C5 ameliorates the development of dextran sulfate sodium (DSS)-colitis in mice

Tomoki Aomatsu, 1, 2 Hirotsugu Imaeda, 1 Kenichiro Takahashi, 1 Takehide Fujimoto, 2 Eiji Kasumi, 1 Hiromitsu Ban, 1 Shigeki Bamba, 1 Atsushi Yoden, 2 Hiroshi Tamai, 2 Yoshihide Fujiyama 1 and Akira Andoh 1, 2*

1 Department of Medicine and 2 Division of Mucosal Immunology, Graduate School, Shiga University of Medical Science, Seta Tsukinowa, Otsu 520-2192, Japan

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The complement system is a potent effector of innate immunity. To elucidate the pathophysiological role of the complement system in inflammatory bowel disease, we evaluated the effects of anti-C5 antibodies on the development of dextran sulfate sodium-induced colitis in mice. Dextran sulfate sodium-colitis was induced in BALB/c mice with intraperitoneal administrations of anti-C5 antibodies (1 μg/body) every 48 h. Tissue samples were evaluated by standard histological procedures. The mucosal mRNA expression of the inflammatory cytokines was analyzed by real-time PCR. Body weight loss in the mice was completely blocked by the administration of anti-C5 antibody. The disease activity index was significantly lower in the anti-C5 antibody-treated mice than the dextran sulfate sodium mice. The colonic weight/length ratio, histological colitis score and mucosal myeloperoxidase activity were significantly lower in the anti-C5 antibody-treated mice than the dextran sulfate sodium mice. The administration of the anti-C5 antibody significantly reduced the mucosal expression of mRNAs for tumor necrosis factor-α, interleukin-1β and interleukin-6. In conclusion, the complement system plays a role in the development of dextran sulfate sodium-induced experimental colitis.

Key Words: inflammatory bowel disease, immunology, DAF

The complement system is part of innate immunity and is one of the major pathogenic event that drives various inflammatory responses in numerous diseases. 1, 2 The complement system consists of three major pathways: the classical, mannose-binding lectin, and alternative pathways. All pathways of complement activation lead to cleavage of the C5 molecule generating C5a and MAC. 3 C5a exerts a predominantly pro-inflammatory activity through interactions with the classical G-protein coupled receptor C5aR (CD88), which is expressed on various immune and non-immune cells. 1 C5b-9 causes cytolysis through the formation of the membrane attack complex (MAC), and sub-lytic MAC and soluble C5b-9 also possess a multitude of non-cytolytic immune functions. These two complement effectors, C5a and C5b-9, both generated from C5 cleavage, are key components of the complement system responsible for propagating and/or initiating pathology in different diseases, including paroxysmal nocturnal hemoglobinuria (PNH), rheumatoid arthritis, ischemia–reperfusion injuries and neurodegenerative diseases.

We have previously demonstrated that intestinal epithelial cells are the local sites of complement biosynthesis. 4, 5 Complement components are also supplied into the gut lumen from the exocrine pancreas and bile system. 6, 7 Furthermore, previous studies have reported the deposition of complement components at the inflamed mucosa of inflammatory bowel disease (IBD) patients, suggesting that complement-mediated tissue injury is involved in the pathophysiology of IBD. 8, 9 The regulatory mechanisms of complement activation are finely balanced, since overactivation of the complement system leads to tissue injury. The regulation of complement activation is mediated by complement-activation regulatory proteins such as decay-accelerating factor (DAF or CD55), membrane cofactor protein (MCP or CD46), and CD59. 10 DAF prevents the assembly of C3 convertases (C4b2a and C3bBb), and also dissociates deposited-C3 convertases. 10 Lin et al. 11 reported that dextran sulfate sodium (DSS)-induced experimental colitis was exacerbated in DAF-knock out mice, indicating that complement activation plays an important role in the development of DSS-colitis.

In this study, to investigate the role of the complement system, we evaluated the effects of neutralizing anti-C5 antibodies on the development of DSS-colitis in mice. Since C5 is a central component of the complement cascade, complement activation leading to the formation of C5a and MAC does not occur in this model.

Materials and Methods

Induction of colitis. Six to eight week-old male BALB/c mice were purchased from Charles River Japan (Kanagawa, Japan). They were acclimatized for one week before the experiment, and were housed individually in a room maintained at 22°C under a 12-h day/night cycle throughout the experiments. Experimental colitis was induced by oral administration of DSS (molecular weight 5000; Wako Pure Chem. Ind. Ltd., Osaka, Japan). Half of the mice were randomized to receive 3.5% (w/v) DSS in their drinking water for 14 days, and the other half of the mice were given regular drinking water. Neutralizing anti-C5 monoclonal antibodies (Eculizumab; Alexion Pharmaceuticals, Inc., Cheshire, CT) (1 μg/body) were administered intraperitoneally every 48 h for the duration of the experiment. The mice were weighed every other day, and were inspected visually for any sign of sickness. Immediately before sacrifice, the stool was tested for blood by the guaiac test. On day 14, all of the mice were sacrificed and their colons were collected for histological analysis and a myeloperoxidase activity assay. This study protocol was approved by the Animal Care and Use Committee of the Shiga University of Medical Science (Otsu, Japan).

*To whom correspondence should be addressed.
E-mail: andoh@belle.shiga-med.ac.jp
Assessment of inflammation in DSS-induced colitis.

Daily clinical assessment of the DSS-induced colitis were performed, including a measurement of food intake and body weight, an evaluation of stool consistency, and the presence of blood in the stools by the guaiac paper test. The stool consistency was assessed using the following four point-scale: 0, normal; 1, soft; 2, very soft but formed; and 3, liquid. The intensity of the guaiac paper test was scored by the following scale: 0, negative; 1, faintly blue; 2, moderately blue; 3, dark blue; and 4, blood visible. A validated clinical disease activity index ranging from 0 to 4 was calculated using the following parameters: stool consistency, presence of fecal blood, and changes in body weight.\(^{12}\)

Histology. A histological examination was performed on three samples of the distal colon from each animal. The samples were fixed in 10% buffered formalin, dehydrated in ethanol, and then embedded in paraffin. Five-micrometer-thick sections were then prepared and stained with hematoxylin and eosin. All of the histological evaluations were performed in a blinded fashion using a validated scoring system.\(^{12}\)

Tissue myeloperoxidase activity. The samples were washed with cold PBS, blotted dry, and were immediately thawed for the myeloperoxidase activity determination using the O-dianisidine method previously described.\(^{13}\) The activity was expressed as the amount of enzyme necessary to generate a change in absorbance of 1.0 per min per gram wet weight of the tissue.

Real-time polymerase chain reaction (PCR). The mRNA expression of Notch ligands in the samples was assessed by real-time PCR analysis. The oligonucleotide primers used in this study are described in Table 1. Real-time PCR was performed using a LightCycler 2.0 system (Roche Applied Science, Tokyo, Japan).

| Gene (NCBI ID)          | Orientation | Sequence (5’-3’)                  |
|------------------------|-------------|-----------------------------------|
| Mouse TNF-α (NM_013693.2) | Forward     | ATGAGCACAGAAAGCATGATC             |
|                        | Reverse     | TACAGGCTTTGTCACCTAGAATT            |
| Mouse IL-1β (NM_008361.3) | Forward     | CAGGATGAGGACTGAGCACC              |
|                        | Reverse     | CTCTGCACTCAAACCTCAC                |
| Mouse IL-6 (NM_031168.1)  | Forward     | GACAAACGCAAGTGCTTCAGAGA           |
|                        | Reverse     | CTAGGGGTGCGAATGAGTCTC              |
| β-actin (NM_007317.3)    | Forward     | GTGGGCCGCCCTTTTACCC               |
|                        | Reverse     | CGGTTGGGCTTTCAGGCTGAGG            |

Table 1. PCR primers used in this study

The PCR was performed using a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The data were normalized versus β-actin for each gene.

Statistical analysis. Statistical analyses were performed using the one-way ANOVA with Scheffe’s post hoc test or the Kruskal-Wallis test when appropriate. A two-way ANOVA for repeated measures was used to test for group and time effects on the clinical data (e.g., disease activity index) over 7 successive days of clinical observation. A p value less than 0.05 was considered to be statistically significant.

Results

To evaluate the role of complement activation in DSS-colitis, the administration of an anti-C5 antibody was started at the same time as the DSS exposure. The administration of the mAb was repeated every 48 h. As shown in Fig. 1, on days 6 after the initiation of DSS-induced colitis, a significant decrease in body weight was observed in the DSS mice compared to the DSS plus anti-C5 antibody mice. The administration of anti-C5 antibody significantly reduced the disease activity index, colon length, and colon weight/length ratio (Fig. 2).

Fig. 1. Changes in the body weight of DSS-colitis mice. The mice were fed DSS over 14 days. The weight of each individual mouse was then followed daily. The data represent means ± SD (n = 5 mice/group). *p<0.05; DSS mice vs DSS plus anti-CS antibody mice.

Fig. 2. Comparison of colitis development. The disease activity index, colon length and colon weight/length ratio on day 14 were compared between groups. The data represent means ± SEM (n = 5 mice/group). *p<0.05; **p<0.01.
was observed in the DSS-treated mice as compared with the control mice or the anti-C5 Ab-treated mice. After this time point, the body weight loss progressed in the DSS mice, but was completely blocked by the administration of anti-C5 antibody. The administration of the mAb did not affect the changes in body weight of control mice.

As shown in Fig. 2, the disease activity index was significantly lower in the anti-C5 antibody-treated mice than in the DSS mice. The total colonic length was significantly shorter in the DSS mice than in the anti-C5-treated mice (Fig. 2). The colonic weight/length ratio was significantly lower in the anti-C5 Ab-treated mice than in the DSS mice (Fig. 2), indicating that a blockade of the complement cascade by the anti-C5 antibody inhibited the progression of the edematous changes of the colon in DSS-colitis.

DSS-colitis is characterized by histological findings such as edema, the infiltration of inflammatory cells into both the mucosa and submucosa, ulceration and mucosal thickening. Our histological analysis indicated that the administration of the anti-C5 antibody markedly ameliorated the severity of the colitis as compared to the DSS mice (Fig. 3). The myeloperoxidase activity was significantly elevated in the DSS mice, but was significantly reduced in the anti-C5-treated mice.

To characterize the regulation of various inflammatory genes during the process of DSS-colitis, total RNA was isolated from the colons of the differently-treated mice, and the mRNA expression of cytokines was evaluated by real-time PCR. As shown in Fig. 4, the mice treated with DSS showed an increased expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6. The administration of the anti-C5 antibody significantly reduced the expression of mRNAs for TNF-α, IL-1β and IL-6.

Discussion

The complement system is a potent effector for both normal immune and inflammatory responses. Activation of the complement system also induces tissue injury. Previous studies have demonstrated local complement activation in the inflamed mucosa of IBD patients, suggesting the involvement of complement activation in the pathogenesis of IBD. A previous study on complement activation reported that DSS-induced experimental colitis was much more severe in decay-accelerating factor (DAF) KO mice. DAF is a representative anti-complement protein expressed on the apical surface of intestinal epithelial cells. The changes in DSS colitis-development in DAF KO-mice indicated that protection from complement activation mediated by DAF expression is an important process in the pathogenesis of DSS-colitis. This observation leads one to speculate that a reduction in complement activity might improve the development of colitis.

In this study, we used anti-C5 antibodies, which exert potent inhibitory activity against complement C5. The activation of C5 is a critical step in the complement cascade, and blockade of C5 activation results in no formation of C5a and MAC. In agreement with our initial expectation that a blockade of the complement cascade would lead to an improvement in colitis development, we found that the development of DSS-colitis was markedly ameliorated by the administration of anti-C5 antibodies; the disease activity and histological changes were significantly more severe in the DSS mice as compared to the anti-C5 antibody-treated mice. Furthermore, the administration of anti-C5 mAb induced a significant decrease of mucosal expression of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6. These findings indicated that the complement system might act as a promoting factor in the development of DSS-colitis.
The complement system consists of non-specific anti-bacterial proteins, and these anti-bacterial proteins play a role in the gut lumen. Previous studies have reported that intestinal epithelial cells are the local source of complement components and that these complement components are also supplied into the gut lumen from the exocrine pancreas and bile system. Therefore, blockade of the complement cascade at the C5 step may modulate the composition of the gut microbiota. The gut microbiota are an important factor which can affect colitis development. For example, the development of spontaneous colitis in genetically engineered mice requires the presence of intestinal bacteria, since under germ-free conditions these animals do not develop colitis. Furthermore, recent studies demonstrated that the gut microbiota participate in the development of mucosal regulatory T cells or Th17 cells, which are key components regulating mucosal inflammation. The gut microbiota might affect the balance between beneficial versus pathogenic bacteria, and thus ameliorate the development of colitis. Further investigation into the changes in the gut microbiota is needed in the future.

In conclusion, we observed that the development of DSS colitis is ameliorated in anti-C5 antibodies-treated mice. This is a novel finding for the complement system in gut immunity. Complement activation sometimes attacks the host tissues and causes tissue injury, but some inflammatory responses which lead to mucosal inflammation may also be blocked by the complement system.

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Abbreviations

DSS dextran sulfate sodium
IBD inflammatory bowel disease
IL interleukin
TNF tumor necrosis factor

Conflict of Interest

The authors disclose no conflicts of interest.

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