Decay-Accelerating Factor Deficiency Increases Susceptibility to Dextran Sulfate Sodium-Induced Colitis: Role for Complement in Inflammatory Bowel Disease

Feng Lin, David Spencer, Denise A. Hatala, Alan D. Levine and M. Edward Medof

*J Immunol* 2004; 172:3836-3841; doi: 10.4049/jimmunol.172.6.3836

http://www.jimmunol.org/content/172/6/3836

References

This article cites 63 articles, 28 of which you can access for free at: http://www.jimmunol.org/content/172/6/3836.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Decay-Accelerating Factor Deficiency Increases Susceptibility to Dextran Sulfate Sodium-Induced Colitis: Role for Complement in Inflammatory Bowel Disease

Feng Lin,* David Spencer,‡ Denise A. Hatala,† Alan D. Levine,‡ and M. Edward Medof 2*

Decay-accelerating factor (DAF or CD55) is expressed on colonic epithelial cells but its function in the mucosa is unknown. In humans, a proportion of DAF-deficient (Cromer INAB) patients develop inflammatory bowel disease (IBD). To evaluate how DAF deficiency may contribute to gut inflammation and thus could play a role in IBD pathogenesis, we compared the severity of dextran sulfate sodium-induced colitis in Daf1 gene-targeted and control mice. Seven days after consuming 3% dextran sulfate sodium in their drinking water, Daf1−/− mice suffered markedly greater weight loss (−24.7 ± 7.5% vs −14.2% ± 4.9%), exhibited uniformly bloody diarrhea as compared with soft stool in control mice, developed shortened colons, and had larger spleens. Histological examination of distal colons showed massively increased neutrophilic and mononuclear cell infiltration, greater epithelial cell destruction, and increased ulcerations. Cytokine production in organ cultures of colonic explants showed increased levels of IL-12 and IL-6. Fourteen days after switching back to regular water, in contrast to the Daf1+/− controls which showed little stool abnormality, all Daf1−/− mice continued to have diarrhea. Organ culture cytokine measurements at this time point, i.e., the end of the recovery phase, showed markedly increased levels of IL-10 (6-fold), IL-12 (4-fold), and IL-6 (2-fold), as well as TNF-α (>10-fold) compared with the controls. Our findings argue that, as shown for IL-10 in Il10−/− mice and IL-2 in Il2−/− mice, DAF control of complement additionally is important in regulating gut homeostasis and consequently its activity may participate in protecting against IBD. The Journal of Immunology, 2004, 172: 3836–3841.

Three surface regulatory proteins are present on the surface membranes of self-cells to protect them from autologous complement-mediated injury during activation of the complement cascade (reviewed in Ref. 1). These regulators are the decay-accelerating factor (DAF1 or CD55), the membrane cofactor protein (MCP or CD46), and the membrane inhibitor of reactive lysis (MIRL or CD59). The first two, DAF and MCP, function early in the cascade to dissociate self-cell-bound C3 convertases (C3bBb and C4b2a) (2–4) and promote the cleavage of covalently bound C3b and C4b fragments upon which the convertases assemble (5, 6). Their cooperative activities (7) prevent amplification of C3 activation and subsequent progression of the cascade to the terminal attack sequence. The third regulator, CD59, functions late in the cascade to block the uptake of C9 by C5b-8 and interferes with the subsequent polymerization of C9. Its activity prevents the formation of membrane attack complexes which bring about cell lysis (8–10). Both DAF and CD59 are membrane-linked by posttranslationally added GPI anchors (9, 11). MCP, although linked by a conventional polypeptide anchor, contains only a short cytoplasmic sequence (12). These anchor structures permit the three regulators to move freely within the plasma membranes of self-cells to efficiently inhibit the progression of the attack sequence wherever complement activation is initiated on their surfaces (4, 13).

Both direct and indirect effects of complement activation have been implicated as playing roles in the tissue injury associated with inflammatory diseases of the gastrointestinal (GI) tract (reviewed in Refs. 14 and 15). Among evidence in support of this, C3b and other activation fragments have been detected on intestinal cells in lesions of chronic inflammatory bowel diseases (IBD) in humans (16, 17). Pathologically, polymorphonuclear cell infiltration, usually a result of C5a generation, characteristically is seen (18). Additionally, immunohistochemical analyses of colonic epithelium in patients with IBD have shown that surface levels of DAF are markedly up-regulated (19). The consequence of this up-regulation is unstudied, but it has been hypothesized (19) that the augmented regulatory activity would afford increased protection against heightened complement system activity that may occur in the bowel diseases.

Mice differ from humans in that rather than one, there are two DAF genes, termed Daf1 and Daf2 (20). Mice also differ in that in place of MCP, they express a rodent-specific regulator termed complement receptor-related protein Y (Crry) which not only has cofactor function of MCP but also decay-accelerating function overlapping that of DAF. The mouse Daf1 gene encodes predominantly GPI-anchored DAF protein (20, 21) and is expressed ubiquitously in all tissues (22–24), whereas the Daf2 gene encodes predominantly a transmembrane DAF variant (20, 25) and is significantly expressed only in testis (20, 22, 24) and splenic dendritic cells (24). The Daf1 gene thus has been considered to be the counterpart of the human DAF gene.

In this report, we used dextran sulfate sodium (DSS) to induce colitis in normal and Daf1 knockout mice (24) and compared clinical symptoms, pathological changes, and the production of cytokines by diseased colons. DSS-induced colitis in mice has been
exploited as a model of superficial intestinal inflammation (26–28). In this model, colonic mucosal inflammation, ulceration, weight loss, and bloody diarrhea develop upon addition of the drug to drinking water. Upon administering DSS in an identical fashion to Daf1+/− and Daf1+/+ littermates, we found markedly greater tissue damage and increased proinflammatory cytokine production in the DAF-deficient animals.

Materials and Methods

DAF knockout mice

Daf1 knockout mice were developed as described previously (24). Briefly, the Daf1 gene on one chromosome was knocked out by homologous recombination and Cre/LoxP-mediated deletion in murine GK129 embryonic stem cells. The recombined embryonic stem cells were microinjected into blastocysts, chimeras generated, and the chimeric mice bred with the C57BL/6 strain. Eight- to 12-wk-old knockout mice and their littermates with mixed background (129/C57BL/6) were used. Mixed sexes were used. The mean weight of the Daf1−/− mice was 19.3 g and of the Daf1−/+ mice was 20.6 g. The mice were maintained in the Case Western Reserve University Animal Resource Center and all experiments were performed according to an approved protocol of the Institutional Animal Care and Use Committee.

Colitis induction

On day 1, 3% DSS (Mw ~40 kDa; ICN Pharmaceuticals, Aurora, OH) was added to the drinking water and intake allowed to proceed ad libitum. Mice were weighed every other day and inspected visually for any sign of sickness. Immediately before sacrifice, stool was tested by guaiac test. At day 7, one-half of the mice were sacrificed by carbon dioxide gassing. The other half of the mice were returned to regular drinking water for an additional 14 days to analyze the recovery process. At that time, i.e., 21 days after the start of the experiment, these mice were sacrificed and colonic tissue was harvested as described for mice on day 7.

Organ culture and cytokine measurements

One-half-centimeter segments of both the distal and proximal colon were cultured on permeable membranes in 5 ml of DMEM in six-well tissue culture dishes. Twenty-four hours after ex vivo culture, culture undernourished of fat, mesentery, and blood vessels, samples were chopped into a slurry, transferred to a 50-ml tube containing 15 ml of HBSS, 10% FBS, and 5 mM EDTA, and the tissue pieces pipetted vigorously. The tubes were incubated at 37°C with continued pipetting every 5 min to remove epithelial cells from the lamina propria. Supernatants then were filtered through a 100-μm mesh (Falcon 2360; Costar, Cambridge, MA) and the flow-through containing colonic epithelial cells was used for RNA isolation.

RNA was isolated using a mini-RNeasy kit (Qiagen, Valencia, CA) and reverse-transcribed with Superscript II and oligo(dT) primers (Invitrogen, San Diego, CA). The resulting reverse transcriptase products were used as a template for qPCR on an Applied Biosystems autosensetime detection unit using the SYBR master kit (Applied Biosystems, Foster City, CA). All reactions were performed in duplicate, and the results were analyzed using Applied Biosystems Prism software normalizing against mouse GADPH PCR products.

Results

In pilot experiments, 3% DSS was added to the drinking water of two pairs of Daf1−/− mice and Daf1+/+ littermates. After 7 days, the mice were weighed, scored for bloody diarrhea, sacrificed, and their colons analyzed by H&E staining. As shown in Table I, markedly greater weight loss and much more severe bloody diarrhea developed in the Daf1−/− mice compared with their Daf1+/+ counterparts. Consistent with the clinical differences, as shown in Fig. 1A, massively increased inflammatory cell infiltration into the colon with accompanying epithelial cell destruction was observed.

Based on these results, a large experiment with an expanded number of mice was conducted. Three percent DSS was administered for 7 days to 20 Daf1−/− mice, and their Daf1+/+ littermates as

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight Loss (%)</th>
<th>Stool Consistency</th>
<th>Blood in Colon</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daf1−/+ 1</td>
<td>15.0</td>
<td>Soft</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daf1−/+ 2</td>
<td>17.6</td>
<td>Soft</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daf1−/− 1</td>
<td>23.0</td>
<td>Diarrhea</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Daf1−/− 2</td>
<td>31.4</td>
<td>Diarrhea</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

*p < 0.05, Daf1−/− vs Daf1+/+. Overall severity of disease varied in different studies, but differences between the Daf1+/+ and Daf1−/− groups were consistent in all studies.

**FIGURE 1.** A, H&E staining of rectal sections 7 days after DSS administration. B, H&E staining of rectal sections after 14 days of recovery, i.e., on day 21. Left, Daf1+/+ mice; right, Daf1−/− mice.

Histological and immunohistochemical analyses

For histology, colon segments were fixed overnight in 10% Formalin, embedded in paraffin blocks, and cut into 5-μm sections. Sections were stained with H&E as described previously (29). For immunohistochemical analysis of DAF expression, formaldehyde-fixed sections were treated with 3% H2O2 to block endogenous peroxidases, heated for Ag retrieval, and stained for 1 h at 20°C with a mixture of rat anti-mouse DAF mAbs 2C6 and 3D5 (30). Following washing, sections were further incubated for 30 min at 20°C with biotinylated (mouse adsorbed) rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA). After washing, staining was developed using an ABC kit (Vector Laboratories). For some studies, cryosections were prepared and immunofluorescence analysis was performed with FITC-labeled goat anti-rat Ig Ab (PharMingen, La Jolla, CA). For immunohistochemical analyses of C3b deposition, pieces of colon were Formalin fixed, paraffin embedded, and 5-μm sections again prepared. The sections were sequentially pretreated with 3% H2O2 and with heat-induced epitope retrieval in 10 mM citrate buffer (pH 6.0). They were then blocked with 5% normal goat serum, after which they were stained for 1 h at 20°C with goat anti-mouse C3 IgG (ICN Biochemicals, Costa Mesa, CA) or nonrelevant control IgG (Jackson ImmunoResearch, West Grove, PA) and secondarily stained with biotinylated rabbit anti-goat antiserum (DAKO, Carpentaria, CA) followed by HRP-conjugated streptavidin (DAKO) and diaminobenzidine. Washed slides were mounted and examined for DAF and for C3b deposition using an Olympus OM2 microscope (31).

Colonic epithelial cell isolation and quantitative PCR (qPCR)

Daf1−/− mice and Daf1+/+ controls were fed 3% DSS for 24 h and then sacrificed. Colons were then harvested and flushed with HBSS. After removal of fat, mesentery, and blood vessels, samples were chopped into a
and IL-12 (pflammatio in the distal part of the /H9251 Daf1 p at each time point for cytokine (IL-12, IL-10, IL-6, IFN-
analyses of colons at 7 and 21days, 0.5-cm segments of both the mice back to regular drinking water. In addition to histological
itantly studied by analyzing colons 14 days after switching the above, and in a further arm of the protocol, recovery was concom-
Vlaration and unhealed epithelium in the distal colons of the mice compared with their
0.14 ± 0.05 g (enlarged).
above, and in a further arm of the protocol, recovery was concom-
entially by guest on July 29, 2017 http://www.jimmunol.org/ Downloaded from
3, DAF expression was prominent on the apical surface of gut epithelia in the Daf1+/+ mice. In contrast, staining was markedly reduced in the Daf1−/− mice, although some staining was detectable (see Discussion). As shown in Fig. 4, at this early time point (before leukocyte infiltration), marked C3b deposition was evident on the mucosal surface of the Daf1−/− mice, whereas there was only minimal deposition on the mucosa of the wild-type controls. As shown in Fig. 3, top right, DAF expression on colonic epithelial cells was up-regulated at this early time point. qPCR analyses of isolated colonic epithelium showed a 2-fold increase in Daf1 mRNA and no change in expression of Daf2 mRNA.

### Discussion

In the GI tract, the representation of components of the complement system is limited under normal conditions. During the course of mucosal damage, however, serum complement components can flow into the lumen in massive amounts (reviewed in Ref. 32). In addition, the exocrine pancreas can secrete large amounts of complement into the GI tract (33). At the same time, gut epithelial cells themselves can synthesize and secrete complement locally (34–36). As indicated (Introduction), evidence for the involvement of complement activation has been obtained in different forms of IBD as well as gastritis (19, 37, 38). In all of these diseases, C3b deposition can be detected on luminal epithelial cells.

DAF, as well as the other intrinsic complement regulators MCP and CD59, have been identified in the GI tract (39, 40). In humans, under constitutive conditions, DAF is expressed sporadically on the luminal surface but, as indicated (Introduction), its expression is up-regulated during inflammation (16, 38, 40). Despite these and the above observations, the importance of intrinsic complement regulatory activity in the GI tract has received little study. In this investigation, through the use of a model of GI inflammation in normal and a knockout (24), we were able to focus on DAF, the first of the intrinsic regulators to be characterized (4).

As indicated (Introduction), we used Daf1−/− mice deficient in the expression of GPI-anchored DAF protein ubiquitously and specifically on gut mucosa. Our detection in the present study of a low level of DAF reactivity in the gut of Daf1−/− mice following DSS treatment is consistent with expression of DAF deriving from the Daf2 gene. Previous studies of mouse tissues employing PCR have shown that low levels of Daf2 mRNA are present in the GI tract (20). Our studies of colonic epithelial cells 24 h following DSS administration showed that Daf2 mRNA was up-regulated and the up-regulation derived entirely from the Daf2 gene.

In general, the roles of autologous C3b deposition, consequent release of C3a and C5a anaphylotoxins, and direct complement-mediated injury to colonic cells during intestinal inflammation are

### Table II. Repeat analysis of the effects of DSS on an expanded series of mice at day 7 (representative 5 of 20)

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight Lossa (%)</th>
<th>Consistency</th>
<th>Blood in Colon</th>
<th>Spleenb</th>
<th>Enlargementb,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daf1+/+ 1</td>
<td>7.2</td>
<td>Diarrhea</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1+/+ 2</td>
<td>13.4</td>
<td>Soft</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1+/+ 3</td>
<td>10.5</td>
<td>Soft</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1+/+ 4</td>
<td>5.2</td>
<td>Diarrhea</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1+/+ 5</td>
<td>6.0</td>
<td>Soft</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1−/− 1</td>
<td>15.7</td>
<td>Diarrhea</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Daf1−/− 2</td>
<td>11.1</td>
<td>Diarrhea</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daf1−/− 3</td>
<td>3.9</td>
<td>Diarrhea</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daf1−/− 4</td>
<td>9.6</td>
<td>Diarrhea</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daf1−/− 5</td>
<td>19.0</td>
<td>Diarrhea</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*p < 0.05.

The mean spleen weight in the Daf1+/+ mice was 0.12 ± 0.04 g (nonswollen) and in the Daf1−/− mice was 0.14 ± 0.05 g (enlarged).

### Table III. Analyses of comparative effects of DSS administration in Daf1−/− and Daf1+/+ mice on day 21

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight Lossa (%)</th>
<th>Spleenb</th>
<th>Colon Length (cm)</th>
<th>Stool Consistency</th>
<th>Blood in Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daf1+/+ 1</td>
<td>13.2</td>
<td>Normal</td>
<td>6.0</td>
<td>Soft</td>
<td>Yes</td>
</tr>
<tr>
<td>Daf1+/+ 2</td>
<td>8.0</td>
<td>Normal</td>
<td>8.5</td>
<td>Soft</td>
<td>Yes</td>
</tr>
<tr>
<td>Daf1+/+ 3</td>
<td>19.7</td>
<td>Normal</td>
<td>5.5</td>
<td>Diarrhea</td>
<td>Yes</td>
</tr>
<tr>
<td>Daf1+/+ 4</td>
<td>15.8</td>
<td>Normal</td>
<td>8.5</td>
<td>Soft</td>
<td>No</td>
</tr>
<tr>
<td>Daf1−/− 1</td>
<td>31.3</td>
<td>Small</td>
<td>5.5</td>
<td>Diarrhea</td>
<td>Yes (enlarged)</td>
</tr>
<tr>
<td>Daf1−/− 2</td>
<td>14.0</td>
<td>Enlarged</td>
<td>8.0</td>
<td>Diarrhea</td>
<td>Yes</td>
</tr>
<tr>
<td>Daf1−/− 3</td>
<td>28.0</td>
<td>Normal</td>
<td>7.0</td>
<td>Diarrhea</td>
<td>Yes</td>
</tr>
<tr>
<td>Daf1−/− 4</td>
<td>25.5</td>
<td>Normal</td>
<td>7.5</td>
<td>Diarrhea</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*p < 0.05.

The mean spleen weight in the Daf1+/+ mice was 0.17 ± 0.05 g (nonswollen) and in the Daf1−/− mice was 0.26 ± 0.11 g (enlarged).

X̄ = 14.2 ± 4.9.

X̄ = 24.7 ± 7.5.
poorly understood. In this study, we demonstrate, for the first time, that in the distal colon of DAF-deficient mice, superficial epithelial injury induced by DSS adsorption is enhanced and that the rate of healing in these mice is retarded. The increased tissue damage in the absence of the protection afforded by DAF-dependent C3 convertase regulation is accompanied by elevated expression of proinflammatory cytokines conventionally secreted by macrophages (41) but potentially also produced by epithelial cells (42). The striking polarity of the damage localized primarily in the distal colon may not be entirely explained by the inhibition of complement by DAF protein (Fig. 3) and raises the possibility that in the absence of DAF, other inflammatory mediators in addition to complement may also propagate the host response in the GI tract. Moreover, the retardation of tissue healing in the absence of DAF raises the possibility that in addition to the generation of the cytokines measured, the elaboration of other components of intestinal metabolism associated with epithelial growth or regeneration (43) could in principle be linked with complement activation. Taken together the results underscore an importance of complement system activity in the initiation, propagation, and repair of intestinal damage in IBDs.

Complement activation fragments have been shown to induce cytokines not only from colonic cells but from a wide range of other cell types. Examples are that C5a anaphylatoxin can induce the release of TNF-α, IL-6, and IL-1 from inflamed colonic tissue (44–46) and the generation of IL-8, IL-6, TNF-α, IL-10, and IL-1 from retinal pigment epithelial cells (47). Moreover, recent studies have shown that C5a receptor antagonists protect against disease in a rat model of IBD (48).

Our analysis of cytokine production by colonic explants showed that the predominant cytokines elevated 7 days after DSS treatment in Daf1+/- mice were IL-10 and IL-6, both of which, as
indicated, are usually products of phagocytic macrophages (41). Since both IL-10 and IL-6 exhibit pro- and anti-inflammatory activities, it is possible that their expression reflects an unregulated response due to C3 and C5 activation. It is noteworthy that IFN-γ levels were not increased in Daf1/−/− mice at either 7 or 21 days, supporting earlier reports that the superficial damage induced by DSS and the repair processes of the gut are T cell independent (49). The expression of IL-10, TNF-α, IL-12, and IL-6 in the rectum of Daf1/−/− mice was substantially increased at 21 days, consistent with the observed lack of epithelial repair in the distal colon. Since IL-12 is considered principally to be a stimulator of IFN-γ production (50), the lack of IFN-γ generation suggests an alternate activity for IL-12 in the healing mucosa. As TNF-α, IL-6, and IL-10 possess proinflammatory as well as wound-healing activities (51), their elevated expression at 21 days in the rectum alone suggests that the inflammatory process remains unchecked, preventing effective healing.

Previous studies (52–55) have shown that DAF is essential to protect blood elements and vascular endothelial cells that are in intimate and prolonged contact with plasma where complement levels are highest. Although early studies (39) showed that DAF is widely distributed extravascularly on virtually all cell types, its physiological importance in these sites has remained obscure. This is particularly true for extracorporal sites such as the gut or sites where complement is normally low or absent. The availability of Daf1 knockout mice has made such analyses now possible. By showing in DSS-induced colitis that 1) much more severe clinical disease and markedly greater pathology develops in mice devoid of colonic DAF protein than in Daf1+/+ control mice and that 2) the heightened clinical and pathological changes are associated with increased and prolonged production of proinflammatory cytokines, we provide strong evidence that this regulation is important in protecting colonic epithelium during inflammation.

Ulcerative colitis, one of the major forms of IBD, initiates in patients as a superficial disease that begins in the rectum and extends proximally, forming a discrete border between inflamed and noninflamed tissue. In contrast, Crohn’s disease, the other major form of IBD, is characterized predominantly by ileocecal skip lesions that occur in both the large and small bowel, with occasional transmural inflammatory infiltrate (56). Although both diseases are classically viewed as T cell mediated, the prevailing yet unproven belief holds that they both are due to the induction of an unbalanced immune response by commensal bacterial flora (57). Because of the heterogeneity in clinical symptoms and histological changes of IBD, it is likely that the initiating events of the disease vary markedly among patients.

DSS-induced colitis has been widely used as an experimental model to study pathogenic mechanisms underlying IBD. The chemical produces diarrhea and weight loss similar to the human disease. It causes focal crypt lesions, mucosal and submucosal inflammation, and granulocyte infiltration as seen in human ulcerative colitis (58). Many of the cytokines that are generated are found in colonic specimens from IBD patients (59). Although the damage to the epithelium induced by DSS does not closely reflect the events that lead to IBD, the resulting ulceration leads to systemic contact with commensal flora and induction of the innate host response. It has been shown that bacterial flora in DSS colitis do not change (60, 61). In response to this bacterial overload, unregulated activation of the complement system in the absence of epithelial expression of DAF may trigger inflammatory cell infiltration into the mucosa, resulting in the overproduction of proinflammatory cytokines such as TNF-α and IL-6. Increased synthesis of IL-10 in our model may reflect an unsuccessful compensatory response by the innate host defense system, although not directly addressed in this report. Continued expression of inflammatory mediators in the absence of DAF at day 21, a time when substantial repair occurred in wild-type mice, may reflect an inability to terminate the innate inflammatory response in the absence of controlled C3 and C5 activation, the latter of which is also regulated by DAF (4).

Of relevance to the issue of complement activation in colonic inflammation is that DAF serves as a receptor to certain microbes that infect colonic mucosa. These include Afa/Dr-adhering Escherichia coli strains (62). Whether the pathogens not only utilize DAF for adhesion but, by virtue of impairing its activity, cause autologous complement-mediated damage to mucosal cells and thereby facilitate their cellular entry or submucosal invasion/spread is unknown.

DAF has been shown to be the erythrocyte surface protein previously designated as the Cromer blood group Ag in RBC typing (63). A small number of patients who lack the Ag and are termed to have the INAB phenotype have been identified (64–67). It is noteworthy that some of these INAB individuals have developed IBD (67, 68). Clinical records, however, are incomplete and the precise correlation remains unknown. This finding, taken together with the results of the present study, argue that like IL-10, IL-2, and TCR, DAF is important in the maintenance of gut homeostasis, presumably in analogy to the other mediators, by preventing inflammation despite the presence of high concentrations of bacteria in the lumen.

Acknowledgments

We thank Dr. C. Paul Morgan (Cardiff, Wales) for anti-mouse DAF mAbs 2C6 and 3D5, Kelly Ferguson, Medical Technologist (American Society of Clinical Pathologists) (University Hospitals of Cleveland, Cleveland, OH) for immunostaining, and Kelly Jerfutz and Ramona Kim for manuscript preparation.

References


Downloaded from http://www.jimmunol.org by guest on July 29, 2017


