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Inhibition of Plasmin Protects Against Colitis in Mice by Suppressing Matrix Metalloproteinase 9–Mediated Cytokine Release From Myeloid Cells

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BACKGROUND & AIMS: Activated proteases such as plasmin and matrix metalloproteinases (MMPs) are activated in intestinal tissues of patients with active inflammatory bowel diseases. We investigated the effect of plasmin on the progression of acute colitis. **METHODS:** Colitis was induced in *Mmp9*$^{-/-}$, *Plg*$^{-/-}$, and C57BL/6 (control) mice by the administration of dextran sulfate sodium, trinitrobenzene sulfonic acid, or CD40 antibody. Plasmin was inhibited in control mice by intraperitoneal injection of YO-2, which blocks its active site. Mucosal and blood samples were collected and analyzed by reverse-transcription polymerase chain reaction and immunohistochemical analyses, as well as for mucosal inflammation and levels of cytokines and chemokines. **RESULTS:** Circulating levels of plasmin were increased in mice with colitis, compared with controls. Colitis did not develop in control mice injected with YO-2 or in *Plg*$^{-/-}$ mice. Colons from these mice had reduced infiltration of Gr1+ neutrophils and F4/80+ macrophages, and reduced levels of inflammatory cytokines and chemokines. Colonic inflammation and colitis induction required activation of endogenous MMP9. After colitis induction, mice given YO-2, *Plg*$^{-/-}$ mice, and *Mmp9*$^{-/-}$ mice had reduced serum levels of tumor necrosis factor and C-X-C motif chemokine ligand 5, compared with control mice. **CONCLUSIONS:** In mice, plasmin induces a feedback mechanism in which activation of the fibrinolytic system promotes the development of colitis via activation of MMP9 or proteolytic enzymes. The proteolytic environment stimulates the influx of myeloid cells into the colonic epithelium and the production of tumor necrosis factor and C-X-C motif chemokine ligand 5. In turn, myeloid CD11b+ cells release the urokinase plasminogen activator, which accelerates plasmin production. Disruption of the plasmin-induced chronic inflammatory circuit therefore might be a strategy for colitis treatment.

Keywords: IBD; Mouse Model; Plasminogen; UC.

Crohn's disease and ulcerative colitis, the major forms of inflammatory bowel disease (IBD) in human beings, result from the interaction of genetic and environmental factors that ultimately promote an immunopathologic process leading to chronic inflammation.[1] Treatment of IBD generally relieves symptoms, but is not curative. Activation of proteases such as matrix metalloproteinases (MMPs), or serine proteases, such as plasmin, can break down the intestinal-epithelial barrier because of their potential to degrade components of the extracellular matrix, resulting in the invasion of inflammatory cells.[2]

MMP9, expressed by epithelial cells, plays an important role in the development of colitis by modulating cell-matrix interaction and wound healing.[3] MMP inhibitor treatment is effective for colitis, but patients have described side effects including severe lethargy and chills. Therefore, the therapeutic effects of more selective inhibitors of disease-associated MMPs currently are under investigation.[4] Another way of controlling the activation of MMPs is through the serine protease plasmin,[5–7] which has been shown to be important for the release of cytokines/chemokines such as Kit ligand, monocyte chemoattractant protein-1, C-X-C motif chemokine ligand 5 (CXCL5), and basic fibroblastic growth factor.[7–11] It is conceivable that the production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) also is regulated via plasmin-mediated MMP activation. TNF-α is induced rapidly in the intestinal mucosa during the initial activation of immune cells, and this induction is linked to disease progression during IBD.[12]

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Abbreviations used in this paper: **Ab, antibody; CXCL5, C-X-C motif chemokine ligand 5; DAI, disease activity index; DSS, dextran sulfate sodium; FDP, fibrin degradation product; IBD, inflammatory bowel disease; IL, interleukin; MMP, matrix metalloproteinase; PA, plasminogen activator; PAP, plasmin-antiplasmin complex; Plg, plasminogen; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; uPA/*Plau*, urokinase-type plasminogen activator; YO-2, *trans*-4-aminomethylcyclohexanecarbonyl-Tyr(*O*-Pic)-octylamide.**

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Plasmin, a key enzyme of the fibrinolytic cascade, can degrade the fibrin clot. It is generated by conversion from its precursor, plasminogen (Plg), by the plasminogen activators (PAs) tissue-type PA (*Plat*), and urokinase-type PA (uPA/*Plau*). Early clinical studies showed that circulating monocytes derived from IBD patients showed increased secretion of PA,[13] and that disease activity depended on up-regulation of uPA in the active stage of the disease.[14]

In the present study, we examined the role of the fibrinolytic system in patients with IBD. We show that genetic and pharmacologic plasmin inhibition prevents the progression of IBD in experimental models of colitis, and ameliorates the disease in part by suppressing the MMP9-dependent influx of inflammatory cells and production of inflammatory cytokines.

Materials and Methods

Animal Studies

Mmp9$^{+/+}$ and *Mmp9*$^{-/-}$ mice and *Plg*$^{+/+}$ and *Plg*$^{-/-}$ mice each were used after 10 back-crosses onto a C57BL/6 background. C57BL/6 recombinase-activating gene 2 (Rag2$^{-/-}$) mice were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan). Animal studies were approved by the Animal Review Board of Juntendo University (Tokyo, Japan).

Induction of Colitis

Experimental dextran sulfate sodium (DSS) (ICN Biomedical molecular weight, 36,000–50,000 daltons; ICN Biomedicals Inc, Ohio, GA) colitis was induced by administering 2% DSS via drinking water on days 0–7. Trinitrobenzene sulfonic acid (TNBS)-induced colitis was induced by colonic injection of 100 μL

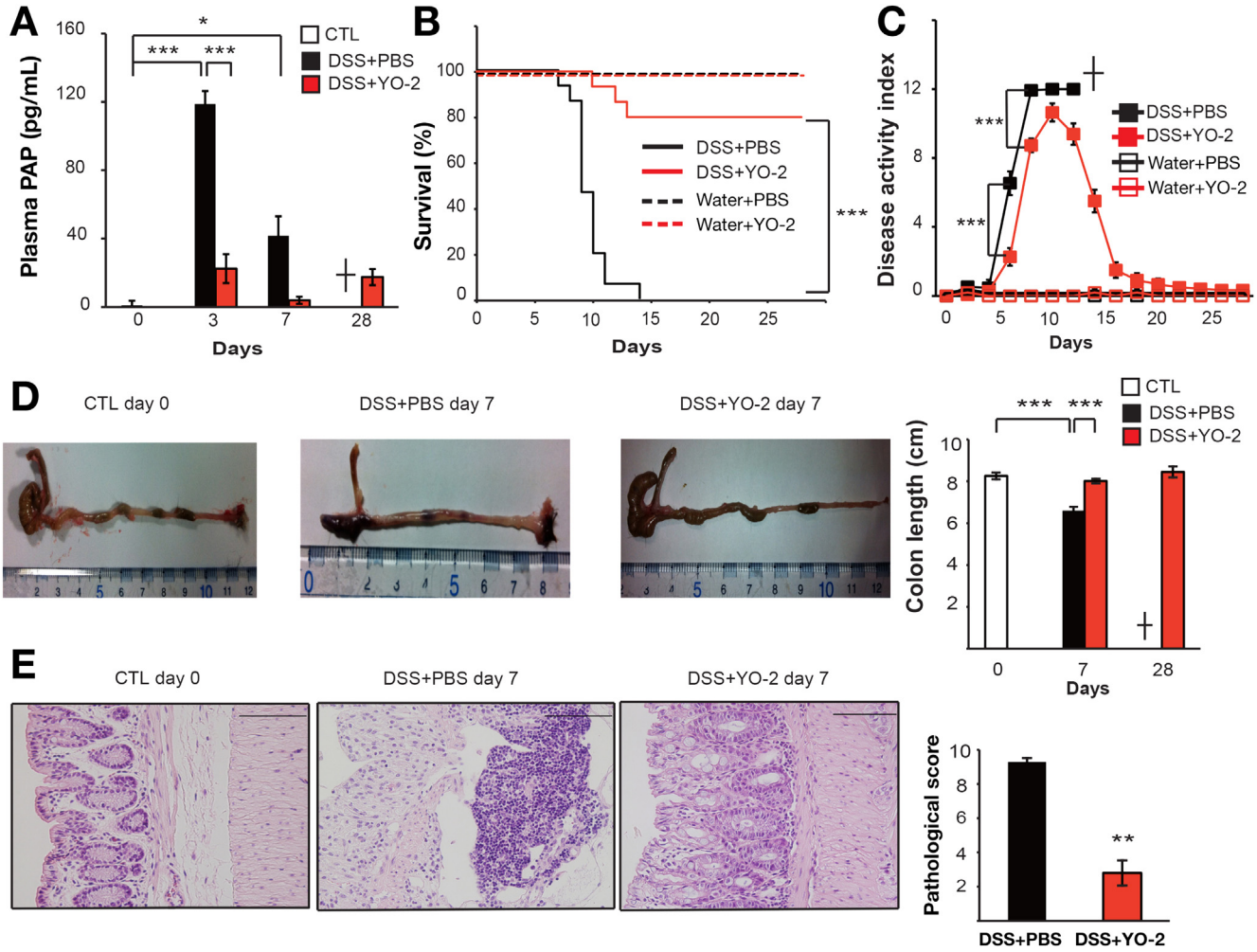

Figure 1. Plasmin inhibition prevents DSS-induced colitis progression. Colitis was induced by DSS and mice were injected with or without YO-2. (*A*) Plasma derived from mice treated with or without YO-2 was analyzed for PAP as a measure of active plasmin by enzyme-linked immunosorbent assay. n = 3/group. (*B*) Percentage survival and (*C*) DAI were determined at the indicated time points in the following treatment groups: 2% DSS + phosphate-buffered saline (PBS), n = 15; 2% DSS + YO-2, n = 15; water + PBS and water + YO-2, n = 5. (*D*) Colon lengths were measured at the indicated time points. n = 9–14/group. (*E*) Representative H&E-stained colon sections are shown. *Scale bars*: 200 μm. Histologic inflammatory scores were determined for each section from DSS-induced mice treated with/without YO-2. n = 3/group. Values represent means ± SEM. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$, determined by a 2-tailed Student *t* test and log-rank test.