Pharmacological Targeting of Neutrophil Serine Proteases Prevents Lethality in Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice

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Abstract

Background and aims: In recent years the number of patients with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis has been on the rise. Neutrophils are thought to be first cells to colonize disease-state colon tissue. Neutrophil elastase (NE) and cathepsin-G (Cat-G) are major secretory products from activated neutrophils and a major contributor to tissue destruction in inflammatory diseases. We predicted that inhibiting neutrophils and their proteases would improve ulcerative colitis.

Methods: Acute colitis was induced in mice by oral administration of 2% DSS for 7 days. In the first experiment, an inhibitor of Gr-1, a key neutrophil protein, was administered. In the second, the respective antagonists for NE and Cat-G, proteinases secreted by neutrophils, were administered, and the conditions of DSS-induced colitis were evaluated for improvement. Blood samples were collected and analyzed for levels of cytokines.

Results: The survival rate and pathology were improved in the Gr-1 inhibition experiments. In addition, administration of inhibitors of NE and Cat-G also resulted in clinical improvement. However, elevation of TNF-α, a key cytokine, was not suppressed in either experiment.

Conclusion: The proteolytic environment of neutrophils is associated with colonic inflammation. Disruption of the neutrophil proteases NE and Cat-G might therefore be a strategy for treatment of colitis regardless of TNF-α suppression.

Keywords: Colitis; Neutrophil; Proteases

Abbreviations: Ab: Antibody; DSS: Dextran Sulfate Sodium; CD: Crohn's Disease; UC: Ulcerative Colitis; FACS: Fluorescen

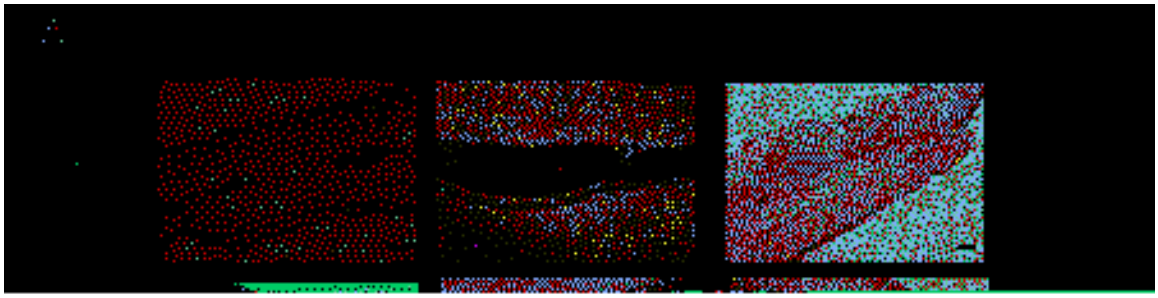

Figure 1:

Figure 2: ONO-5046 suppresses the development of murine DSS-

the pathogenesis of acute lung injury, hypercytokinemia can develop, in which neutrophils are activated to accumulate in the lungs and release NE. HXe NE injures pulmonary vascular endothelial cells and alveolar epithelial cells, causing increased pulmonary vascular permeability. ONO-5046 improves acute lung injury by selectively inhibiting NE [15]. Moreover, we conŽrmed that ONO-5046 overcame the role of MMP9, but NE was not present on the upstream protease of MMP9 (data not shown). However, as Morohoshi et al. have shown, ONO-5046 might actually have the potential to be a new therapeutic approach for patients with active UC [5].

On the other hand, Cat-G also induced the conversion of proMMP-1 to active MMP-1 in photoaging in hairless mice [16]. Another Cat-G inhibitor was shown to reduce neutrophil inžux into sites of inžammation and counteract asthma pathophysiology [17]. We likewise demonstrated that a Cat-G inhibitor ameliorated the DSS-induced inžammation. In addition, NE- and Cat-G-deŽcient mice showed a decreased response in a rheumatoid arthritis model. HXis defect was accompanied by a decrease in local production of TNF-α and IL-1β [18]. It is generally accepted that the mTNF-α/sTNF-α ratio is regulated mainly through shedding of cytokines from the cell surface by activated ADAM17. Other proteases present at inžammatory sites, such as Cat-G and NE, may also contribute to TNF-α shedding [19]. HXough TNF-α of our colitis model could not be regulated by inhibition of Cat-G and NE, HXis phenomenon is considered to depend on the particular disease.

In actual clinical trials, while mild cases of IBD may respond to aminosalicylates for induction and maintenance of remission, for management of severe IBD the current situation is far from satisfactory, especially in cases of steroid-dependent or steroid-refractory IBD, or in patients who are intolerant to corticosteroids. HXe widespread use of anti-TNF-α agents has changed the treatment paradigm in the management of patients with IBD [20]. HXese therapies are associated with a higher rate of induction of remission, but agents usually reserved for more severe disease have been known to fail in approximately 40% of patients [9,10]. HXus, new target therapy is required such as anti-integrin therapy or inhibition of proinžammatory cytokines such as IL13, IL-17, and IL12/23p40 [9,21,22] in humans but also, for instance, plasmin inhibitor, MMP inhibitor, cannabinoids and palmitoylethanolamide in mice [4,23,24]. In conclusion, our data suggest that serine proteases are activated during colitis progression. HXis proteolytic environment controls cell inŽltration into colonic tissues, but not the production and secretion of the proinžammatory cytokine TNF-α. Our Žndings suggest that the targeting of NE and Cat-G is an attractive therapeutic candidate for TNF-α Abs-refractory IBD.

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Study concept and design, SM; Data acquisition, HR, SM, TU,; Analysis and interpretation of data, HR, SM, TU; Draring of the manuscript, SM; Critical revision of the manuscript, SM,HK, MT, YK, YT, KS.

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HXe authors have no conžicts of interest to declare.

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