

Article

Dextran sulfate sodium-induced colitis mice up-regulated extracellular matrix tenascin-C

Md. Shafiqul Islam^{1, 2,*}, Masatoshi Hori² and Hiroshi Ozaki²

¹Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

²Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan

*Corresponding author: Md. Shafiqul Islam, D.V.M., Ph.D., Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. E-mail: shafiqpharma@yahoo.co.uk

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Abstract: Tenascin-C, an extracellular matrix glycoprotein, expresses high level during embryogenesis and almost absent during the normal postnatal life. However, it is re-appeared in a diverse condition such as tissue injury and in the stroma of various carcinomas. In this study, we investigated the appearance of tenascin-C in dextran sulfate sodium (DSS)-induced colitis in mice. DSS induced colitis mice demonstrated severe mucosal damage, with distortion and loss of crypts, depletion of goblet cells and infiltration of macrophages particularly F4/80 positive macrophages, granulocytes and lymphocytes in the colon tissues. These DSS inflamed colon expressed a high and dense level of tenascin-C in the severe damaged areas, whereas, moderate staining was observed in the moderate inflamed areas. DSS-induced colitis mice significantly increased macrophages infiltration in the colon tissues. These results suggested that tenascin-C extracellular matrix re-appeared in the colon tissues during inflammation.

Keywords: tenascin-C; DSS; colitis; macrophage

1. Introduction

Tenascin-C (TnC) is an extracellular matrix express high level during embryogenesis and almost absent during normal postnatal life with some basal level detectable in a varieties of tissues. But, these molecules are re-appeared in a diverse condition such as tissue injury, wound healing, vascular disease, tumorigenesis and metastasis. Tenascin-C is a modular, multifunctional extracellular matrix (ECM) glycoprotein that is associated with tissue injury and repair (Patel *et al.*, 2011). It was discovered originally in gliomas, muscle tissue and in the nervous system, and called by different names: myotendinous antigen, glial/mesenchymal ECM protein, cytотactин, J1 220/200, neuronectin and hexabrachion (Chiquet-Ehrismann *et al.*, 2003; Patel *et al.*, 2011). Recent scientific evidences suggested that mucosal TnC is increased in ulcerative colitis and Crohn's disease, especially in areas of ulceration(Geboes *et al.*, 2001), and sera of patients with active ulcerative colitis and Crohn's disease(Riedl *et al.*, 2001). On the other hand, recent researches on TnC knockout mice were evidenced that deletion of tenascin-C gene exacerbates colitis in mice (Islam *et al.*, 2014). Then the important question arises what type of cells are responsible for the constitutive and pathological expression of tenascin-C in colon tissues. During colitis, a mixture of cells of many different lineages including epithelial, lymphocytes, resident macrophages, subepithelial fibroblast and fibroblast like cells are involved either for pro-inflammatory or anti-inflammatory phenomena. Recently scientists have focused that intestinal subepithelial myofibroblast could be an important sources of tenascin-C molecules in the colon tissues in mice (Islam *et al.*, 2014). Tenascin-C expression was correlates with the pattern of inflammation in DSS-induced colitis mice. In this study, we have

focused on colitis inflammation and tenascin-C up regulation. We have found that DSS-induced colitis up-regulated tenascin-C in the inflamed and damaged mucosal areas.

2. Materials and Methods

2.1. Animals

Animal care and treatment were conducted in accordance with the institutional guidelines of the University of Tokyo. Experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Tokyo (approval code: P07-138).

2.2. Induction of colitis in mice and evaluation of disease activity index

Experimental colitis was induced by giving mice 1% (C57BL/6N) (w/v) DSS (MP Biomedicals Inc., Osaka, Japan) in drinking water *ad libitum* for 8 days. In addition, we used 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced C57BL/6N colitis mice. TNBS 150 mg kg⁻¹ body weight was administered intrarectal under anesthesia and sampling was done after 2 days of TNBS administration.

2.3. Histopathology

For histopathological analysis, a representative sample from the mid-part of the colon was fixed in 4% paraformaldehyde, embedded in paraffin, sectioned (4 µm), stained with haematoxylin and eosin, and examined at × 200 magnification.

2.4. Immunofluorescent staining of colon tissues of mice

The representative specimens from mid-colon (4-5µm) were permeabilized with Triton X-100 (0.2%), blocked by normal goat serum (10%) and then incubated with the following primary antibodies; rat anti-TnC (1: 500) and rat anti-F4/80 (1:200) respectively over night at 4°C. After washing with PBS, the specimens again incubated with the appropriate secondary antibody in a dilution of 1:1000 for 2 hours and counter-stained with DAPI (Molecular probes). Images were obtained using an Eclipse E800 fluorescence microscope (Nikon, Tokyo, Japan).

2.5. Statistical analysis

All experiments were performed with a minimum of triplicate replication, and values are expressed as mean ± SEM. Statistical significance (**P < 0.01) was determined by using the Student's *t* test.

3. Results

3.1. DSS-induced colitis inflammation in mice

Ulcerative colitis and Crohn's disease are two major forms of inflammatory bowel disease (Melgar *et al.*, 2005) which affects a million of people worldwide and are characterized by chronic uncontrolled inflammation of intestinal mucosa (Papadakis *et al.*, 2000). DSS-induced colitis mice are commonly practiced to investigate the pathogenesis of inflammatory bowel disease. We used 1% (w/v)DSS in drinking water in C57BL/6 mice *ad libitum* for 8 days to establish an acute and sub-acute colitis model mice. Significant weight loss and diarrhea were observed during the DSS treatment (data not shown) in mice. Histopathological examination of the colon of mice given DSS in drinking water showed mucosal inflammation involving all layers of the bowel wall, with a marked increase in the thickness of muscle layer, crypts distortion and, in some sections, loss of crypts were evident. Extensive granulation tissues with the presence of monocytes and lymphocytes, depletion of goblet cells were apparent in the mucosa in untreated mice (Figure 1A-a-b). Colon photographs showed that DSS-induced colon was inflamed along with bloody stools and shortening of colon length, whereas, control animal showed healthy colon appearance (Figure 1B).

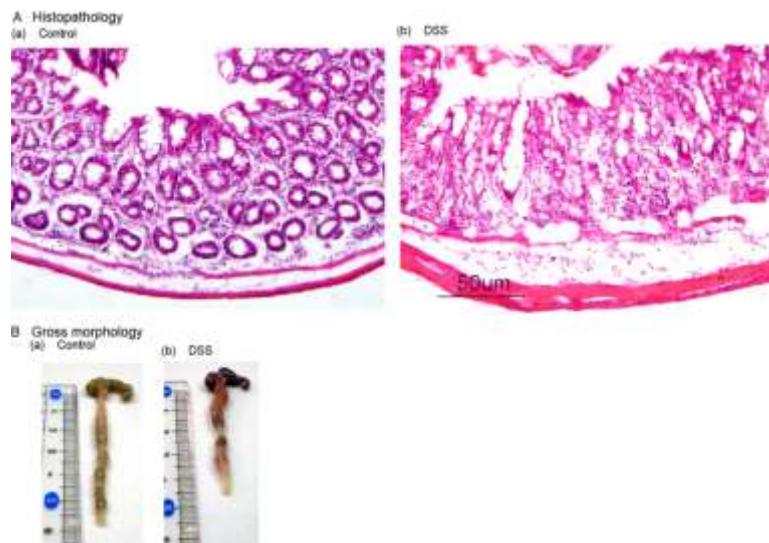


Figure 1. DSS induced colitis in C57BL/6 mice. Experimental colitis was induced by 1% DSS in drinking water *ad libitum* for 8 days. (A) Histology (haematoxylin and eosin staining) of full thickness of mid-colon are shown in (a) non-treatment: control, (b) DSS induced colitis. (B) Gross appearance of colon is shown (a) non-treatment: control, (b) 1% DSS mice. n=3-4, Bar=50μm.

3.2. DSS-induced colitis increased Tenascin-C expression in colon tissues

The extracellular matrix molecule tenascin-C is highly expressed during embryonic development, tissue repair and in pathological situations such as chronic inflammation and cancer. Extracellular matrix TnC expression is induced at the site of inflammation. DSS-induced inflammation was categorized as moderate and severe inflamed areas. Our immunohistochemistry analysis exhibited that in control mice, tenascin-C was found in the muscle layers and in a mucosal layer, only a small amount of tenascin-C was detected in submucosa at a subsurface epithelial region of colon (Figure 2a). On the other hand, in the inflamed colon tissue treated with DSS, the immunohistochemical analyses showed moderate tenascin-C expression at the tissue damage (Figure 2b) or inflamed areas and densely expression in the severe inflamed areas (Figure 2c).

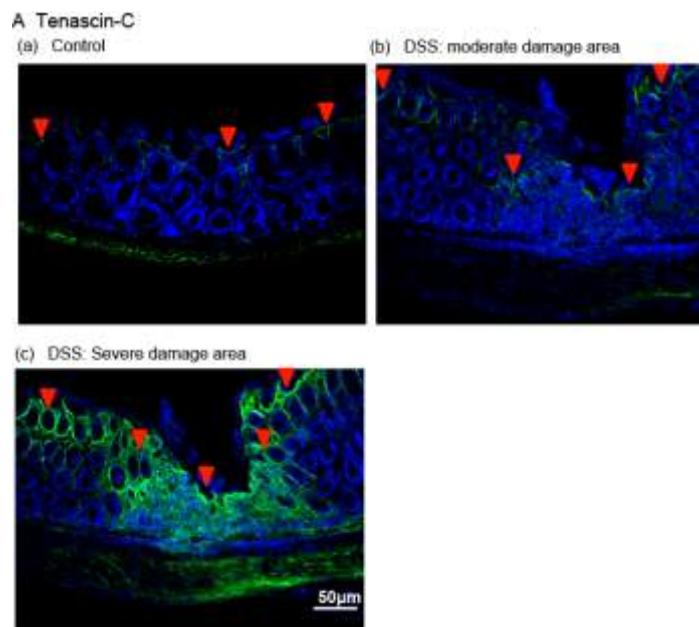


Figure 2. DSS induced colitis increased tenascin-C. Experimental colitis was induced by 1% DSS in drinking water (*ad libitum*) for 8 days. (A) Immunofluorescent staining of mid-colon tissue for tenascin-C (a) untreated control, (b) moderately inflamed areas of DSS-induced colitis and (c) severely inflamed areas of DSS-induced colitis. Arrow indicates the expression of tenascin-C (Green color). Nucleus is stained with DAPI (Blue color). n=3-4, Bar=50μm.

3.3. DSS-induced colitis increased F4/80 macrophages in colon tissues

F4/80 macrophages were analyzed by immunohistochemistry staining in mid-colon both in healthy control and DSS-inflamed mid-colon. In healthy control only a few number of F4/80 positive cells were found mainly in the mucosal part of colon. DSS-induced colon showed a huge number of positive cells particularly in the inflamed areas including mucosal and muscular layer (Figure 3A-a-b). Quantitative analysis of macrophages counts also indicated that in DSS-induced colitis significantly ($P<0.01$) increased macrophages infiltration (Figure 3B).

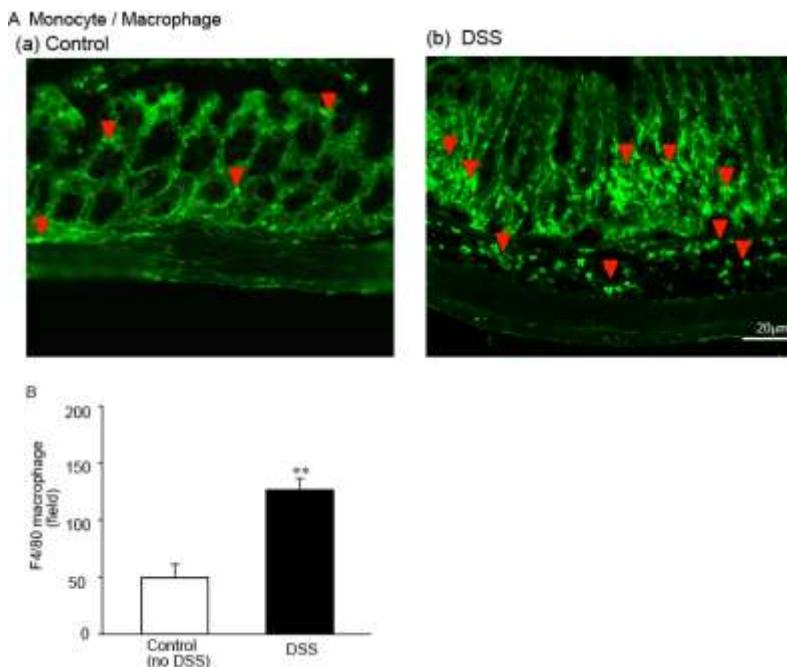


Figure 3. DSS induced colitis increased F4/80 macrophages. Experimental colitis was induced by 1% DSS in drinking water (*ad libitum*) for 8 days. (A) Immunofluorescent staining of mid-colon tissue for F4/80 positive cells (a) untreated control, (b) DSS-induced colitis. (B) Histogram showing significantly increased number of F4/80 positive cells in DSS colitis mice. ** $P<0.01$. Arrow indicates the F4/80 positive cells (Green color). Nucleus is stained with DAPI (Blue color). n=3-4, Bar=50μm.

4. Discussion

There are many *in vivo* and *in vitro* disease models are used to investigate the pathophysiology of inflammatory bowel disease. Among the models of inflammatory bowel disease (IBD), oral administration of DSS has been widely used to study the mechanisms of colonic inflammation (Islam *et al.*, 2008). However, as a spontaneous model, a SAMP1/Yit 20weeks old mouse is considered a model for human Crohn's disease (Islam *et al.*, 2014). Extracellular matrices play important roles in colon homeostasis including inflammation, protection, proliferation, cell migration, and repair and so on. Besides these, the composition of the extracellular matrix plays an important role in the mechanism of metastasis of colon cancer cells (Ohtaka *et al.*, 1996). In this study, we used inflammatory bowel disease model mice to investigate the pathophysiology of extracellular matrix, tenascin-C. This is of great importance that tenascin-C express high level during embryogenesis and almost absent during normal postnatal life with some basal level detectable in a varieties of tissues. But, these molecules are re-appeared in a diverse condition such as tissue injury, wound healing, vascular disease, tumorigenesis and metastasis. Our DSS-induced colitis mice expressed pathology of colon such as infiltration of inflammatory cells, loss of crypts in some portion of colon areas, loss of goblet cells were evident, shortening of colon length etc. (Figure 1). We then investigated whether these changes are associated with extracellular matrix tenascin-C. We found that tenascin-C is highly expressed during DSS-induced inflammation at the site of inflammation. But there was a variation of expression of tenascin-C on the basis of severity of inflammation. Immunohistochemistry analysis demonstrated that healthy colon tissues expressed tenascin-C in the muscle layers and mucosal layer and a small amount was detected in sub-mucosa at a subsurface epithelial region of colon (Figure 2). On the other hand, DSS colitis mice revealed moderate tenascin-C expression at the tissue damage or inflamed areas and densely expression in the severe inflamed areas (Figure 2). Recent scientific evidences suggested that mucosal tenascin-c is increased in ulcerative colitis and Crohn's disease, especially in

areas of ulceration, and sera of patients with active ulcerative colitis and Crohn's disease. On the other hand, recent researches on tenascin-C (-/-) mice were evidenced that deletion of tenascin-C gene exacerbates colitis in mice (Islam *et al.*). Many other scientists also reported that DSS-induced colitis greatly expressed F4/80 macrophages in the colon tissues (Peng *et al.*, 2010).

5. Conclusions

DSS-induced colitis up-regulated tenascin-C in the inflamed and damaged mucosal areas and expression was correlates with the pattern of inflammation in DSS-induced colitis mice.

Conflict of interest

None to declare.

References

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