Beneficial Effect of Glatiramer Acetate Treatment on Syndecan-1 Expression in Dextran Sodium Sulfate Colitis

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ABSTRACT

Syndecan-1, the most abundant heparan sulfate proteoglycan in the gastrointestinal tract, is reduced in the regenerative epithelium in inflammatory bowel disease (IBD). This study explored the effects of the immunomodulator glatiramer acetate (GA; Copaxone) treatment on syndecan-1 expression in dextran sodium sulfate (DSS)-induced colitis. Acute and chronic colitis was induced in C57BL/6 mice by 2 and 1.5% DSS in tap water, respectively. GA was applied subcutaneously, 2 mg per mouse per day, starting on the day of DSS induction until the mice were sacrificed. Syndecan-1 expression was assessed by immunohistochemistry. The effect of adoptive transfer of GA-specific T cells as an organ-specific therapy was also evaluated. Syndecan-1 expression was significantly lower in both colitis groups compared with that in naive mice (p < 0.0001). GA attenuated clinical scores and pathological manifestations of colitis and led to the reinstatement of normal levels of syndecan-1. After adoptive transfer, GA-specific cells homed to the surface epithelium of the distal colon, accompanied by the augmentation of syndecan-1 staining in their vicinity. We concluded that syndecan-1 expression is reduced in DSS-induced colitis and could be a potential prognostic factor in IBD. Treatment with GA exerts not only an anti-inflammatory effect but also a possible beneficial effect in stabilizing the intestinal epithelium barrier and tissue repair in DSS colitis. GA may be applied as a novel drug for IBD, shifting treatment from immunosuppression toward immunomodulation.

Introduction

Inflammatory bowel disease (IBD) is thought to result from an inappropriate and ongoing activation of the mucosal immune system in response to the presence of normal luminal flora. This aberrant response most likely is facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system (Podolsky, 2002). The organization and maintenance of mucosal integrity are mediated to a large extent by cell adhesion molecules that function between both the adjacent cells and the extracellular matrix to provide support for tissue repair through their close interplay with growth factors (Principi et al., 2006). Syndecans are a class of heparan sulfate proteoglycans (HSPGs) that mediate both cell adhesion and growth factor binding via glycosaminoglycan side chains (Bartlett et al., 2007). Of the four known syndecan core proteins, syndecan-1 (CD138) is the best characterized for its binding to a variety of components of the extracellular matrix, including: collagen type I, III, and V as well as fibronectin, thrombospondin, and basic fibroblast growth factor. It is also the most relevant HSPG in the gastrointestinal tract, because it is expressed on the basolateral surface of columnar epithelial cells (Carey, 1997; Day and Forbes, 1999; Götte, 2003). Syndecan-1 functions as an integral membrane protein that participates in cell proliferation, cell migration, and cell matrix interactions (Carey, 1997; Bartlett et al., 2007). It is therefore not surprising that altered syndecan-1 expression has been detected in many different tumor types (Inki et al., 1994; Pulkkinen et al., 1997; Hirabayashi et al., 1998; Kumar-Singh et al., 1998; Seidel et al., 2000; Leivonen et al., 2004), including hepatocellular and gastric carcinoma (Matsumoto et al., 1997; Wiksten et al., 2001). A decrease in syndecan-1 immunoreactivity has been reported in the...
majority of human colorectal adenocarcinomas in correlation with the stage of the disease and local lymph node metastasis (Hashimoto et al., 2008).

The role of syndecan-1 in the intestinal barrier was clarified further by studies in syndecan-1 knockout mice. One of the studies describes that the loss of syndecan-1 increases the permeability of the epithelium, resulting in protein-losing enteropathy, similarly to the defective barrier function that is typical for IBD (Bode et al., 2008). However, the actual involvement of syndecan-1 in an experimental model of IBD has not been explored except for a recent report (in Chinese), of which only an abstract is available (Wang et al., 2008). The normal healing process in IBD during restitution is disrupted by inflammation. This may be due to the loss of growth factors, cell adhesion molecules (e.g., syndecan-1), or both, causing a reduced rate of healing. Evidence for a reduction of syndecan-1 expression in the regenerating epithelium that overlies inflamed tissue was reported almost 10 years ago in patients with IBD (Day et al., 1999, 2003). It is well known that in IBD there is an imbalance between proinflammatory and anti-inflammatory factors, and this loss of homeostasis is critical in mediating epithelial cell injury (Fiochhi, 1998). However, the direct role of syndecan-1 in the pathogenesis of IBD has not been investigated.

The synthetic copolymer glatiramer acetate (GA; Copoxone; copolymer-1), an approved drug for the treatment of multiple sclerosis with only minor side effects (such as irritation at injection sites and, rarely, transient vasomotor response) and a high safety profile (Aharoni et al., 1997), was demonstrated to exert its therapeutic activity in experimental autoimmune encephalomyelitis/multiple sclerosis by modulating the immune response at different levels of specificity, resulting in an anti-inflammatory effect (Fridkis-Harel et al., 1994; Aharoni et al., 1997). In recent years, it has been demonstrated in our laboratory that GA treatment ameliorated the pathological manifestation of IBD in several animal models, including trinitrobenzene sulfonic acid- and dextran sodium sulfate (DSS)-induced colitis (Aharoni et al., 2005, 2006). In addition, a spontaneous model of colitis in C57BL/6J mice showed a clinical benefit after GA treatment (Aharoni et al., 2006). Improvement of clinical symptoms, such as weight loss and intestinal bleeding, was accompanied by reduced expression of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ), and enhanced levels of regulatory anti-inflammatory cytokines, such as transforming growth factor β (TGF-β) and IL-10 (Aharoni et al., 2005, 2006). Furthermore, it was reported that adaptively transferred GA-specific T cells traveled to injury sites and secreted TGF-β in situ, leading to the suppression of DSS-induced colitis (Aharoni et al., 2007).

In view of the critical role of syndecan-1 in maintaining the normal intestinal epithelial barrier and enabling tissue repair, it was of interest to investigate the relationship between syndecan-1 expression and clinical status in DSS-induced colitis as well as the effect of GA treatment on syndecan-1 levels. We report here with that the expression of syndecan-1 at the surface epithelium that overlies inflamed colon tissue is reduced significantly in both acute and chronic DSS-induced colitis. We also demonstrate that after GA treatment the normal expression level of syndecan-1 is restored. Furthermore, adoptive transfer of GA-specific lymphocytes led to increased syndecan-1 levels in the vicinity of transplanted cells. These results suggest that GA not only exerts an anti-inflammatory effect but also has a possible beneficial effect in the stabilization of the intestinal epithelial barrier and tissue repair in DSS colitis.

Materials and Methods

Mice. C57BL/6 mice were purchased from Harlan (Jerusalem, Israel), and ROSA26 transgenic mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Female mice, 8 to 10 weeks of age weighing 18 to 21 g, were kept under specific pathogen-free conditions and were used for the induction of DSS-induced colitis. All of the experiments were approved by the Institutional Animal Care and Use Committee.

Induction of DSS Colitis. Acute colitis was induced by supplementing acidified drinking water with 2% (w/v) DSS (mol. wt. 36,000–50,000; MP Biomedicals, Eschwege, Germany) for 4 to 5 days. Chronic colitis was induced by applying three cycles of 1.5% DSS (w/v) for 5 days with intervals of 5 days of plain drinking water. Control mice received acidified drinking water during the study period. Animals were sacrificed at the indicated days by carbon dioxide. We did not observe differences in the water consumption among the various experimental groups (7.0–7.5 ml per mouse per day), excluding the possibility of volume-associated deviation.

Glatiramer Acetate. GA consists of acetate salts of synthetic polypeptides containing four amino acids: L-alanine, L-glutamate, L-lysine, and L-tyrosine (Arnon and Sela, 2003). GA from batch 242902007, with an average molecular mass of 7850 Da, obtained from Teva Pharmaceutical Industries (Petah Tikva, Israel), was used throughout the study. GA treatment was administered by daily injections, 2 mg/mouse (100 mg/kg), subcutaneously in phosphate-buffered saline (PBS), starting at the day of DSS induction until the mice were sacrificed.

Experimental Design. In each experiment, mice were assigned randomly to three groups: DSS (DSS-induced colitis mice), DSS + GA (DSS-induced colitis mice with coadministration of GA), or control (mice received distilled water without DSS or GA) with identical average body weight. In the first experiment, an additional control group was included in which normal mice were treated by the same dose of GA (2 mg per day per mouse) for 5 days. Animals induced with acute disease were sacrificed 7, 10, or 14 days after DSS induction. Animals in the chronic disease group were sacrificed 26 days after DSS induction.

Adaptive Transfer. Short-term GA-specific T cell lines originating from ROSA26 mice expressing β-galactosidase (β-gal) were prepared as described previously (Aharoni et al., 2007). Activated GA-specific lymphocytes (30 × 10⁶ cells/mouse) were injected into the peritoneum of mice with DSS-induced colitis at the day of disease induction. Mice were sacrificed 10 days after adoptive transfer.

Assessment of DSS Colitis. In all of the experiments, body weight, rectal bleeding, and survival were monitored daily. Intestinal bleeding was quantified by Hemocult test (Beckman Coulter, Fullerton, CA). When the mice were sacrificed, the large intestine was collected and evaluated for colon length and microscopic colonic damage.

Microscopic Scoring. Proximal, medial, and distal portions of colon and cecum were fixed overnight in 2.5% paraformaldehyde at 4°C. Paraffin-embedded sections were stained with hematoxylin and eosin. The evaluation was based in part on that used elsewhere (Dielman et al., 1998) and adapted to the pathology manifested in our laboratory. The degree of histological damage and inflammation was graded in a blinded fashion by an expert pathologist (O.B.). The following manifestations were included in the evaluations: inflammation (0, none; 1, mild; 2, moderate; 3, severe; 4, accumulation of inflammatory cells in the gut lumen), distribution of lesions (0, none; 1, focal; 2, multifocal; 3, nearly diffuse; 4, diffuse), and depth of inflammation and layers involved (0, none; 1, mucosa only; 2, mucosa...
and submucosa; 3, limited transmural involvement; 4, transmural). The overall histological score was the sum of the four evaluations (maximal score of 12). In addition, we evaluated the nature of the mucosal inflammation (acute versus chronic) by the visualization of distorted crypt architecture, crypt atrophy, and acute/chronic inflammatory infiltrates.

**Immunohistochemistry. Syndecan-1 staining.** Immunostaining for syndecan-1 was performed on formalin-fixed, paraffin-embedded tissues using an avidin–biotin–peroxidase complex technique. Sections (4-μm-thick) were deparaffinized by xylene (2 × 15 min), hydrated through descending strengths of alcohol (100, 95, and 70% ethanol), and rinsed in PBS. Endogenous peroxidase activity was blocked by incubating specimens in 6% hydrogen peroxide in methanol with 30% hydrochloric acid for 20 min. Antigen retrieval was performed by boiling sections for 15 min in 10 mM citrate buffer at pH 6.0. Sections then were blocked with 20% normal horse serum in PBS for 1 h followed by overnight incubation (at room temperature) with the primary rat anti-mouse syndecan-1 antibody (1:200; clone 281-2; BD Pharmingen, San Diego, CA) in PBS containing 2% normal horse serum. After being washed in PBS, sections were incubated for 1 h in biotinylated anti-rat antibody (1:100; Vector Laboratories, Burlingame, CA). The ABC complex from the Vectastain kit (Vector Laboratories) was used for detection, followed by staining with 0.04% diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) for 5 min and counterstaining with hematoxylin.

**Adaptive transfer staining.** Colon sections were fixed in 2.5% phosphate-buffered paraformaldehyde and sectioned (16 μm) by sliding microtome. To study the presence and location of the injected β-galactosidase (lacZ)-expressing cells in the colon, free-floating sections were fixed in 0.5% glutaraldehyde (Sigma) for 2 min, washed in 1× PBS, and incubated for 12 h at 37°C with (5-bromo-4-chloro-3-indolyl-D-galactopyranoside). Sections then were immunostained with 0.04% diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) for 5 min and counterstaining with hematoxylin.

**Quantification.** Syndecan-1 protein expression was quantified by a light microscope (E600; Nikon, Tokyo, Japan) equipped with Plan Apo objectives connected to a CCD camera (DMX1200F; Nikon). Digital images (1 mm²) of five sections of the surface epithelium area in the distal colon of each mouse were collected. Each section then was divided into five subareas, and the integral optical density (IOD) was analyzed using Image-Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD). An average of 25 subareas for each mouse is presented.

**Statistical Analysis.** Data are expressed as the mean ± S.E.M. of values obtained from the indicated number of mice. Weight, bleeding, survival, colon length, and IOD measurements were compared using the two-tailed independent t test. Histological score was compared using the two-tailed Mann-Whitney U test. p values of <0.05 were considered significant. It should be noted that in experiments involving small numbers of animals that the statistical values should be taken with caution.

**Results**

**Effect of GA Treatment on Clinical Manifestations of DSS-Induced Colitis.** The clinical symptoms in both acute and chronic IBD models as well as the effect of GA treatment are depicted in Figs. 1 and 2. DSS was used to induce both acute (single cycle) and chronic (three cycles of a lower dose) models of colitis. In complete accord with our previously reported results (Aharoni et al., 2005, 2006, 2007), both acute and chronic DSS-induced colitis mice demonstrated weight loss of approximately 20% of their original body weight; in contrast, DSS + GA mice groups in both models dropped an average of only 5% of their baseline body weight (Figs. 1A and 2A). Intestinal bleeding, a pathological manifestation of DSS colitis evaluated by the Hemoccult test, revealed severe bleeding in both models (Figs. 1B and 2B). GA treatment significantly reduced intestinal bleeding. Thus, on day 9 (in the acute group) and on day 13 (in the chronic group), when most of the DSS mice (100 and 80%, respectively) suffered from bleeding, only 40% of the DSS + GA mice demonstrated positive Hemoccult tests (Figs. 1B and 2B). For the effect on morbidity, by day 15 only 25% of the acute DSS group survived, whereas in the DSS + GA group 75% survived (Fig. 1C). In the chronic DSS model, there was essentially no mortality (only one animal from the DSS + GA group died without any manifestation of colitis) (Fig. 2C). Disease was assessed by two additional parameters—colon length and histological evaluation of the colon. As demonstrated, according to these parameters, a severe effect was observed in the two DSS models (Figs. 1, D–F, and 2, D–F), whereas GA treatment ameliorated clinical disease and histological damage, thus corroborating our previous observations (Aharoni et al., 2006).

**Expression of Syndecan-1 in Acute DSS Colitis Tissue Samples.** Representative mice from each group were sacrificed 7, 10, and 14 days after DSS induction. For each time point, control, DSS, and DSS + GA were tested (a total of nine mice per the experiment). Their colons were analyzed by specific histochemical staining for the detection of syndecan-1. Figure 3 depicts a characteristic staining from each time point. As shown in normal tissue samples from control mice (Fig. 3A), intense and homogeneous epithelial staining of syndecan-1 is visible in the surface epithelium and along the length of the crypts. Staining was located at the basolateral surface of the columnar epithelium. A very similar pattern is observed in the intestine of the control normal mouse treated with GA (Fig. 3B). Stained colon tissues from mice with acute DSS colitis displayed a marked reduction in syndecan-1 membraneous staining advancing with disease progression with time, predominantly in the superficial epithelium as compared with the colon of control mice. This result is depicted in Fig. 3, C, E, and G, for samples of colons resected at days 7, 10, and 14, respectively, after DSS induction. As shown, syndecan-1 staining in the crypts of epithelium surrounding inflamed tissue was heterogeneous compared with that of control samples, suggesting that tissue injury in this model is localized in focal inflammatory areas. In contrast, colon samples taken at the same time points from the DSS + GA mice exhibited intense and homogeneous epithelial staining of syndecan-1 in surface epithelium (Fig. 3, D, F, and H) as well as in the crypts resembling normal colon (Fig. 3, A and B).

**Expression of Syndecan-1 in Chronic DSS Colitis Tissue Samples.** Animals from the control and DSS groups as well as the DSS + GA mice were sacrificed on day 26 after three cycles of DSS, and their colons were analyzed for syndecan-1 histochemical staining (n = 4 per group). Figure 4 depicts a representative staining of tissue obtained from two control mice, three mice from the DSS colitis group, and three...
mice from the DSS + GA group. Normal tissue samples in this experiment as well showed intense and homogeneous epithelial staining of syndecan-1 (Fig. 4, A and B). Chronic DSS colitis colon tissues displayed a drastic reduction in the level of syndecan-1 staining and heterogeneity in its membranous distribution, predominantly in the surface epithelium (Fig. 4, C, E, and G). In contrast, similarly to the results in the acute DSS model, colon samples from the GA-treated mice in the chronic DSS model displayed intense and homogeneous epithelial staining of syndecan-1 in surface epithelium (Fig. 4, D, F, and H) and resemble the normal mice samples.

**Quantification of Syndecan-1 in Both Acute and Chronic DSS Colitis.** To validate our immunohistochemistry results, we quantified the stained distal colons from both experiments (acute and chronic colitis) using Image-Pro Plus 4.1 software. For each experimental group, the IOD for 25 samples from each mouse was analyzed, and a comparison was made between the average scores obtained (Fig. 5A). For the acute colitis experiment, the results in the DSS mice group demonstrate a highly significant reduction in the IOD compared with that of the controls that further decreased with disease progression (for day 14, \( p < 0.0001 \)). GA treatment significantly enhanced syndecan-1 staining via IOD evaluation (\( p < 0.0001 \)). In the chronic colitis experiment, the results from four individual mice as well as their average score on day 26 demonstrated a highly significant reduction in the IOD between the control mice and the DSS group (Fig. 5B; \( p < 0.0001 \)). The difference in the IOD between the DSS + GA mice and the DSS mice was highly significant as well (\( p < 0.0001 \)). Quantification of syndecan-1 staining provides additional validity to our studies.
Effect of Adoptively Transferred GA-Induced Cells on Syndecan-1 Levels in the Intestinal Epithelium.

In a previous publication, we demonstrated that adoptive transfer of GA-specific cells is effective in ameliorating the symptoms of acute colitis, and these intraperitoneally injected cells migrated and accumulated at sites of damage within the colon (Aharoni et al., 2007). To explore whether such treatment will affect the levels of syndecan-1, we performed an adoptive transfer experiment in which GA-specific T cells were established from ROSA26 transgenic mice that ubiquitously express β-gal in their cells and thus could be detected immunohistologically in situ.

β-gal-positive cells from GA-specific lines obtained after one in vitro stimulation with GA were transferred intraperitoneally to mice in which acute DSS colitis was induced. The results depicted in Fig. 6 demonstrate that β-Gal-positive cells were abundantly present in the colon 10 days after their adoptive transfer into DSS-induced mice. The presence of β-Gal-positive cells (blue) at the surface epithelium of the distal colon was accompanied by the augmentation of syndecan-1 (red) staining in their vicinity (Fig. 6C). Areas with increased levels of syndecan-1 coincided with the presence of GA-specific cells. In this experiment as well, normal tissue samples showed intense and homogeneous epithelial staining of syndecan-1 (Fig. 6A), whereas colon tissues from DSS mice displayed severe reduction and only sporadic syndecan-1 membranous staining (Fig. 6B).

Discussion

The therapeutic potential of GA for the treatment of IBD has been shown previously by demonstrating its effect in several acute and chronic colitis murine models. In our cur-
rent study, we further evaluated the effect of GA treatment on the expression of syndecan-1 in the colon of mice induced with acute DSS. Specific staining for syndecan-1 (brown) located in the intestinal epithelium basolateral membrane of a control mouse (A), a control GA-treated mouse (B), DSS mice (C, E, and G), and DSS + GA mice (D, F, and H). Representative images from colons harvested on days 7 (C and D), 10 (E and F), and 14 (G and H) after DSS induction (a total of nine mice per experiment; magnification, 40×).

DSS-induced colitis is the experimental animal model for human IBD that is utilized increasingly in mice and other rodents due to its simplicity and reproducibility (Okayasu et al., 1990). DSS provided in the drinking water produces epithelial barrier breakdown and leads to well characterized histological changes in the colon, including ulceration, infiltration of inflammatory cells into the lamina propria, and focal crypt damage (Okayasu et al., 1990). Therefore, the DSS model provides a unique opportunity to examine therapeutic approaches to target the innate and adaptive immune systems (Dieleman et al., 1994). In the acute DSS model, it appears that inflammation is not the primary event but is secondary to crypt loss that evolves into a chronic colitis with similar manifestations to those in the chronic model induced by multiple cycles of DSS (Cooper et al., 1993). It also may be associated with failure to maintain intestinal epithelial barrier integrity, which can be fatal (Ford, 2006). Syndecan-1 was shown to play a major role in maintaining murine and human intestinal epithelial barrier function (Bode et al., 2008) and in ulcer healing (Day and Forbes, 1999). Furthermore, loss of syndecan-1 has been observed in the ulcerated mucosa of patients with IBD (Day et al., 1999, 2003). This loss may lead to a reduced rate of tissue repair and to sustained chronic inflammation (Day and Forbes, 1999), thus implying a correlation between the low levels of syndecan-1 and the pathogenesis of IBD.

The results of this study illustrate that indeed, in both acute and chronic DSS colitis, the expression of syndecan-1 is reduced significantly on the surface intestinal epithelium. Daily GA treatment, which ameliorates IBD symptoms (Aharoni et al., 2005, 2006), prevented the reduction of syndecan-1 levels in parallel to its beneficial therapeutic effect on
Benificial Effect of GA Treatment on Syndecan-1 in DSS

the clinical manifestations of the disease. Moreover, adoptive transfer of GA-specific T cells that reach the gut and suppress disease (Aharoni et al., 2007) was accompanied by augmented syndecan-1 expression in the colon—enhancement that led to syndecan-1 levels not significantly different from the levels observed in control mice. Furthermore, areas of increased syndecan-1 levels coincide with the presence of GA-specific cells in the colon. These results imply that the transplanted GA-induced cells are the mediators for the higher syndecan-1 levels observed.

In the acute DSS model, the rapid establishment of symptoms (within the first few days after DSS ingestion) suggests that innate immunity might play a central role in the generation of the lesion, manifested by elevated expression of macrophage-derived cytokines such as TNF-α, IL-1β, and IL-6 (Dieleman et al., 1998; Melgar et al., 2005) but not the T cell cytokines IL-3 or IFN-γ (Dieleman et al., 1994). There are some indications for a correlation between these proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and the down-regulation of syndecan-1 expression (Day et al., 2003). Furthermore, the immediate elevation of TNF-α followed by the secretion of IFN-γ resulted in a synergistic reduction of syndecan-1 expression in the intestinal epithelial cells (Henry-Stanley et al., 2006; Bode et al., 2008). In turn, reduced syndecan-1 expression amplifies the effects of TNF-α and IFN-γ, leading to a positive feedback of further syndecan-1 loss and perpetuation of the chronic inflammatory state (Bode et al., 2008).

An inappropriate activation of the intestinal immune system predominantly involves CD4+ T helper 1 (Th1) cells that create an imbalance between proinflammatory and anti-inflammatory processes that play a pivotal role in the pathogenesis of IBD (Shanahan, 2001). In previous studies in our laboratory, we illustrated that DSS colitis results in increased levels of TNF-α, IFN-γ, and the Th1 T-box transcription factor in colon samples. GA treatment diminished colonic Th1 cytokine levels (Aharoni et al., 2006) and macrophage production of IL-1β (Weber et al., 2007) while inducing expression of TGF-β, IL-10, and the Th2 transcription factor GATA-3 (Aharoni et al., 2006). In the present study, we confirm these results, namely, GA treatment improves the clinical state of DSS colitis mice and histological damage in their colon, and further demonstrate that GA treatment prevents the suppression of syndecan-1 levels in both acute and chronic colitis (Figs. 3 and 4). Therefore, it is plausible that GA acts by deviating CD4+ pathogenic T cells from Th1 to Th2 phenotypes, thus hindering the synergistic effect of TNF-α and IFN-γ and consequently maintaining the syndecan-1 level almost to that of control mice. In addition, GA up-regulates TGF-β expression (Th2/3) (Aharoni et al., 2007), which is a general inducer of epithelial syndecan-1 (Hayashida et al., 2006). Whether these changes are primary or secondary to the inflammation, lower levels of syndecan-1 will exacerbate inflammation and complicate the resolution

Fig. 5. Quantification of syndecan-1 in both acute and chronic DSS colitis. The intensity of specific syndecan-1 staining was evaluated by measuring the IOD of its expression. Digital images (1 mm²) of five sections from each mouse of the surface epithelium area in the distal colon were evaluated. Each section was divided into five subareas, and the IOD of the staining was analyzed using the Image-Pro Plus 4.1 software. An average of 25 subareas per mouse is presented. Quantification results of acute DSS colitis (A) (days 7, 10, and 14) and chronic DSS colitis (B) (day 26; individual mouse as well as an average of four mice per group) are presented. ***, p < 0.0005, significant effect GA treatment versus DSS mice; ###, p < 0.0005, significant effect of DSS versus control mice.

Fig. 6. Colocalization of adoptively transferred GA-specific T cells and syndecan-1 staining in the intestinal epithelium. β-Gal expressing GA-specific T cells were established from ROSA26 transgenic mice. Cells obtained after one in vitro stimulation with GA were adoptively transferred to C57BL/6 mice on the day of DSS induction. Ten days after cell injection, colons were excised, sectioned (20 μm), and examined for the presence of labeled cells (blue) and syndecan-1 immunostaining (red). Representative pictures of control mouse (A), DSS-induced colitis mouse (B), and DSS-induced colitis mouse treated with GA-specific cells (C) are demonstrated.
of lesions; hence, restoring appropriate barrier function remains a worthwhile therapeutic objective in IBD. Adaptive transfer of GA-specific cells also leads to the amelioration of DSS colitis (Aharoni et al., 2007) and, as shown here, to increased syndecan-1 levels in the vicinity of the transplanted plants. Because the transplanted cells were shown to express TGF-β in situ and not TNF-α, they could support syndecan-1 production in the gut epithelium. Thus, GA or GA-specific T cell treatment shifts the cytokine profile and leads to an up-regulation of syndecan-1 expression in DSS colitis. In addition, clinical improvement and restoration of syndecan-1 levels are observed shortly after disease induction, suggesting that the innate arm is involved directly. Therefore, it is possible that the beneficial effect of GA in experimental colitis models originates also from its modulation of the innate immune response.

The pathophysiology of intestinal inflammation in IBD is multifactorial. Increased epithelial permeability, due to barrier dysfunction, plays a central role in the inflammatory process. Syndecan-1 is the predominant HSPG on intestinal epithelial cells; its main functions are tissue repair and maintenance of the normal intestinal epithelial barrier (Day and Forbes, 1999; Day et al., 1999; Bode et al., 2008). Loss of syndecan-1, which has been observed in the colons of IBD patients, may be involved in impaired intestinal healing (Day et al., 1999). Heparin has been suggested as a treatment for IBD patients, but it has serious side effects (Gaffney et al., 1994). In the present study, we demonstrate the credibility of the DSS colitis model by revealing syndecan-1 reduction in the epithelium of mice with DSS colitis. In addition, we showed that GA treatment prevented the decrease of syndecan-1 levels, which may indicate a possible link to the improved regeneration and stabilization of the epithelial barrier concomitant with its effect in ameliorating the pathological manifestations of DSS colitis. Thus, GA with its high safety profile may provide the benefit of a novel drug for IBD that has a different therapeutic mechanism, namely, modulating rather than suppressing the immune response. One benefit of GA therapy could be the sustained expression of syndecan-1 within the normal epithelial architecture. These findings might encourage clinical trials for the evaluation of GA treatment for human IBD, mainly Crohn's disease.

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Authorship Contributions

Participated in research design: Yablecovitch, Shabat-Simon, Aharoni, and Arnon.

Conducted experiments: Yablecovitch, Shabat-Simon, Aharoni, and Eilam.

Contributed new reagents or analytic tools: Eilam and Brenner.

Performed data analysis: Yablecovitch and Shabat-Simon.

Wrote or contributed to the writing of the manuscript: Yablecovitch, Shabat-Simon, Arnon and Eilam.

References


Beneficial Effect of GA Treatment on Syndecan-1 in DSS


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