Effect of Adiponectin on Murine Colitis Induced by Dextran Sulfate Sodium

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Background & Aims: Adiponectin, an adipose tissue–derived hormone, exhibits anti-inflammatory properties and has various biological functions, such as increasing insulin sensitivity, reducing hypertension, and suppressing atherosclerosis, liver fibrosis, and tumor growth. The aim of the present study was to determine the effect of adiponectin on intestinal inflammation. **Methods:** We investigated the effect of adiponectin on dextran sulfate sodium (DSS)-induced colitis by using adiponectin-knockout (APN-KO) mice and an adenovirus-mediated adiponectin expression system. We also examined the contribution of adiponectin deficiency to trinitrobenzene sulfonic acid (TNBS)-induced colitis. In vitro, we examined the effect of adiponectin on intestinal epithelial cells. **Results:** After administration of 0.5% DSS for 15 days, APN-KO mice developed much more severe colitis compared with wild-type mice. The messenger RNA expression levels of chemokines were significantly higher in the colonic tissues of DSS-treated APN-KO mice compared with wild-type mice, accompanied by increased cellular infiltration, including macrophages. Adenovirus-mediated supplementation of adiponectin significantly attenuated the severity of colitis, but there were no differences in the severity of TNBS-induced colitis between the 2 groups. Adiponectin receptors were expressed in intestinal epithelial cells, and adiponectin inhibited lipopolysaccharide-induced interleukin-8 production in intestinal epithelial cells. **Conclusions:** Adiponectin is protective against DSS-induced murine colitis, probably due to the inhibition of chemokine production in intestinal epithelial cells and the following inflammatory responses, including infiltration of macrophages and release of proinflammatory cytokines.

Inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, are disorders of unknown etiology characterized by chronic relapsing inflammation of the gastrointestinal tract.[1] The current literature suggests that various immune, genetic, and environmental factors influence both the initiation and progression of colitis.[2–4] During the past half century, the incidence of inflammatory bowel disease has markedly increased in developed countries,[2–4] where a marked increase in the proportion of people with obesity and/or obesity-associated metabolic syndrome has become a social problem.[5,6] This suggests that changes in lifestyle may contribute to the development of inflammatory bowel disease and obesity.

There is ample evidence that adipose tissue produces and secretes a variety of biologically active molecules[5,6] (conceptualized as adipocytokines[7] and include adiponectin,[8] leptin,[6] plasminogen activator inhibitor 1,[7] and tumor necrosis factor α[9]). A series of studies have shown that dysregulated production of these adipocytokines participates in the pathogenesis of obesity-associated metabolic diseases. Adiponectin is an adipocyte-specific secretory factor that we identified in human complementary DNA.[8] The mouse homologue of adiponectin was independently cloned as ACRP30 or AdipoQ.[10,11] We and others have shown that adiponectin has a wide array of biological functions, such as increasing insulin sensitivity in the liver and skeletal muscles,[12,13] preventing atherosclerosis,[14] and inhibiting fatty liver[15] and liver fibrosis.[16] Recent data have pointed to the anti-inflammatory effects of adiponectin, especially in endothelial cells[17] and macrophages.[18–20] In addition, we recently reported that the hypertrophied mesenteric adipose tissue of patients with Crohn's disease produces and secretes high levels of adiponectin, and the expression level of adiponectin in mesenteric adipose tissue inversely correlates with disease severity.[21] This suggests that adiponectin might have a potential role in the suppression of colitis.

Intestinal epithelial cells produce a variety of immunomodulatory substances, including the chemokine interleukin (IL)-8, and play crucial roles in regulating the inflammatory response in the pathogenesis of inflammatory bowel disease.[22,23] Production of the chemokine macrophage inflammatory protein (MIP)-2, which acts in the mouse in a manner similar to IL-8 in humans, in intestinal epithelial cells has been implicated as an important mechanism of dextran sulfate sodium (DSS)-induced murine colitis.[24] It has been reported that adiponectin inhibits IL-8 secretion from the endothelial cells,[25] but whether it affects IL-8 production in intestinal epithelial cells has not yet been defined.

In the present study, we investigated the effects of adiponectin on experimental colitis induced by DSS or trinitrobenzene sulfonic acid (TNBS) using adiponectin-knockout (APN-KO) mice and an adenovirus-mediated adiponectin expression system. To determine the direct effect of adiponectin on intestinal epithelium, we examined the effect of adiponectin on IL-8 production in HT-29 cells in vitro.

Materials and Methods

Animals

APN-KO mice were generated as described previously and backcrossed to the C57BL/6J strain for more than 5 gen-

Abbreviations used in this paper: Ad-APN, adenovirus-adiponectin; Ad-β-gal, adenovirus-β-galactosidase; APN-KO, adiponectin-knockout; DAI, disease activity index; DSS, dextran sulfate sodium; IL, interleukin; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RT-PCR, reverse-transcription polymerase chain reaction; TNBS, trinitrobenzene sulfonic acid; WT, wild-type.

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erations.[13] The control C57BL/6J mice were purchased from Clea Japan (Tokyo, Japan) and maintained for 1–2 weeks in the same circumstances before starting DSS treatment. Mice were maintained on a 12-hour light/dark cycle under pathogen-free conditions and had ad libitum access to a standard diet and water until reaching the desired age (8–10 weeks).

Induction of Colitis by DSS

Experiment 1. Male mice were treated with 0.5% DSS (mol wt, 36–50 kilodaltons; MP Biomedicals, Aurora, OH) dissolved in sterile filter-purified distilled water (Millipore Corp, Bedford, MA). There was no restriction regarding the dose taken, and the DSS solution was provided ad libitum for 15 days. Fresh DSS solutions were prepared on day 5 and day 10. Control mice (not treated with DSS) received sterile filter-purified distilled water alone.

To determine the optimum dose of DSS and duration of treatment, we performed several preliminary experiments. When treated with 2.5% DSS for 7 days, both APN-KO and wild-type (WT) mice developed colitis that was too severe to allow us to see any differences between the 2 groups. Then, we tried another protocol with a lower dose of DSS for a longer duration. When treated with 1% DSS for 10 days, the disease activity index (DAI) of APN-KO mice tended to be higher than that of WT mice, although the difference was not statistically significant. Next, when treated with 0.5% DSS for 15 days, WT mice developed only minimal colitis and APN-KO mice developed significantly severe colitis. We stopped treatment and analyzed all mice on day 15, when one APN-KO mouse died of colitis. Based on these data in our preliminary studies, we judged that a protocol of 0.5% DSS for 15 days was optimal for examining the susceptibility of APN-KO mice to DSS-induced colitis.

Experiment 2. To investigate the disease severity during recovery and the survival rate, male mice were treated with 2.5% DSS for 5 days and water for the following 7 days.

Calculation of DSS Load

DSS load for all DSS-treated mice was calculated using the following formula: *Load = ([Total Drinking Volume {mL} × DSS {mg} per 100 mL]/Initial Body Wt [g])*. The drinking volume was recorded every 5 days using a calibrated water bottle.

Assessment of Inflammation in DSS-Induced Colitis

Daily clinical evaluations included measurement of body weight, observation of stool consistency, and determination of the presence of blood in the stool by a guaiac paper test (ColoScreen; Helena Laboratories Inc, Beaumont, TX). A previously validated clinical DAI ranging from 0 to 4 was scored using the parameters of weight loss, stool consistency, and the presence or absence of fecal blood.[26]

Experiment 1. On day 15, mice were killed, colons removed, and colon length and weight measured before processing for histopathologic analysis and inflammatory-related gene messenger RNA (mRNA) expression.

Experiment 2. We evaluated the DAI and the survival rate.

Histopathologic Analysis of DSS-Induced Colitis

Five samples were collected from each animal from each region of the colon (proximal, middle, and distal colon) for histopathologic examination. The samples were fixed in 10% buffered formalin and embedded in paraffin and then assessed in a blinded fashion using a scoring system described previously.[27] Briefly, 3 parameters were measured: severity of inflammation (0, none; 1, slight; 2, moderate; 3, severe), extent of injury (0, none; 1, mucosal; 2, mucosal and submucosal; 3, transmural), and crypt damage (0, none; 1, basal one third damaged; 2, basal two thirds damaged; 3, only surface epithelium intact; 4, entire crypt and epithelium lost). The score of each parameter was multiplied by a factor reflecting the percentage of tissue involvement (×1, 0–25%; ×2, 26%–50%; ×3, 51%–75%; ×4, 76%–100%), and all numbers were summed. The combined histopathologic score ranged from 0 to 40.

Immunohistochemical Analysis

The monoclonal antibody F4/80 (Serotec, Oxford, England) was used to track macrophage infiltration. After a limited trypsin (Sigma-Aldrich, St. Louis, MO) digestion for 10 minutes, endogenous peroxidase activity was blocked using 0.3% H_2O_2 in methanol for 30 minutes. Sections were incubated with 1.5% (vol/vol) normal sheep serum to reduce nonspecific reactions and then incubated overnight with biotinylated rat anti-mouse F4/80 (1:200) at 4°C. Further steps were performed according to the instructions provided on the labeling of the Vectastain Elite ABC (avidin/biotin complex) System (Vector Laboratories, Burlingame, CA). Sections were then washed in water, counterstained with methyl green, dehydrated, and mounted.

Preparation and Administration of Adenovirus

Adenovirus producing the full-length mouse adiponectin was prepared by using the Adenovirus Expression Vector Kit (Takara, Kyoto, Japan). To test the effect of adiponectin, 5×10^7 plaque-forming units of adenovirus-adiponectin (Ad-APN) or adenovirus-β-galactosidase (Ad-β-gal) were injected into the jugular vein of mice 2 days before the administration of DSS. On day 17 after the virus injection (on day 15 after administration of DSS), mice were killed for analysis.

Immunoblotting

Immunoblotting analysis of adiponectin was performed as described previously.[13] The protein bands were quantified using an imaging densitometer (FluorChem; Alpha Innotech Corp, San Leandro, CA).

Cell Cultures and Preparation of Recombinant Adiponectin

In the present study, we used several human intestinal epithelial cell lines (CaCo-2, T-84, SW-480, and HT-29 cells) and a human hepatoma cell line (HepG2 cell). All cell lines were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured at 37°C using 5% CO_2 in the following media supplemented with 10% fetal bovine serum (Sigma Chemical Co, St Louis, MO) and 100 U/mL each of penicillin and streptomycin (Nacalai Tesque, Kyoto, Japan): Dulbecco's