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Catestatin decreases macrophage function in two mouse models of experimental colitis

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ABSTRACT

Mucosal inflammation in patients with inflammatory bowel disease (IBD) is characterized by an alteration of prohormone chromogranin A (CgA) production. The recent demonstration of an implication of CgA in collagenous colitis and immune regulation provides a potential link between CgA-derived peptides (catestatin, CTS) and gut inflammation.

Colitis was induced by administration of dextran sulfate sodium or 2, 4 dinitrobenzenesulfonic acid to C57BL/6 mice. Treatment with human (h)CTS or its proximal or distal part was started one day before colitis induction and colonic inflammatory markers were determined. Pro-inflammatory cytokines were evaluated in peritoneal isolated and bone marrow derived macrophages (BMDMs); p-STAT3 level was studied. Serum levels of CgA and CTS were assessed in experimental colitis and in a separate study in IBD patients and healthy controls.

We show that sera from IBD patients and that in experimental colitis conditions the colonic level of mouse (m)CgA and mCTS are significantly increased. Moreover, *in vivo* treatment with human (h)CTS reduces the disease onset and suppresses exacerbated inflammatory responses in preclinical settings of colitis associated with an increase of p-STAT3. *In vitro*, hCTS treatment decreases proinflammatory cytokine release by peritoneal macrophages and BMDMs and increases p-STAT3 levels.

These results support the hypothesis that CTS is increased during colitis and that hCTS modulates intestinal inflammation *via* the macrophage population and through a STAT-3 dependent pathway in a murine model of colitis. Identification of the molecular mechanism underlying the protective role of this peptide may lead to a novel therapeutic option in IBD.

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1. Introduction

Inflammatory bowel diseases (IBDs), consisting of Crohn's disease (CD) and ulcerative colitis (UC), are characterized by a chronic relapsing and remitting course that results from intestinal inflammation [1]. The apparent therapeutic benefit of biological therapy (tumor necrosis factor-alpha (TNF-α)—neutralizing antibody) [2], corticosteroids and thiopurines underscores the importance of the dysregulated immune response. However, some patients are resistant to these drugs, and all of these therapeutic agents have adverse side effects [3,4]. For the most ill patients, monoclonal antibodies to TNF-α are used, however, these agents are expensive, require indefinite use, and concern for infectious and potentially even malignant complications [5] limit physicians' decisions to introduce these agents earlier in the treatment paradigm. Therefore, new cost effective agents need to be developed.

Mucosal changes in IBD are reflected by mucosal and transmural inflammation accompanied by a prominent infiltrate

Abbreviations: BMDM, bone marrow-derived macrophages; CTS, catestatin; CgA, chromogranin A; CD, Crohn's disease; CRP, C-reactive protein; DAI, disease activity index; DNBS, 2,4 dinitrobenzene sulfonic acid; DSS, dextran sodium sulphate; EC, enterochromaffin cells; IBD, inflammatory bowel diseases; IL, interleukin; LPS, lipopolysaccharide; MPO, myeloperoxidase; STAT3, signal transducer and activator of transcription 3; TNF, tumor necrosis factor; UC, ulcerative colitis.

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of activated cells from both the innate and adaptive immune systems. The release of inflammatory mediators, including pro-inflammatory cytokines from immune cells [6,7], mediates tissue injury and exacerbation of IBD. In addition to immune cells, inflammation in the gut is associated with an alteration in enterochromaffin (EC) cells releasing mainly serotonin and chromogranin-A (CgA) [8].

The human CgA gene consists of eight exons separated by seven introns and has been mapped to chromosome 14q32.16. It translates to a 457 amino acids protein containing a signal peptide of 18 amino acids associated with a mature human CgA protein of 439 amino acids. After chromaffin cells, EC cells are the main source of CgA in the gut [9] which is an important enteric mucosal signaling molecule influencing gut physiology [10,11]. The overall homology for CgA in different vertebrates is around 40%, but the most highly conserved regions occur at the N and C-termini, which show up to 88% sequence homology. Cell- and tissue-specific processing of CgA has been described in the rat, mouse and human GI tract [12]. The presence of numerous pairs of basic amino acids indicate potential sites for cleavage by prohormone convertases 1/3 or 2, carboxypeptidase E/H [13], consistent with evidence that CgA may serve as a prohormone for shorter bioactive fragments [14]. Proteolytic fragments of CgA exert a broad spectrum of regulatory activities on the cardiovascular, endocrine and immune systems [15]. Among its highly conserved C-terminal regions, CgA gives rise to main peptides of biological importance: the antihypertensive peptide catestatin (human CTS; $hCgA_{352–372}$) [16–18] and its short version cateslytin (human CTL; $hCgA_{352–366}$), which have immune regulation properties [19] and regulate smooth muscle cell proliferation [20]. Besides the physiological context, CgA or CgA-derived peptides (CgDPs) may play an important role in the immune interaction in relation to inflammation.

Recently, links between the serum CgA concentration and outcome in patients admitted with systemic inflammatory response syndrome, periodontitis, diabetic retinopathy [21] and rheumatoid arthritis [22] have been reported. Moreover, elevated levels of CgA have been found in patients with IBD [23], lymphocytic colitis [24], and collagenous colitis [25], where at long term the disease can lead to the development of colon cancer.

Patient with IBD have been reported to have an increased risk of colorectal cancer [26,27], and risk factors include the severity of inflammation, a family history of colon cancer, and disease duration [28]. It is suspected that chronic inflammation, immune cell activation, and specific release of molecules promoting cell migration are implicated in the long-term development of dysplasia and colon carcinoma. For decades, CgA has been used as a marker for colorectal carcinoma and transitional mucosa [15]. Thus, a close relation between chronic inflammation, immune cell activation and release of cell migration-promoting molecules should exist. A recent work from Rumio et al. demonstrated that the conserved N-terminal fragment of CgA (vasostatin-1) has a protective effect in mice developing acute and chronic colitis [29].

Macrophages play a key role in host defense against bacterial pathogens that stimulate them *via* the activation of toll-like receptors. Macrophage activation results in the secretion of proinflammatory cytokines such as TNF-α, interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) and in the induction of a Th-1 cytokine response, with the production of interferon-gamma (INF-γ). In this context, macrophages are considered to be classical proinflammatory effector cells [30]. Human studies revealed that there is a significant increase in the numbers of macrophages within the inflamed tissue and the peripheral blood of patients with CD or UC [31,32]. In addition, macrophage depletion in the dextran sulfate sodium (DSS) colitis model almost completely inhibited experimental colitis [33]. Recent data has demonstrated that CTS can modulate monocyte migration [19].

Therefore, to evaluate the potential of hCTS and to decipher the sequence of the peptides implicated, herein we evaluated the effect of hCTS and its proximal and distal sequence in two murine models of colitis and evaluated their effects on macrophages. We report that treatments with hCTS, or its derived sequences, significantly ameliorate disease severity and inhibit inflammation in the context of DSS- and 2,4-dinitrobenzene sulfonic acid (DNBS)-induced experimental colitis. This therapeutic efficacy is mediated through the regulation of peritoneal macrophages cytokine production *via* an intracellular mechanism that implicates the signal transducer and activator of transcription 3 (STAT-3) protein.

2. Material & methods

2.1. Animals

Male C57BL/6 (7–9 weeks old) mice were purchased from Charles Rivers (Canada) and maintained in the animal care facility at the University of Manitoba under specific pathogen-free conditions. All experiments were approved by the University of Manitoba Animal Ethics Committee (10-073) and conducted under the Canadian guidelines for animal research.

2.2. Peptides used

hCTS ($hCgA_{352–372}$: SSMKLSFRARAYGFRGPGPQL [17]); modified hCTS ($shCgA_{352–372}$: SLPRRQLPSSAGMRGGKFAYF); hCTS, proximal sequence ($hCgA_{352–366}$: SSMKLSFRARAYGFR), and hCTS, distal sequence ($hCgA_{360–372}$: ARAYGFRGPGPQL) were used (gift from Dr. Metz-Boutigue or purchased from Biopeptide Co., Inc, San Diego, CA). The peptides were used at different doses ranging from 0.5 to 1.5 mg/kg/day as reflected by previous published data related to the use of peptide for intra-rectal injection [34].

2.3. DSS and 2,4-DNBS colitis

DSS (molecular weight [*MW*], 40 kDa: MP Biomedicals, Soho, OH) was added to the drinking water at a final concentration of 5% (wt/vol) for 5 days [33,35]. Controls were time-matched and consisted of mice that received normal drinking water only. Mean DSS consumption was noted per cage each day. For the DNBS study, mice were anaesthetized using Isoflurane® (Abbott, Toronto, Canada). PE-90 tubing (10 cm long; ClayAdam, Parisppany, NJ) that was attached to a tuberculin syringe (BD, Mississauga, Canada) was inserted 3.5 cm into the colon. Colitis was induced by administration of 100 μl of 4 mg of DNBS solution (ICN Biomedical Inc. Aurora, OH) in 30% ethanol (Sigma, Mississauga, Canada) and left for 3 days [36]. For the DNBS study mice were supplied with 6% sucrose in their drinking water to prevent dehydration.

2.4. Assessment of colitis severity—Disease activity index (DAI)

DAI scores have historically correlated well with the pathological findings in a DSS-induced model of IBD [37]. DAI is the combined score of weight loss, stool consistency, and bleeding. Scores were defined as follows: weight: 0, no loss; 1, 5–10%; 2, 10–15%; 3, 15–20%; and 4, 20% weight loss; stool: 0, normal; 2, loose stool; and 4, diarrhoea; and bleeding: 0, no blood; 2, presence of blood; and 4, gross blood. Blood was assessed using the Hemoccult II test (Beckman Coulter, Oakville, Canada). DAI was scored from day 0 to day 5 during DSS treatment.

2.5. Macrophage isolation

Five or three days after the beginning of the DSS or the DNBS induction, respectively, resident peritoneal cells were collected as

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