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Prolongation of carrageenan-induced inflammation in human colonic epithelial cells by activation of an NFκB-BCL10 loop

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ABSTRACT

Carrageenan, a sulfated polysaccharide that is widely used as a food additive, induces inflammatory responses in animal models and human cells. The carrageenan-induced inflammatory cascades involve toll-like receptor (TLR)4- and B-cell leukemia/lymphoma (BCL)10-dependent activation of NF-κB, leading to increased IL-8 production. Translocations involving BCL10 in the mucosa-associated lymphoid tissue (MALT) lymphomas are associated with constitutive activation of NF-κB. This report presents a mechanism by which carrageenan exposure leads to prolonged activation of both BCL10 and NF-κB in human colonic epithelial cells. Study findings demonstrate that nuclear RelA and RelB bind to an NF-κB binding motif in the BCL10 promoter in human colonic epithelial NCM460 and HT-29 cells. *In vitro* oligonucleotide binding assay, non-radioactive gel shift assay, and chromatin immunoprecipitation (ChIP) indicate binding of RelA and RelB to the BCL10 promoter. Prolonged inflammation follows activation of the BCL10-NFκB inflammatory loop in response to carrageenan, shown by increased BCL10, RelA, and IL-8 for 36 to 48 h and increased RelB for 24 h following withdrawal of carrageenan after 12 h. In contrast, exposure to dextran sulfate sodium, which does not cause inflammation through TLR4 and BCL10 in the colonic epithelial cells, did not provoke prolonged activation of inflammation. The carrageenan-enhanced BCL10 promoter activity was blocked by caffeic acid phenethyl ester (CAPE) and MB-132 which inhibit NF-κB activation. These results indicate that NF-κB binding to the BCL10 promoter can lead to prolonged activation of the carrageenan-induced inflammatory cascade by a transcriptional mechanism involving an NF-κB-BCL10 loop.

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1. Introduction

1.1. Background about carrageenan exposure

Carrageenans are highly sulfated polysaccharides that are obtained from red seaweeds (Rhodophycae). They have been widely used for decades as a thickener, stabilizer, or emulsifying agent in many processed foods in the Western diet, including dairy products, processed meats, soymilk, and infant formula, and are also used in a variety of other products, such as cosmetics, toothpaste, room deodorizers, and pharmaceuticals. Current data suggest an average consumption of 250 mg/day of carrageenan in the United States. Multiple studies in mammals have demonstrated that carrageenan exposure predictably causes inflammation, including development of ulcerations, polyps, colitis, and colorectal tumors, and carrageenan has been used in thousands of cell-based and animal experiments to cause inflammation, primarily to study mediators of inflammation and anti-inflammatory therapeutics [1–3].

1.2. Carrageenan stimulates TLR4-BCL10 mediated pathway of inflammation

In human colonic epithelial cells and murine models, we have reported that carrageenan triggers innate immune pathways of inflammation in which TLR4 and BCL10 are critical [4–8]. The inflammatory response initiated by carrageenan exposure activated both canonical, involving RelA (p65) and p50, and non-canonical, involving RelB and p52, pathways of NF-κB activation. We have

Abbreviations: BCL10, B-cell leukemia/lymphoma 10; CAPE, caffeic phenethyl ester; CARMA, caspase recruitment domain membrane-associated guanylate kinase; CBM, CARMA-BCL10-MALT complex; CGN, carrageenan; ChIP, chromatic immunoprecipitation; DSS, dextran sulfate sodium; Hsp, heat-shock protein; MALT, mucosa-associated lymphoid tissue; IκB, inhibitor of κB; IKK, inhibitor of IκB kinase; IL-8, Interleukin-8; IRAK, Interleukin-β receptor associated kinase; NF, NF-κB consensus oligonucleotide; NF-κB, nuclear factor kappaB; NFE, experimental NF-κB binding site in BCL10 promoter; NFEM, mutated experimental NF-κB binding site in BCL10 promoter; NIK, NF-κB inducing kinase; PAF, platelet-activating factor; ROS, reactive oxygen species; TLR4, Toll-like receptor 4; Ub, ubiquitin

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demonstrated that carrageenan induces inflammatory responses via three cascades: 1) a TLR4, BCL10, IkappaB kinase (IKK)γ, and phospho-IκBα-mediated activation of RelA; 2) a TLR4, BCL10, phospho-NF-κB-inducing kinase (NIK), IKKα-mediated activation of the non-canonical pathway leading to nuclear translocation of p52 and RelB; and 3) a reactive oxygen species (ROS)-mediated pathway requiring Hsp27 and IKKβ (Fig. 1) [4–8]. Recent work also demonstrated that carrageenan-induced inflammation caused glucose intolerance, insulin resistance, and impaired insulin signaling in mouse and cell-based studies [9]. These effects are consistent with the role of TLR4-induced inflammation reported in diabetes and carrageenan stimulation of TLR4-mediated inflammatory cascades [10].

1.3. BCL10 is associated with constitutive activation of NF-κB in the MALT lymphomas

BCL10, which encodes a cytosolic protein composed of 233 amino acids, has a pivotal role in the innate immune-mediated pathways of inflammation that require TLR4. The BCL10 gene (locus 1p22) was originally identified from a recurrent breakpoint t(1;14)(p22;q32) found in gastric mucosa-associated lymphoid tissue (MALT) lymphomas that was associated with constitutive activation of NF-κB [11,12]. BCL10 was shown to be an adaptor protein that mediated canonical NF-κB signaling in T and B lymphocytes. Subsequently, a critical role for BCL10 in non-myeloid cells was identified, including mediation of the inflammatory cascade in response to carrageenan, lipopolysaccharide, platelet-activating factor (PAF), lysophosphatidic acid, and angiotensin II [4,13–17]. Experiments with BCL10 silencing and mutation in human colonic epithelial cells demonstrated a requirement for BCL10 in the production of canonical and non-canonical activation of NF-κB, involving NF-κB components RelA and RelB, respectively [6,7,18]. In this report, we present a transcriptional mechanism by which carrageenan initiates an inflammatory loop, involving the up-regulation of BCL10 expression and prolonged activation of canonical and non-canonical NF-κB pathways of inflammation. This transcriptional effect is based on our previous identification of a putative NF-κB binding sequence in the BCL10 promoter that was activated following exposure to PAF [17].

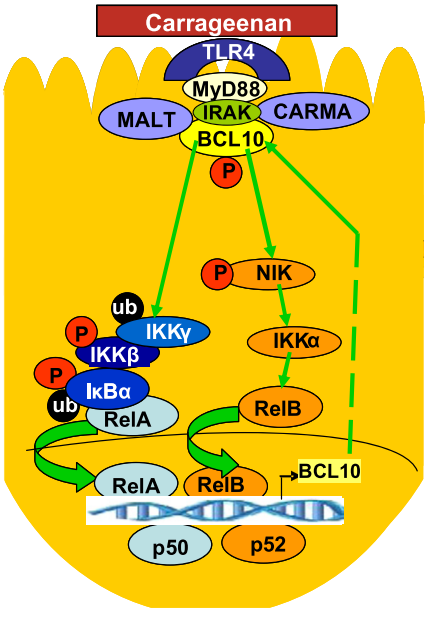

Fig. 1. Schematic illustration of carrageenan-stimulated inflammatory signaling pathways in human colonocytes. Schematic illustration indicates the presence of a signaling loop following exposure to carrageenan. Carrageenan through TLR4 and BCL10 leads to nuclear translocation/activation of NF-κB, both RelA and RelB. In turn, these nuclear factors bind to the putative NF-κB binding element in the BCL10 promoter, stimulating increased expression of BCL10, which again leads to increased nuclear translocation/activation of NF-κB through effects on the IKK signalosome.

2. Materials and methods

2.1. Cell culture of colonic epithelial cells

NCM460 cells, a human colonic epithelial cell line derived from normal colonic mucosa, were grown in M3:10™ media (INCELL, San Antonio, TX) and maintained at 37 °C in a humidified 5%CO_2 environment with media changes at 2-day intervals [19]. The HT-29 (ATCC #HTB-38) cell line, a human colonic adenocarcinoma cell line, was grown in DMEM media with 10% FBS.

2.2. BCL10 promoter activity by luciferase assay

A 1310 bp promoter region of BCL10 gene (NM_003921) that was cloned previously into pGL2 plasmid (Promega, Fitchburg, WI) was transiently transfected into NCM460 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) [17]. Twenty-four hours post-transfection, cells were treated with λ-carrageenan (1 μg/ml; Sigma Chemical Company, St. Louis, MO) for 24 h. Promoter activity was measured utilizing firefly luciferase assay kit (Promega) as described previously and expressed as relative luciferase units (RLU)/mg protein [17]. Putative transcription factor binding sites in the promoter region were identified using TFSEARCH and Motif Search (http://motif.genome.jp/), as previously described [17].

2.3. BCL10 and phospho-BCL10 by ELISA

A standardized BCL10 ELISA was used to determine the BCL10 content following stimulation by carrageenan, with or without caffeic acid phenethyl ester (CAPE), an inhibitor of NF-κB activation or MG-132, a proteasomal inhibitor [4,20,21]. Cells were pre-treated with CAPE (50 μM × 1 h) or MG-132 (20 μM × 2 h), and then in combination with carrageenan (1 μg/ml × 24 h). Phospho-BCL10 was detected by cell-based ELISA using phospho(Ser138)-BCL10 antibody, as previously described [6,7].

2.4. RelA, RelB and c-Rel binding to BCL10 promoter by oligonucleotide-based ELISA

Nuclear extracts were prepared from control or carrageenan-treated NCM460 cells by a nuclear extraction kit (Active Motif, Carlsbad, CA). Sense and antisense oligonucleotides encompassing the putative NF-κB binding region in the BCL10 promoter and corresponding mutated constructs were commercially synthesized. After being annealed, the double-stranded oligonucleotides [NF-κB (NF) consensus oligonucleotide: 5′-GGGACTTTCC-3′; NF-κB Experimental binding site in BCL10 promoter (NFE): 5′-GGAAACGCCC-3′; NFE Mutated (NFEM): 5′-G**TCC**ACGCCC-3′] were coated onto the wells of 96-well microtiter plates according to the reported procedure [22]. Treated and control nuclear extract samples were added to the coated wells and incubated for 1 h. NF-κB components (RelA, RelB or c-Rel) bound to the coated oligonucleotides were captured by anti-RelA, anti-RelB or anti-c-Rel antibodies and detected by an anti-rabbit-HRP-conjugated IgG (Active Motif). Color development was performed with hydrogen peroxide/TMB chromogenic substrate, and intensity of the developed color proportionately represented the quantity of NF-κB component in each sample. The sample values were normalized with the total cell protein determined by protein assay kit (Pierce, ThermoFisher Scientific, Rockford, IL).