Impact of intrarectal chromofungin treatment on dendritic cells-related markers in different immune compartments in colonic inflammatory conditions

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Abstract

BACKGROUND

Chromofungin (CHR: chromogranin-A 47-66) is a chromogranin-A derived peptide with anti-inflammatory and anti-microbial properties. Ulcerative colitis (UC) is characterized by a colonic decrease of CHR and a dysregulation of dendritic CD11c+ cells.

AIM

To investigate the association between CHR treatment and dendritic cells (DCs)-related markers in different immune compartments in colitis.

METHODS

A model of acute UC-like colitis using dextran sulphate sodium (DSS) was used in addition to biopsies collected from UC patients.

RESULTS

Intrarectal CHR treatment reduced the severity of DSS-induced colitis and was associated with a significant decrease in the expression of CD11c, CD40, CD80, CD86 and interleukin (IL)-12p40 in the inflamed colonic mucosa and CD11c,
Bernstein CN has been on the advisory boards for Abbvie Canada, Amgen Canada, Bristol Myers Squibb Canada, Janssen Canada, Roche Canada, Sandoz Canada, Takeda Canada, Pfizer Canada and consulted to Takeda and Mylan Pharmaceuticals. He has received educational grants from Abbvie Canada, Pfizer Canada, Takeda Canada, Janssen Canada. He has been on speaker’s panel for Abbvie Canada, Medtronic Canada and Janssen Canada. The other authors declare that they have no conflicts of interest.

Data sharing statement: All data generated or analyzed during this study are included in this published article.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Supported by Crohn’s and Colitis Canada, No. P46601; Canadian Foundation for Innovation, No. P39902; Children’s Hospital Research Institute of Manitoba, No. P45040; Natural Sciences and Engineering Research Council, No. P41124; and Manitoba Health Research Council, No. P41118 (to Ghia JE).

Country/Territory of origin: Canada

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report’s scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Open-Access: This article is an open-access article that was selected by an in-house editor and

CD80, CD86 IL-6 and IL-12p40 within the mesenteric lymph nodes and the spleen. Furthermore, CHR treatment decreased CD80 and CD86 expression markers of splenic CD11c+ cells and decreased NF-κB expression in the colon and of splenic CD11c+ cells. In vitro, CHR decreased CD40, CD80, CD86 IL-6 and IL-12p40 expression in naïve bone marrow-derived CD11c+ DCs stimulated with lipopolysaccharide. Pharmacological studies demonstrated an impact of CHR on the NF-κB pathway. In patients with active UC, CHR level was reduced and showed a negative linear relationship with CD11c and CD86.

CONCLUSION

CHR has protective properties against intestinal inflammation via the regulation of DC-related markers and CD11c+ cells. CHR could be a potential therapy of UC.

Key Words: Chromofungin; Chromogranin-A; Colitis; Cytokines; Dendritic cells; Gut hormones

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Core Tip: Ulcerative colitis (UC) is characterized by a colonic decrease of chromofungin (CHR: chromogranin-A 47-66) and a dysregulation of CD11c+ dendritic cells (DC). Using a UC-like model (dextran sulphate sodium) and biopsies collected from UC patients, we demonstrated a protective effect of CHR via the regulation of DC-related markers and CD11c+ cells at the colonic, mesenteric lymph node and spleen levels, through a potential effect via the NF-κB pathway. In patients with active UC, CHR level showed a negative linear relationship with CD11c+ marker. CHR could be a potential therapy of UC, but larger samples and additional experiments are needed.

Citation: Kapoor K, Eissa N, Tshikudi D, Bernstein CN, Ghia JE. Impact of intrarectal chromofungin treatment on dendritic cells-related markers in different immune compartments in colonic inflammatory conditions. World J Gastroenterol 2021; 27(47): 8138-8155

URL: https://www.wjgnet.com/1007-9327/full/v27/i47/8138.htm

DOI: https://dx.doi.org/10.3748/wjg.v27.i47.8138

INTRODUCTION

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn’s disease (CD), disorders in which the gastrointestinal (GI) tract becomes ulcerated and inflamed[1,2]. To date, the exact etiopathology and mechanism behind them are unknown. It is believed that genetic predisposition and environmental factors lead to an abnormal mucosal immune response in the intestinal lining[3-5]. Over the last decade, the diseases have increased worldwide, but the greatest prevalence is evident in western countries[6,7]. Although treatments and therapeutic strategies are evolving quickly, treatments are still inadequate for a substantial percent of those with active IBD, and some therapies may have serious adverse side effects. Therefore, IBD needs new therapeutic approaches associated with higher efficacy and limited side effects[8].

IBD is characterized by an ongoing inflammatory process manifested by an increase of immune cell infiltration and accompanied by an up-regulation of pro-inflammatory cytokines, including interleukin (IL)-6 and IL-12p40[9]. The continuing release of mediators damages the intestinal epithelium, leading to abnormal activities of enterochromaffin cells (EC), goblet cells and Paneth cells, and impaired tissue repair[10,11]. UC is represented by an overactivation of the adaptive immune system represented by T cells which is considered a consequence of abnormal activation of innate immune cells[12] such as dendritic cells (DCs)[10,11,13-17].

DCs are produced by the bone marrow and can be located in the mucosal tissues or circulate in the lymph tissues and the blood[18]. DCs interaction and antigen presentation to T cells and their subsequent polarization can be induced within mesenteric lymph node (MLNs) and the spleen[19]. In UC, as in other chronic inflammatory diseases, DC activation is represented by an up-regulation of the CD80, CD86, and CD11c markers and is associated with an increased release of IL-6 and IL-12p40 to
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Received: March 12, 2021  
Peer-review started: March 12, 2021  
First decision: May 1, 2021  
Revised: May 12, 2021  
Accepted: December 7, 2021  
Article in press: December 7, 2021  
Published online: December 21, 2021  
P-Reviewer: Watanabe T  
S-Editor: Gao CC  
L-Editor: A  
P-Editor: Gao CC

promote a Th1 cell polarization[20,21]. In UC, the IL-12p40 pathway’s importance has been confirmed by genome-wide association studies, identifying the IL-12 genes as a major contributor[22,23]. Intracellularly, the production of IL-12p40 is regulated by different pathways, including NF-κB. In IBD, a significant increase in NF-κB p65 has been observed in the gut epithelial and immune cells isolated from colonic biopsies [24]. More specifically related to DCs, the NF-κB pathway plays a crucial role in maintaining their intracellular activation responsible for T-cell polarization[25]. Therefore, the excessive pro-inflammatory cytokine production can in part be related to the overactivation of DCs in the gut[26]. Nevertheless, in certain conditions, the level and expression of DCs activation markers are not quite equivalent when comparing the different immune compartments (i.e. colon, MLN, spleen)[19].

Chromogranin-A (CHGA) is the second most prominent protein found in the secretory granules of ECs in the GI tract. For decades, CHGA has been known to be associated with IBD, but few data have demonstrated a plausible mechanism of association due to the lack of a specific CHGA receptor[27]. An increase in the total number of endocrine cells defined as CHGA-immunoreactivity has been described in several cohorts of patients with active IBD[28]. In parallel, the serum CHGA concentration has been reported to be significantly increased compared to controls[29-32]. CHGA is considered a prohormone and can be the precursor of several bioactive peptides having a wide variety of functions in the human body affecting the cardiovascular, endocrine, neuroendocrine and immune systems[33]. One of the peptides generated, CHR (CHGA\textsubscript{C-\textit{aa}}), encoded by Exon-IV located at the N-terminal section of CHGA, has been demonstrated to depict anti-fungal and anti-microbial activities[34]. Recently, in the context of UC and experimental colitis, new data highlighted an essential anti-inflammatory role through epithelial cells and peritoneal macrophage regulation[25], but no data exist regarding the broader effect within the immune system and its different compartments.

Taken together, we hypothesized that CHR regulates DCs activation in the context of UC experimental colitis, and we evaluated CHR’s effects on different DCs-related markers at various immune compartments and defined a potential intracellular pathway implicated.

**MATERIALS AND METHODS**

**Human participants**

Biopsies were collected from participants undergoing colonoscopy with known UC or with no IBD. A total of four biopsies were taken from inflamed sites identified endoscopically in persons with active UC (n = 10) and from normal tissue from healthy participants (n = 10). Biopsy collection was approved by the University of Manitoba Research Ethics Board. Participants were ≥ 18 years. All concomitant IBD therapies, including 5-amino salicylates, corticosteroids, and methotrexate, were permitted in active UC patients. Healthy control individuals had no history of abdominal afflictions, especially IBD. For more details, see Kermarrec et al[20]. The individuals in the control group were not on any regular medication at the time of the study. The University of Manitoba Research Ethics Board [HS14878 (E)] approved the study.

**Animal and ethics statement**

All the experiments were conducted under protocols # 15-010 and #19-014, approved by the University of Manitoba Ethics Committee under Canadian animal research guidelines. In-house groups of 6 male C57BL/6 mice (six to eight weeks old) with bodyweight between 20 g to 25 g were used. All animals were kept in the specific pathogen-free barrier facility maintained at the University of Manitoba animal care facility. Animals were kept in a 12-h dark/light cycle and fed ad libitum.

**Peptide**

CHGA\textsubscript{C-\textit{aa}}- RILSLRHQNLKELQDLAL[35-39] were synthesized by a solid-phase method and purified by reverse-phase high-performance liquid chromatography to reach < 98% purity (Pepmic Co., Suzhou, China). As previous studies from our laboratory demonstrated an absence of effect of the scramble version[25], this group was not added to our experimental plan.

**Dextran sulphate sodium-induced experimental colitis and experimental plan**

Based on previously published data, CHR’s dose was adjusted to 2.5 mg/kg per day
The 1% of phosphate buffer saline (PBS, 1%) (Life Technology, Grand Island, NY, United States) was given intra-rectally to the controls [40], all the injections of both CHR and PBS started one day before the induction of experimental colitis and lasted for 5 d. Dextran sulphate sodium (DSS) (molecular weight, 40 kDa; MP Biomedicals, Soho, OH, United States) was added to regular drinking water and freshly replaced every two days at a concentration of 5% (wt/vol) for 5 d till the mice are sacrificed [41]. The average consumption of DSS was noted per cage each day. Animals were randomized, 6 mice per group were assigned for each experiment. Time matching of the controls was done with mice receiving normal drinking water only.

Assessment of DSS-induced colitis
Disease activity index (DAI) included a combined score of weight loss, stool consistency, and bleeding was recorded daily [42] from day zero to day five during DSS treatment. Blood in the stool was evaluated using the Hemoccult II test (Beckman Coulter, Oakville, ON, Canada). Colon length and macroscopic scores [42] were assessed on sacrifice day.

Spleen and MLN isolation
After the induction of DSS colitis on sacrifice day, the spleen and MLN were collected and digested in 2 mg/mL collagenase D (Roche Diagnostics, Meylan, France) dissolved in RPMI 1640 (Life Technologies) at 37 ºC for 30 min associated with a 10 min intermittent shaking. To stop the reaction and disrupt the DC-T cell complexes, the cell suspension was supplemented with 5 mmol/L EDTA (Sigma, Mississauga, ON, Canada) during the last 5 min. The homogenate was then passed through a 70-mm cell strainer (VWR, Mississauga, ON, Canada) and then washed with RPMI-1640 at 1200 rpm for 5 min. Red blood cells (RBC)’s lysis was done using ACK lysis buffer (150 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA; Life Technologies).

MACS CD11c sorting
EasySep™ Mouse CD11c Positive selection kit (Stemcell Technologies) was used according manufacturer’s instructions. The cells were then collected, counted and used for stimulatory experiments.

Isolation and stimulation of bone marrow-derived DCs
Six to eight weeks naïve mice were sacrificed by cervical dislocation. In sterile conditions, the femur and tibia were removed and soaked in RPMI-1640 medium [containing 10% heat-inactivated fetal bovine serum (FBS), 25 mg/mL gentamicin, 2 mmol/L L-glutamine] (Life Technologies). Bone marrow cell extraction was performed by cutting both ends of the bones and flushing them with 1 mL of sterile RPMI-1640 into a sterile Petri dish. The cell suspension was collected and centrifuged for 5 min at 1000 rpm. The supernatant was discarded, and pellets were resuspended in RBC ACK lysis buffer (150 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA; Life Technologies) for the lysis of RBC. After the second centrifugation, the pellet was resuspended in RPMI-1640 and counted [43]. The purity of the cells was then verified by flow cytometry. Granulocyte-macrophage induced colony-stimulating factor (GM-CSF, Cedarlane, Burlington, ON, Canada) induced culture of bone marrow-derived DCs (BMDCs) were used for the cell culture. Cells were suspended at a concentration of 1 × 10^6 cells/mL in culture plates containing complete RPMI 1640 medium and stimulated with 20 ng/mL of GM-CSF. The cell culture was then put in an incubator at 37 ºC with 5% CO2. GM-CSF cultured media was replaced on the 3rd and 6th day. On the 8th day, the semi-suspended cells were collected with gentle pipetting, and the loosely attached cells were scraped using a cell scraper generating mature cells [43,44]. The CD11c+ cells (1 × 10^6 per well) isolated via MACS were cultured in complete RPMI 1640 medium (Life Technologies) containing 10% heat-inactivated FBS, 25 mg/mL gentamicin and 2 mmol/L L-glutamine (Life Technologies) in 12-well plates in the presence or absence of 10^6 M of CHR for 12 h. After 12 h, they were stimulated with 100 ng/mL of lipopolysaccharide (LPS, Sigma) for 24 h. The cell culture was collected for quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) analysis, and ELISA assessed the supernatant. To determine the intracellular pathway, pharmaceutical blocker/activator, betulinic acid (NF-κB activator, 10 μmol/L; Sigma) or BAY 11-7082 (NF-κB inhibitor, 10 μmol/L; Sigma) were added to the culture medium for 24 h at the same time of LPS.

qRT-PCR
Total RNA from colonic tissue, MLN, splenocytes, CD11c+ splenic and CD11c+ BMDC
isolated cells was extracted using TRIZol™ Plus RNA Purification Kit (Life Technologies) and reverse-transcribed using SuperScript VILO cDNA Synthesis Master Mix (Life Technologies). Gene expression was measured by a qRT-PCR using a Roche Light Cycler 96 Real-Time System and Power SYBR green master mix (Life Technologies). Differences in the threshold cycle (ΔCt) number between the target genes and the optimal reference gene Eukaryotic elongation factor 2 (Eef2)[45] were used to calculate differences in expression. Human and mouse primer sequences used are provided in Tables 1 and 2.

RT2-profiler qPCR array
RT2 profiler™ PCR array mouse dendritic and antigen-presenting cell (Qiagen Inc, Toronto, ON Ref #330231) was performed for the quantitative PCR according to the manufacture instructions to profile the expression of 84 essential genes. Changes in gene expression between different experimental groups and the heat maps (colour-coded graphs with groups in columns and genes in rows) were generated using the web-based program of RT2 profiler PCR array data analysis.

Protein quantification
Enzyme-linked immunosorbent assays (ELISAs) were used for protein quantification from full-thickness tissue homogenates and/or supernatants from the cell cultures. Mouse commercial ELISA kits were used to detect IL-6 and IL-12p40 (R & D Systems, Inc., Minneapolis, United States).

Statistical analysis
Data are expressed as the mean ± SE. Unpaired Mann-Whitney U test was applied to compare between two groups. To compare between more than two groups, One-Way ANOVA followed by a post-hoc test was used. The analysis DAI repeated measure Two-Way ANOVA followed by a post-hoc test was used. To analyze the association between different markers studied, Spearman’s correlation test was used. The statistical two-tailed significance level was determined at P = 0.05. GraphPad Prism software (version 6; GraphPad Software, Inc, La Jolla, CA, United States) was used to compute the statistics.

RESULTS

In colitic mice, in vivo CHR treatment decreased colonic inflammatory macro- and micro-scopic scores
As reported previously[40], we confirmed CHR’s anti-inflammatory effect on experimental colitis induction. CHR treatment (2.5 mg/kg/day, i.r.) resulted in a marked reduction in the external DAI (Figure 1A). No difference was seen within the first two days, but starting day 3, a significant difference was detected, culminating at a 3-fold reduction on day 5 when compared with the colitic group. We also confirmed the beneficial effect of the CHR treatment on the macroscopic score (P < 0.0002) when distal colonic sections were examined (Figure 1B).

In colitic mice, in vivo CHR treatment decreases whole colonic section DCs-related surface and functional markers
RT-PCR array analysis of DCs-related membrane and functional markers was conducted on whole colonic sections. As presented in Figure 1C, whole colonic samples isolated from mice treated with CHR demonstrated a significant downregulation of surface and intracellular markers, notably: CD86 4.43-fold (P < 0.04, n = 6 per group), CD80 4.21-fold (P < 0.04, n = 6 per group) and NF-kB1 11.01-fold (P < 0.02, n = 6 per group). To confirm the data, gene expression level using a single target qRT-PCR technique was used. Development of colitis induced a significant colonic increase of mRNA expression of surface markers 86 (P = 0.0089, n = 6 per group) and CD80 (P = 0.0005) when compared with the non-colitic PBS group (Figure 2A and B). Treatment with CHR significantly decreased the two markers (P = 0.0001 and P < 0.0001 respectively, n = 6 per group). Besides, two others DC-related markers not present in our initial array were quantified. Induction of colitis induced a significant colonic increase of CD40 (P < 0.0001, n = 6 per group) and CD11c (P < 0.0001, n = 6 per group) when compared with the non-colitic PBS group. The same pattern of significant decrease was found for CD40 (P = 0.0002, n = 6 per group) and CD11c (P < 0.0001, n = 6 per group) (Figure 2C and D) in colon treated with CHR when compared with the
untreated group. Besides, a significant increase in colonic mRNA expression and protein quantification of IL-12p40 \( (P < 0.0001, n = 6 \text{ per group}) \) were detected in colitic conditions when compared with the untreated group, and CHR treatment decreased those two markers \( (P < 0.0001 \text{ and } P < 0.0043 \text{ respectively, } n = 6 \text{ per group}) \) (Figures 2E and F) when compared with the untreated group.

**In colitic mice, in vivo CHR treatment decreases MLN DCs-related surface and functional markers**

Next, we assessed the regulation of the different markers within the MLN. Induction of colitis was associated with a significant increase of CD86 \( (P < 0.0001, n = 6 \text{ per group}) \), CD80 \( (P < 0.0001, n = 6 \text{ per group}) \) and CD11c \( (P < 0.0001, n = 6 \text{ per group}) \) mRNA expression (Figures 3A-C) when compared with non-colitic PBS group. In colitic mice, when compared with PBS group, CHR treatment decreased significantly CD86 \( (P < 0.0001, n = 6 \text{ per group}) \) \( \text{(Figure 3A)} \), CD80 \( (P < 0.0001, n = 6 \text{ per group}) \) \( \text{(Figure 3B)} \) and CD11c \( (P < 0.0001, n = 6 \text{ per group}) \) \( \text{(Figure 3C)} \) mRNA expression. Also, induction of colitis induced a significant increase of IL-12p40 \( (P < 0.0001, n = 6 \text{ per group}) \) and IL-6 \( (P < 0.0001, n = 6 \text{ per group}) \) within the MLN when compared with the PBS group, and CHR treatment significantly decreased those two markers \( (P < 0.0001, n = 6 \text{ per group}) \) \( \text{(Figure 3D and E)} \) when compared with untreated group. No significant changes were observed for CD40 (data not shown).

**In colitic mice, in vivo CHR treatment decreases splenocytes DCs-related surface and functional markers**

We assessed the expression of the different markers within the spleen cells. Induction of colitis was associated with a significant increase of splenocytes CD86 \( (P < 0.0001, n = 6 \text{ per group}) \), CD80 \( (P < 0.0001, n = 6 \text{ per group}) \) and CD11c \( (P < 0.0001, n = 6 \text{ per group}) \) mRNA expression (Figures 4A-C) when compared with PBS group. In colitic

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### Table 1 Human primers sequences

| Gene  | Forward (5'-3') | Reverse (3'-5') |
|-------|----------------|----------------|
| INFg  | GCCTATCTGTCACCATCTCATC | CCTCTGGCTGCTGGTATTTAT |
| CCR7  | GTTCAAATCTCAGGTCCCTCTC | TACCCTGGTGATCCTCATCTTA |
| CD74  | CCCAACGAGAACGCCAATGT  | CATGGCCGGTAAGCTGATA |
| IL12p40 | ACCAGACGATGAGGTCTTTTA | CCCTTTGTGACAGGTGTACTG |
| CD86  | CAGACCACATTCCTTGGATCA  | CCGCTTCTTCTTCTTCCATTTC |
| TBP   | CCCGAAACGCCGAATATAATCC | AATCAGTGCCGTGGTTCGTG |
| CD11C | ACTGACTCCTCCTGGATTTCA | TGGGCTTCTGTCCCTTCAAGTAAT |
| IL12A | ATCCAGAGAGACCTCTTTTCATAAC | CCACCTGGTACATCTTCAAGTC |
| IL23A | CAGGTCACATTACATGGGATTC | GCAGTTCTTAATGGCTTCCTG |

### Table 2 Mouse primers sequences

| Gene | Forward (5'-3') | Reverse (3'-5') |
|------|----------------|----------------|
| IL6  | CTTGAACCTTCCAAAGATGGC | TGCACCAGGCAAGTCTCCTCA |
| CD86 | TTACGAAGACCCACGAGATG | ACTACCAGCTCACTCAGGCT |
| CD11c | CCAAGACATCGTGTTCCTGATT | ACAGCTTTAACAAAGTCCAGCA |
| Eef2  | GTGCATCTCCTCCTGGGATTA | CATCCTTGCGAGTGTCAGTGA |
| CD80  | TGGGCCGATGAATAACAGTC | GTTTCTCTGCTTGCCTTCAAG |
| CD40  | GTGCACAGGAGGATGGTAAAG | AAAGCAGTTCCAGGGTTCAG |
| IL12p40 | TGGTTTGCCATCGTTTTGCTG | ACAGGTGAGGTTCACTGTTTCT |
| CCR7  | CAGAGATCGGCTCCTACTT | GCAGTTCTTAATGGCTTCCTG |
| IFNg  | CTCTTCCTCATGGCTGTCTT | TTCTTCCACATCTATGGCCACTT |
Figure 1 Chromofungin (chromogranin-A 47-66) treatment decreases dextran sulphate sodium-induced experimental colitis and colonic colonic cluster of differentiation markers and NF-κB gene expression in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-C: External disease activity index recorded over the period of 5 d (A), macroscopic scores at sacrifice day (B), CD86 and 80 and NF-κB gene expression profile by RT² profiler PCR array at sacrifice day (C). Unpaired two-tailed Mann-Whitney U and Two-way repeated measures or One-way ANOVA followed by multiple comparison tests were used to analyze the data, and adjusted P equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, \( n = 6 \) mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

In colitic mice, in vitro CHR treatment decreases splenic CD11c+ cells DCs-related surface and functional markers

Next splenic CD11c+ cells were isolated from the colitic group treated or not with CHR via the MACS technique. As presented in Figure 5, RT²-PCR array analysis demonstrated a significant downregulation of DC-related surface and intracellular markers after CHR treatment: CD86 (4.0-fold) \((P < 0.0001, n = 6 \text{ per group})\), CD80 (3.8-fold) \((P < 0.0001, n = 6 \text{ per group})\) and CD40 (2.0-fold) \((P < 0.0001, n = 6 \text{ per group})\) when compared with the PBS group and CHR treatment was associated with a significant decrease of IL-2p40 \((P < 0.0001, n = 6 \text{ per group})\) and IL-6 \((P < 0.0001, n = 6 \text{ per group})\) when compared with the colitic PBS group. No significant changes were observed for CD40 (data not shown).

CHR decreases DC-related markers in BMDCs treated with LPS or NF-κB activator

Finally, we determined the intracellular pathway implicated. In BMDCs CD11c+ cells, treatment with NF-κB stimulator significantly increased the mRNA expression of CD86 \((P = 0.0088, n = 6 \text{ per group})\), CD80 \((P = 0.0026, n = 6 \text{ per group})\), CD40 \((P = 0.0119, n = 6 \text{ per group})\) and CHR treatment abolished the increase (Figures 6A-C). LPS treatment increased the mRNA expression of the same three markers, and treatment with NF-κB inhibitor or CHR abolished the increase. CHR treatment decreased the harmful effect of LPS stimulation even in the presence of the NF-κB activator.
Chromofungin (chromogranin-A 47-66) treatment decreases colonic cluster of differentiation markers and interleukin-12 level in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-F: CD86 (A), CD80 (B), CD40 (C), CD11c (D), interleukin (IL)-12p40 mRNA expression (E), IL-12p40 protein level (F). mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction and protein level by ELISA. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted \( P \) equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, \( n = 6 \) mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

In parallel, the NF-κB stimulator increased significantly the protein concentration of IL-6 \( (P<0.0001, n=6 \text{ per group}) \) and IL-12p40 \( (P<0.0001, n=6 \text{ per group}) \) and CHR treatment significantly decreased the level of the two inflammatory proteins (Figure 6D and E). LPS treatment increased significantly the level of IL-6 \( (p<0.0001, n=6 \text{ per group}) \) and IL-12p40 \( (P<0.0001, n=6 \text{ per group}) \), and NF-κB inhibitor or CHR treatments decreased the status of the two markers. CHR treatment decreased the deleterious effect of LPS stimulation even in the presence of an NF-κB activator.

**Relationship between CHGA-Exon-IV and DCs-related markers in colonic biopsies of active UC patients**

Colon biopsies from UC patients were analyzed using qRT-PCR to assess the relationship between CHGA-Exon-IV and CD11c+ cells-related markers. We used our previously published data on Exon-IV[25] to perform a new correlation analysis. Our data demonstrated an upregulation of surface markers related to DCs (Figure 7A and...
Chromofungin (chromogranin-A 47-66) treatment decreases mesenteric lymph node cluster of differentiation markers and cytokine levels in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-E: CD86 (A), CD80 (B), CD11c (C), interleukin (IL)-12p40 (D), IL-6 mRNA expression (E). mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted $P$ equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, $n=6$ mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

**DISCUSSION**

Overactivation of the immune system through the dysregulation of DCs and its subsequent T-cell polarization is one of the cytokine storm features seen in inflamed mucosa[46]. Over the last five years, a lot of attention has been given to CHGA and its derived peptides in regulating the immune components. However, little is known as to
Figure 4 Chromofungin (chromogranin-A 47-66) treatment decreases splenocyte cluster of differentiation markers and interleukin-12 level in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-E: CD86 (A), CD80 (B), CD11c (C), interleukin (IL)-12p40 (D), IL-6 mRNA expression (E) from splenic cells. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted $P$ equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, $n = 6$ mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

how those peptides can modulate innate immune cells in different immune compartments. Using an experimental colitis model mimicking UC and colonic biopsies obtained from participants with active UC compared with biopsies from healthy controls, we assessed CHR's impact in regulating DCs-related markers. Our findings suggest a novel effect by which CHR promotes a down-regulation of DCs-related functional markers via the NF-κB pathway within different immune compartments.

In our UC-like experimental colitis model, we first confirmed the beneficial effect of CHR on qualitative and quantitative inflammatory markers described in our previous publications[25,27,40]. Because of the peptide's specific action demonstrated in our previous study on different cytokines like TNF-α[25,40], we did not consider using a scramble version; this can be regarded as a limitation. However, in a few experimental plans (not shown), the scramble version was used, and no significant effect was seen. With CHR treatment, DAI and macroscopic scores were significantly downregulated, and in addition to the decrease previously seen in IL-6[25], there was a significant decrease in the concentration of IL-12p40 at the mucosal level. This decrease was also
Figure 5 Chromofungin (chromogranin-A 47-66) treatment decreases dextran sulphate sodium-induced experimental colitis and colonic cluster of differentiation markers and NF-κB gene expression in dextran sulphate sodium-induced experimental colitis. CD86 and 80 and NF-κB gene expression profile by RT² profiler polymerase chain reaction array at sacrifice day. One-way ANOVA followed by multiple comparison tests were used to analyze the data, and adjusted P equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, n = 6 mice/group.

seen at the mRNA level, suggesting the peptide’s ability to down-regulate pro-inflammatory markers at the protein and gene levels. These data confirm GWAS studies, which have shown the importance of several APC-related cytokines, most prominently, the ones related to DCs: IL-6, IL-12 which have a critical role during the development of UC[47].

In our study, experimental colitis induced a significant colonic increase of DCs-associated markers CD86, CD80, CD40 and CD11c mRNA expression, and CHR treatment decreased all of them. DCs play a critical role during inflammation[48] as they activate and differentiate T cells and play an essential role in regulating adaptive immunity by releasing IL12p40[20]. At the colonic level, CD11c+ cells show a crucial role in IBD progression, colitis being associated with an increased level of CD11c+cells[46]. In parallel, in inflammatory conditions like asthma, arthritis, other markers like CD86, 80 and 40 are highly expressed in CD11c+ cells[49,50]. T-cell activation can be blocked by suppressing CD86 and CD80[51,52] and the release of IL-12p40 or 6[53,54]. Therefore, our data support the hypothesis that disruption of intestinal DCs-related markers can contribute to the perpetuation of the inflammatory process seen in IBD and that attenuation of DC’s activation could lead to a better outcome.

DCs are essential in detecting antigens at the mucosal level and presenting them within other immune compartments, where, within the MLN, their roles in the regulation of immune activation are pivotal[55]. Human DCs, specifically myeloid (mDCs) CD11c+ DC-SIGN+, are characterized by producing many cytokines and further activating the adaptive immune cells[56-58]. In the context of gut inflammation, increased CD11c+ cells’ level is found in the draining MLN and associated with a marked enhancement of CD11c+HLADR+ DC[46]. In IBD patients, plasmacytoid DCs isolated from MNL exhibit overexpression of CD86, CD80 and an increase of IL-12p40 and IL-6 is seen[55]. In our study, in the context of experimental colitis, our data confirmed an up-regulation of CD86 and CD80[51,52] and the release of IL-12p40 or 6[53,54]. Therefore, our data support the hypothesis that disruption of intestinal DCs-related markers can contribute to the perpetuation of the inflammatory process seen in IBD and that attenuation of DC’s activation could lead to a better outcome.

In addition to the MLN, the spleen plays a significant role in the crosstalk between the innate and adaptive immune systems. Within the spleen, CD11c+ DCs act as first responder cells during the immune response development[59]. Studies have shown that surface costimulatory molecules CD86, 80, and 40 present on isolated splenic CD11c+ cells are modified during the inflammatory process[60]. Using splenocytes isolated cells from colitic mice, our study confirmed these elements by depicting a decreased expression of CD86, CD80 and CD11c, and IL-12p40 and IL-6 levels when treated with CHR. We were also able to confirm the importance of the CD11c+ population, as our RT²-PCR array demonstrated a significant decrease of CD86 and 80 in that population. This was consistent with our data presented in our next experimental plan when we determined the direct impact of CHR on the CD11c+ BDMC cell population. Again here, like within the MLN, no effect was visible on the expression of CD40.
Figure 6 Chromofungin (chromogranin-A 47-66) treatment decreases lipopolysaccharide-stimulated bone marrow-derived CD11c\(^+\) cells' cluster of differentiation markers and cytokine-associated level via the NF-κB pathways. Bone marrow-derived CD11c\(^+\) cells using MACS technique were isolated and cultured with granulocyte-macrophage colony-stimulating factor for 8 d until full maturation. Cells were treated with CHR (10\(^{-6}\) M/mL) for 12 h and then stimulated with lipopolysaccharide (100 ng/mL) in the presence or absence of NF-κB activator/stimulator (10 u/mL) for 24 h. A-E: CD86 (A), CD80 (B), CD40 (C), mRNA expression and IL-12p40 (D), IL-6 (E) medium protein level. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction and protein levels were quantified by ELISA. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted \(P\) equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, \(n = 6\) mice/group. LPS: Lipopolysaccharide; CHR: Chromofungin.
Chromofungin and immune compartments

Figure 7 Chromofungin (CHGA Exon-IV) correlates negatively with cluster of differentiation 86 and 80 mRNA expression. A: mRNA expression of CD86 in the colonic tissue of healthy individuals \((n = 10)\) and participants with active ulcerative colitis (UC) \((n = 10)\); B: Correlation analysis between biopsy CHGA Exon IV and CD86 mRNA expression; C: mRNA expression of CD80 in the colonic tissue of healthy individuals \((n = 10)\) and participants with active UC \((n = 10)\); D: Correlation analysis between biopsy CHGA Exon IV and CD80 mRNA expression. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. Student t-test and Mann-Whitney test and Spearman's correlation were used to analyze, and adjusted \(P\) equal to or smaller than 0.05 is believed to be significant. UC: Ulcerative colitis.

In the context of acute and chronic inflammatory conditions, several inflammatory cytokines are regulated by NF-κB, and previous studies have reported that blocking NF-κB in mice can be considered as a potential treatment for preventing gut inflammation\cite{61-65}. NF-κB plays an essential role in regulating the expression of CD86, CD80, CD40 and CD11c\cite{66-68}. In our study, the RT-PCR array performed on colonic colitic samples, and splenic CD11c+ cells demonstrated a down-regulation of NFκB1 after CHR treatment. Consistent with our in vivo data, mimicking an inflammatory activation state, mouse LPS-stimulated CD11c+ BMDCs showed an increase of CD86, 80, 40 mRNA expression. The difference in CD40 expression between the colon, the MLN, spleen, and the CD11c+ BMDCs can be explained by a potential weak stimulus of CHR in that marker in an in vivo condition in contrast to a strong effect when the peptide had a direct action on the CD11c+ BMDCs, as presented by other when deciphering the impact of commensal bacteria on DC cells in different immune compartments\cite{69}.

In addition, the presence of CHR abolished colitis in the same way as the NF-κB inhibitor. To confirm NF-κB as a potential target, using an NF-κB stimulator, we demonstrated the impact of CHR's inhibitory effect on the detrimental impact induced by the stimulator. Our in vitro evaluation also showed a significant decrease in IL-6 and IL-12p40 in LPS-stimulated CD11c+ BMDCs. As CHR can decrease the effect caused by the LPS and NF-κB stimulator, we can postulate that the targeted protein resides at one of the NF-κB targets. These data agree with human data, showing that anti-IL-12p40 monoclonal antibodies have demonstrated excellent clinical efficacy in a group of UC patients\cite{70}. In the clinical and experimental models of colitis, NF-kB has been demonstrated to be a major immune regulator\cite{61,62,65} when inflammation is developed. However, at that stage, we cannot confirm if the effect of CHR is a direct or an indirect effect on NFkB. Many upstream pathways can be modulated, as for example, TLR4\cite{71}, ER stress\cite{71}. However, there are some early indications that the phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt) pathway maybe be of interest\cite{72}, as CHR can produce a cardioprotective action by regulating the PI3K pathways\cite{37}. Thus, considering the previous literature related to NF-κB and IBD, our results support the concept that CHR can potentially decrease intestinal inflammation by regulating the NF-κB pathway in CD11c+ cells.
We previously demonstrated that CHGA (Exon-IV) was highly down-regulated in UC patients showing that the disease is affecting the levels of CHGA (Exon-IV)[27,73]. As a novel finding, in colonic biopsies from UC participants, we demonstrated that CD11c and its related costimulatory surface markers CD86 were significantly upregulated, leading to a potential enhancement of colitis. Furthermore, in active UC participants, we observed a negative correlation between the CHGA (Exon-IV) with CD86 markers. These alterations can be explained by previous data, which demonstrated changes at the level of the ECs during the inflammatory process[32]. Our small sample size likely led to our inability to show a significant difference in CD11c bearing cells.

Some limitations exist in our study. Regarding the overall impact of CD11c+ cell deactivation, we did not demonstrate a direct impact on T-cell activation, a regulation that remains elucidated via the use of a co-culture model. Further studies are also required to investigate the functional consequences of CHR on other immune cells or epithelial cells and angiogenesis, all implicated in the pathogenesis of colitis. Moreover, we acknowledge that CD11c+ cells isolated from the lamina propria need to be studied, and new studies should define if DC expressing CD11c markers are the main target of CHR. Additional biopsies need to be added to determine a compelling correlation between various markers, and a proteomics approach should be used for protein quantification in the colon, and all correlation analyses should be repeated accordingly. Several other factors might lead to CHR’s protective effect, which we have observed in our study. For example, previous studies demonstrated a link between gut microbiota and experimental colitis and human IBD, and there is evidence showing that CHR can have anti-microbial function[34,35]. Therefore, in our in vivo model, i.r. injection could have induced a microbial change in favour of beneficial bacteria; further metagenomic analyses are needed to study this aspect, but that would not account for the beneficial effect seen on CD11c+ BMDCs.

CONCLUSION

In summary, our study shows that treatment with CHR led to lower colonic inflammation, which was associated with decreased levels of CD11c+-associated cytokines through an NF-κB-dependent mechanism. Hence, CHR seems to act as an NF-κB blocker, reducing primary APCs’ activation and ultimately decreasing the intestinal inflammatory process. CHR alone had no side effect on the mice or the cells, indicating CHR’s relative safety at the studied dose. This previously unknown spatial impact of CHR in colonic inflammation may help broaden research done by other groups on the overall effect of CHGA and its derived peptides on immune regulation in the context of IBD. This might lead to future novel CHR-based therapies in IBD.

ARTICLE HIGHLIGHTS

Research background
Ulcerative colitis (UC) is a disorder in which the gastrointestinal tract becomes ulcerated and inflamed. Although treatments and therapeutic strategies are evolving quickly, treatments are still inadequate for a substantial percentage of those with active UC, and some therapies may have serious adverse side effects. Therefore, UC needs new therapeutic approaches associated with higher efficacy and limited side effects. In UC, there is an overactivation of innate immune cells such as macrophages and dendritic cells (DCs). Recently, in the context of UC and experimental colitis, new data highlighted an essential anti-inflammatory role of chromofungin (CHR), a chromogranin-A derived peptide, on peritoneal macrophages.

Research motivation
To evaluate CHR’s effects on different DCs at various immune compartments and to define a potential intracellular pathway implicated.

Research objectives
To investigate the association between CHR treatment and DCs-related markers in colitis.
Research methods
Participants with active UC and a model of acute UC-like colitis using dextran sulphate sodium were used. We used cell culture and quantitative reverse transcription-polymerase chain reaction to analyze the relative expression levels of CD11c, CD80, CD86, interleukin (IL)-6 and IL-12p40 within the colonic samples, mesenteric lymph nodes and the spleen.

Research results
In a preclinical setting, CHR treatment the expression of CD11c, CD40, CD80, CD86 and IL-12p40 in the inflamed colonic mucosa and CD11c, CD80, CD86, IL-6 and IL-12p40 within the mesenteric lymph nodes and the spleen. In addition, CHR treatment decreased CD80 and CD86 expression markers of splenic CD11c+cells and decreased NF-kB expression in the colon and splenic CD11c+ cells. In vitro, CHR decreased CD40, CD80, CD86, IL-6, and IL-12p40 expression in naive bone marrow-derived CD11c+DC stimulated lipopolysaccharide. Using a pharmacological approach, we demonstrated the impact of CHR on the NF-kB pathway. In a clinical setting, in patients with active UC, CHR level was reduced and showed a negative linear relationship with CD11c and CD86.

Research conclusions
CHR has protective properties against intestinal inflammation, potentially through the regulation of DC-related markers and CD11c+ cells.

Research perspectives
Although we have demonstrated that CHR may have a potential therapeutic interest, additional markers and detailed mechanisms of action need to be determined in a large sample.

REFERENCES

1. Nemati S, Teimourian S. An Overview of Inflammatory Bowel Disease: General Consideration and Genetic Screening Approach in Diagnosis of Early Onset Subsets. *Middle East J Dig Dis* 2017; 9: 69-80. [PMID: 28638582] DOI: 10.15171/mejdd.2017.54
2. Rocchi A, Benchimol EI, Bernstein CN, Bitton A, Feagan B, Panaccione R, Glasgow KW, Fernandes A, Ghosh S. Inflammatory bowel disease: a Canadian burden of illness review. *Can J Gastroenterol* 2012; 26: 811-817. [PMID: 23166905] DOI: 10.1155/2012/984575
3. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; 474: 307-317. [PMID: 21677747] DOI: 10.1038/nature10209
4. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014; 157: 121-141. [PMID: 24679331] DOI: 10.1016/j.cell.2014.03.011
5. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; 361: 2066-2078. [PMID: 19923578] DOI: 10.1056/NEJMr804647
6. Bernstein CN, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut* 2008; 57: 1185-1191. [PMID: 18515412] DOI: 10.1136/gut.2007.122143
7. Goh K, Xiao SD. Inflammatory bowel disease: a survey of the epidemiology in Asia. *J Dig Dis* 2009; 10: 1-6. [PMID: 19236540] DOI: 10.1111/j.1751-2980.2008.00355.x
8. Beaurgerie L, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Boulhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F; CESAME Study Group. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; 374: 1617-1625. [PMID: 19837455] DOI: 10.1016/S0140-6736(09)61302-7
9. Gyires K, Tóth ÉV, Zádori SZ. Gut inflammation: current update on pathophysiology, molecular mechanism and pharmacological treatment modalities. *Curr Pharm Des* 2014; 20: 1063-1081. [PMID: 23782146] DOI: 10.2174/138161281399990417
10. Ince MN, Elliott DE. Immunologic and molecular mechanisms in inflammatory bowel disease. *Surg Clin North Am* 2007; 87: 681-696. [PMID: 17560420] DOI: 10.1016/j.suc.2007.03.005
11. Korzenik JR, Podolsky DK. Evolving knowledge and therapy of inflammatory bowel disease. *Nat Rev Drug Discov* 2006; 5: 197-209. [PMID: 16518373] DOI: 10.1038/nrd1896
12. Huang Y, Chen Z. Inflammatory bowel disease related innate immunity and adaptive immunity. *Am J Transl Res* 2016; 8: 2490-2497. [PMID: 27398134]
13. Xavier RJ, Podolsky DK. Unraveling the pathogenesis of inflammatory bowel disease. *Nature* 2007; 448: 427-434. [PMID: 17653185] DOI: 10.1038/nature06005
14. Strober W. Immunology. Unraveling gut inflammation. *Science* 2006; 313: 1052-1054. [PMID: 16931742] DOI: 10.1126/science.1131997
Two chromogranin a-derived peptides induce calcium entry in human neutrophils by calmodulin-

S, Delalande F, Van Dorsselaer A, Schneider F, Helle K, Aunis D, Prévost G, Metz-Boutigue MH.

Zhang D

DOI:

chromogranin A-(47-66)-derived peptide.

DOI:

chromogranin A (47-66)-derived peptide.

Lugardon K, Eissa N, Wang H, Kapoor K, Diarra A, Gounni AS, Bernstein CN, Ghia JE. Semaphorin-3E attenuates intestinal inflammation through the regulation of the communication between splenic CD11c+ and CD4+ CD25+ T-cells. Br J Pharmacol 2019; 176: 1235-1250 [PMID: 30736100 DOI: 10.1111/bph.14614]

Steinbach EC, Plevy SE. The role of macrophages and dendritic cells in the initiation of inflammation in IBD. Inflamm Bowel Dis 2014; 20: 166-175 [PMID: 23794993 DOI: 10.1097/MIB.0b013e3182d86dca]

Abraham C, Cho JH. IL-23 and autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. Annu Rev Med 2006; 57: 97-110 [PMID: 18976050 DOI: 10.1146/annurev.med.60.051407.123757]

Shih DQ, Targan SR, McGovern D. Recent advances in IBD pathogenesis: genetics and immunobiology. Curr Gastroenterol Rep 2008; 10: 568-575 [PMID: 19006613 DOI: 10.1007/s11894-008-0104-x]

Neurath MF, Plevy SE. The role of macrophages and dendritic cells in the initiation of macrophage and dendritic cell subsets in inflammatory bowel disease. World J Gastroenterol 2006; 12: 3872-3878 [PMID: 17063110 DOI: 10.3748/wjg.v11.i13.1987]

Massironi S, Zilli A, Cavaletti F, Conte D, Peracchi M. Chromogranin A and other enteroendocrine markers in inflammatory bowel disease. Neuropeptides 2016; 58: 127-134 [PMID: 26804239 DOI: 10.1016/j.nepep.2016.01.002]

Sidhu R, Drew K, McAlindon ME, Lobo AJ, Sanders DS. Elevated serum chromogranin A in a patient with active ulcerative colitis and suppresses pro-inflammatory macrophage function through the inhibition of NF-κB signaling. Biochem Pharmacol 2017; 145: 102-113 [PMID: 28827109 DOI: 10.1016/j.bcp.2017.08.013]

Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2018; 12: 907-915 [PMID: 30024302 DOI: 10.1007/18747124.2018.105035]

Eissa N, Hussein H, Hendy GN, Bernstein CN, Ghia JE. Chromogranin-A and its derived peptides and their pharmacological effects during intestinal inflammation. Biochem Pharmacol 2018; 152: 315-326 [PMID: 29656116 DOI: 10.1016/j.bcp.2018.04.009]

Massironi S, Zilli A, Cavaletti F, Conte D, Peracchi M. Chromogranin A and other enteroendocrine markers in inflammatory bowel disease. Neuropeptides 2016; 58: 127-134 [PMID: 26804239 DOI: 10.1016/j.nepep.2016.01.002]

Sidhu R, Drew K, McAlindon ME, Lobo AJ, Sanders DS. Elevated serum chromogranin A in a patient with active ulcerative colitis and suppresses pro-inflammatory macrophage function through the inhibition of NF-κB signaling. Biochem Pharmacol 2017; 145: 102-113 [PMID: 28827109 DOI: 10.1016/j.bcp.2017.08.013]

Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2018; 12: 907-915 [PMID: 30024302 DOI: 10.1007/18747124.2018.105035]

Eissa N, Hussein H, Hendy GN, Bernstein CN, Ghia JE. Chromogranin-A and its derived peptides and their pharmacological effects during intestinal inflammation. Biochem Pharmacol 2018; 152: 315-326 [PMID: 29656116 DOI: 10.1016/j.bcp.2018.04.009]

Massironi S, Zilli A, Cavaletti F, Conte D, Peracchi M. Chromogranin A and other enteroendocrine markers in inflammatory bowel disease. Neuropeptides 2016; 58: 127-134 [PMID: 26804239 DOI: 10.1016/j.nepep.2016.01.002]

Sidhu R, Drew K, McAlindon ME, Lobo AJ, Sanders DS. Elevated serum chromogranin A in a patient with active ulcerative colitis and suppresses pro-inflammatory macrophage function through the inhibition of NF-κB signaling. Biochem Pharmacol 2017; 145: 102-113 [PMID: 28827109 DOI: 10.1016/j.bcp.2017.08.013]

Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2018; 12: 907-915 [PMID: 30024302 DOI: 10.1007/18747124.2018.105035]

Eissa N, Hussein H, Hendy GN, Bernstein CN, Ghia JE. Chromogranin-A and its derived peptides and their pharmacological effects during intestinal inflammation. Biochem Pharmacol 2018; 152: 315-326 [PMID: 29656116 DOI: 10.1016/j.bcp.2018.04.009]

Massironi S, Zilli A, Cavaletti F, Conte D, Peracchi M. Chromogranin A and other enteroendocrine markers in inflammatory bowel disease. Neuropeptides 2016; 58: 127-134 [PMID: 26804239 DOI: 10.1016/j.nepep.2016.01.002]

Sidhu R, Drew K, McAlindon ME, Lobo AJ, Sanders DS. Elevated serum chromogranin A in a patient with active ulcerative colitis and suppresses pro-inflammatory macrophage function through the inhibition of NF-κB signaling. Biochem Pharmacol 2017; 145: 102-113 [PMID: 28827109 DOI: 10.1016/j.bcp.2017.08.013]

Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2018; 12: 907-915 [PMID: 30024302 DOI: 10.1007/18747124.2018.105035]

Eissa N, Hussein H, Hendy GN, Bernstein CN, Ghia JE. Chromogranin-A and its derived peptides and their pharmacological effects during intestinal inflammation. Biochem Pharmacol 2018; 152: 315-326 [PMID: 29656116 DOI: 10.1016/j.bcp.2018.04.009]

Massironi S, Zilli A, Cavaletti F, Conte D, Peracchi M. Chromogranin A and other enteroendocrine markers in inflammatory bowel disease. Neuropeptides 2016; 58: 127-134 [PMID: 26804239 DOI: 10.1016/j.nepep.2016.01.002]
regulated calcium independent phospholipase A2. PLoS One 2009; 4: e4501 [PMID: 19225567 DOI: 10.1371/journal.pone.0004501]

37 Filice E, Pasqua T, Quintieri AM, Cantafio P, Scarvello F, Amodio N, Cerra MC, Marban C, Schneider F, Metz-Boutigue MH, Angelone T. Chromofungin, CgA7-66-derived peptide, produces basal cardiac effects and postconditioning cardioprotective action during ischemia/reperfusion injury. Peptides 2015; 71: 40-48 [PMID: 26151429 DOI: 10.1016/j.peptides.2015.06.013]

38 Etienne O, Gaspier C, Taddei C, Voegel JC, Aunis D, Saaf F, Metz-Boutigue MH, Bolletato-Bellemir AL, Egles C. Antifungal coating by biofunctionalized polyelectrolyte multilayer films. Biomaterials 2005; 26: 6704-6712 [PMID: 15992921 DOI: 10.1016/j.biomaterials.2005.04.068]

39 Metz-Boutigue MH, Gounon Y, Strub JM, Lugardon K, Aunis D. Antimicrobial chromogranins and proenkephalin-A-derived peptides: Antibacterial and antifungal activities of chromogranins and proenkephalin-A-derived peptides. Ann N Y Acad Sci 2003; 992: 168-178 [PMID: 12794056 DOI: 10.1111/j.1749-6632.2003.tb03147.x]

40 Eissa N, Hussein H, Kermarrec L, Grover J, Metz-Boutigue ME, Bernstein CN, Ghia JE. Chromofungin Ameliorates the Progression of Colitis by Regulating Alternately Activated Macrophages. Front Immunol 2017; 8: 1131 [PMID: 28957133 DOI: 10.3389/fimmu.2017.01131]

41 Okayasu I, Hatakeyama S, Yamada M, Okhusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 1999; 106: 694-702 [PMID: 1688811 DOI: 10.1016/S0016-5085(0)90290-6]

42 Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993; 69: 238-249 [PMID: 8530599]

43 Wang W, Li J, Wu K, Azhathi B, Ruxiati M. Culture and Identification of Mouse Bone Marrow-Derived Dendritic Cells and Their Capability to Induce T Lymphocyte Proliferation. Med Sci Monit 2016; 22: 244-250 [PMID: 26802068 DOI: 10.12659/msm.890951]

44 Okwor I, Jia P, Uronna JE. Interaction of Macrophage Antigen 1 and CD40 Ligand Leads to IL-12 Production and Resistance in CD40-Deficient Mice Infected with Leishmania major. J Immunol 2015; 195: 3218-3226 [PMID: 26304989 DOI: 10.4049/jimmunol.1500922]

45 Eissa N, Hussein H, Wang H, Rabbi MF, Bernstein CN, Ghia JE. Stability of Reference Genes for Messenger RNA Quantification by Real-Time PCR in Mouse Dextran Sodium Sulfate Experimental Colitis. PLoS One 2016; 11: e0156289 [PMID: 27244258 DOI: 10.1371/journal.pone.0156289]

46 Magnusson MK, Brynjolfsson SF, Dige A, Uronen-Hansson H, Börjesson LG, Bengtsson JL, Gadjonsson S, Öhman L, Agnolt J, Svjall V, Agace WW, Wick MJ. Macrophage and dendritic cell subsets in IB: ALDH+ cells are reduced in colon tissue of patients with ulcerative colitis regardless of inflammation. Mucosal Immunol 2016; 9: 171-182 [PMID: 26080709 DOI: 10.1038/mi.2015.48]

47 Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014; 14: 329-342 [PMID: 24751956 DOI: 10.1038/nri3661]

48 Kugathasan K, Roediger EK, Small CL, McCormick S, Yang P, Xing Z. CD11c+ antigen presenting cells from the alveolar space, lung parenchyma and spleen differ in their phenotype and capabilities to activate naïve and antigen-primed T cells. BMC Immunol 2008; 9: 4 [PMID: 18700962 DOI: 10.1186/1471-2172-9-4]

49 Balanescu A, Radu E, Nat R, Regalia T, Bojinca V, Predescu V, Predeteau D. Co-stimulatory and adhesion molecules of dendritic cells in rheumatoid arthritis. J Cell Mol Med 2002; 6: 415-425 [PMID: 12417058 DOI: 10.1111/j.1582-4934.2002.tb00520.x]

50 Lambrechts BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. Immunity 2009; 31: 412-424 [PMID: 19766084 DOI: 10.1016/j.immuni.2009.08.008]

51 Li JG, DU YM, Yan ZD, Yan J, Zhuansun YX, Chen R, Zhang W, Feng SL, Ran PX. CD80 and CD86 knockdown in dendritic cells regulates Th1/Th2 cytokine production in asthmatic mice. Exp Ther Med 2016; 11: 878-884 [PMID: 26980066 DOI: 10.3892/etm.2016.2989]

52 Lim TS, Goh JK, Mortellaro A, Lim CT, Hämmelinger GJ, Ricciard痴-Castagnoli P. CD80 and CD86 differentially regulate mechanical interactions of T-cells with antigen-presenting dendritic cells and B-cells. PLoS One 2012; 7: e45185 [PMID: 23024807 DOI: 10.1371/journal.pone.0045185]

53 Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011; 1813: 878-888 [PMID: 21296109 DOI: 10.1016/j.bbcan.2011.01.034]

54 Schurich C, Raine C, Morris V, Ciurtin C. The role of IL-12/23 in T cell-related chronic inflammation: implications of immunodeficiency and therapeutic blockade. Rheumatology (Oxford) 2018; 57: 246-254 [PMID: 28241488 DOI: 10.1093/rheumatology/kex180]

55 Hostmann A, Kapp K, Beutner M, Ritz JP, Loddenkemper C, Ignatius R, Podhajsky P, Amon D, Dau H, Zeitz M, Uldrich R. Dendritic cells from bone marrow and the lung express functional type I interferon receptor in inflammatory and non-inflammatory bowel diseases: subsets and function of plasmacytoid dendritic cells. Immunology 2013; 139: 100-108 [PMID: 23278129 DOI: 10.1111/imn.12060]

56 Risoanu SC, Smolentev V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. Science 1999; 283: 1183-1186 [PMID: 10024247 DOI: 10.1126/science.283.5405.1183]

57 Cella M, Jarrossay D, Facchetti F, Leardi B, Nakajima H, Lambrecht BN, Colonna M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med 1999; 5: 919-923 [PMID: 10426316 DOI: 10.1038/11360]

58 Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Med 2005; 23: 275-306 [PMID: 15771572 DOI: 10.1146/annurev.med.56.012304.104263]
Nam HW, Ahn HJ, Yang HJ. Pro-inflammatory cytokine expression of spleen dendritic cells in mouse toxoplasmosis. *Korean J Parasitol* 2011; 49: 109-114 [PMID: 21738265 DOI: 10.3347/kjp.2011.49.2.109]

Slawek A, Maj T, Cholmamska-Soaya A, CD40, CD80, and CD86 costimulatory molecules are differentially expressed on murine splenic antigen-presenting cells during the pre-implantation period of pregnancy, and they regulate regulatory T-cell abundance, peripheral cytokine response, and pregnancy outcome. *Am J Reprod Immunol* 2013; 70: 116-126 [PMID: 23445188 DOI: 10.1111/aji.12108]

Feng J, Guo C, Zhu Y, Pang L, Yang Z, Zou Y, Zheng X. Baicalein down regulates the expression of TLR4 and NFKB-p65 in colon tissue in mice with colitis induced by dextran sulfate sodium. *Int J Clin Exp Med* 2014; 7: 4063-4072 [PMID: 25550915]

Sánchez-Fidalgo S, Villegas I, Rosillo MA, Aparicio-Soto M, de la Lastra CA. Dietary squalene supplementation improves DSS-induced acute colitis by downregulating p38 MAPK and NFKB signaling pathways. *Mol Nutr Food Res* 2015; 59: 284-292 [PMID: 25387687 DOI: 10.1002/mnfr.201400518]

Zhang J, Cao L, Wang H, Cheng X, Wang L, Zhu L, Yan T, Xie Y, Wu Y, Zhao M, Ma S, Wu M, Wang G, Hao H. Ginsenosides Regulate PXR/NF-κB Signaling and Attenuate Dextran Sulfate Sodium-Induced Colitis. Drug Metab Dispos 2015; 43: 1181-1189 [PMID: 25986830 DOI: 10.1124/dmd.115.063800]

Lubbad A, Oriowo MA, Khan I. Curcumin attenuates inflammation through inhibition of TLR-4 receptor in experimental colitis. *Mol Cell Biochem* 2009; 322: 127-135 [PMID: 19002562 DOI: 10.1007/s11010-008-9949-4]

Andresen L, Jergensen VL, Perner A, Hansen A, Eugen-Olsen J, Rask-Madsen J. Activation of nuclear factor kappaB in colonic mucosa from patients with collagenous and ulcerative colitis. *Gut* 2005; 54: 503-509 [PMID: 15753535 DOI: 10.1136/gut.2003.024165]

Yoshimura S, Bondeson J, Foxwell BM, Brennan FM, Feldmann M. Effective antigen presentation by dendritic cells is NF-kappaB dependent: coordinate regulation of MHC, co-stimulatory molecules and cytokines. *Int Immunol* 2001; 13: 675-683 [PMID: 11312255 DOI: 10.1093/intimm/13.5.675]

Sun Q, Hosoki K, Boldogh I, Sur S. Allergic Extracts Require TLR4 to Activate and Increase Expression of CD40, CD80 and CD86 on Bone Marrow-Derived Dcs. *J Allergy Clin Immunol* 2015; 135: AB9

Shih VF, Davis-Turak J, Macal M, Huang JQ, Ponomarenko J, Kearns JD, Yu T, Fagerlund R, Asagiri M, Zuniga EI, Hoffmann A. Control of RelB during dendritic cell activation integrates canonical and noncanonical NF-κB pathways. *Nat Immunol* 2012; 13: 1162-1170 [PMID: 23086447 DOI: 10.1038/ni.2446]

Fink LN, Froikhaer H. Dendritic cells from Peyer's patches and mesenteric lymph nodes differ from spleen dendritic cells in their response to commensal gut bacteria. *Scand J Immunol* 2008; 68: 270-279 [PMID: 18565117 DOI: 10.1111/j.1365-3083.2008.02136.x]

Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG; CERTIFI Study Group. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; 367: 1519-1528 [PMID: 23075178 DOI: 10.1056/NEJMoa1203572]

Dow W, Zhang J, Sun A, Zhang E, Ding L, Mukherjee S, Wei X, Chou G, Wang ZT, Mani S. Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling. *Br J Nutr* 2013; 110: 599-608 [PMID: 23506745 DOI: 10.1017/S0007114512005594]

Bai D, Ueno L, Vogt PK. Akt-mediated regulation of NFKB signaling. *J Exp Med* 2009; 208: 2863-2870 [PMID: 19609947 DOI: 10.1084/jem.20090476]

Eissa N, Hussein H, Kermarroc L, Ali AY, Marshall A, Metz-Boutigue MH, Hendy GN, Bernstein CN, Ghia JE. Chromogranin-A Regulates Macrophage Function and the Apoptotic Pathway in Murine DSS colitis. *J Mol Med (Berl)* 2018; 96: 183-198 [PMID: 29274006 DOI: 10.1002/jm.21740-1613-6]
