Bacterial Sugar Calms Macrophages: Gpr109a Mediates Induction of the Anti-Inflammatory Cytokine IL-10 in Macrophages When Stimulated with Helicobacter hepaticus Supernatant

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ABSTRACT
Interleukin 10 (IL-10) is an anti-inflammatory cytokine, and its polymorphism is linked to inflammatory bowel diseases (IBDs) in humans. Helicobacter hepaticus, a member of mouse gut microbiota, can induce colonic inflammation in absence of an intact IL-10 signaling, and its secretions were found to induce IL-10 production. H. hepaticus was found to secrete a polysaccharide that signals through Toll-like receptor 2 (TLR2) to induce IL-10 production. Gpr109a is a protein receptor that induces production of anti-inflammatory cytokines. It is hypothesized that H. hepaticus induction of IL-10 through TLR2 is mediated by Gpr109a. To test the hypothesis, bone-marrow derived macrophages (BMDMs) of WT and Gpr109a KO mice were stimulated by H. hepaticus supernatant then IL-10 mRNA and protein levels were determined. WT BMDMs were found to produce significantly more IL-10 mRNA and protein compared to Gpr109a KO BMDMs. In addition, it was found that higher levels of IL-10 are induced in presence of Gpr109a when TLR2 is stimulated with Pam3csk. Immunoblot assays suggest that Gpr109a plays a critical role in the activation of ERK1/2 downstream of TLR2 signaling leading to induction of IL-10. These findings will be valuable in delineating the molecular mechanism of Gpr109a signaling and investigating Gpr109a role in preventing H. hepaticus-induced colitis and intestinal cancers.

INTRODUCTION
Gut Microbiome
The microbiome is defined as the overall collection of microbiota that resides inside humans or on their skin surface (Ursell et al., 2012). The human microbiota performs essential functions that define and contribute to the physiology of the host and therefore they share a relationship of symbiosis (Eloe-Fadrosh & Rasko, 2013). In the human microbiome literature, symbiosis is defined as either a commensal relationship, wherein the interaction is decidedly beneficial for one side of the interaction, or mutualistic, where all organisms involved gain beneficial outcomes (Eloe-Fadrosh & Rasko, 2013). In exchange for an environment and nutrients needed for their survival, intestinal bacteria provide metabolic functions such as aiding in digestion as they assist in degradation of complex carbohydrates and producing some essential vitamins such as vitamin K (Li et al., 2008, Honda & Littman, 2016). In addition, they provide immunologic functions by protecting the gastrointestinal (GI) tract from pathogen colonization (O’Hara & Shanahan, 2006).

The more the microbiome is studied, the more it is thought of as a vital organ of the body. It is estimated that we cohabit with 10^{14} bacteria, most of which reside within our intestines (Li et al., 2008, Honda & Littman, 2016). The human genome is less than 1% of the overall microbiome genome, and the microbiome genes produce thousands of molecules that replace many of the functions of the host which influences the host’s health and survival (Bull & Plummer, 2014, Rath & Dorrestein, 2012, Vyas & Ranganathan, 2012). The composition of the intestinal flora has important health implications, and it can be altered with lifestyle, diet, and age (Hopkins et al., 2001). Reductions in these organisms in the large bowel may be related to increased disease risk in elderly people (Hopkins et al., 2001).

Immune Interactions with the Microbiota
Gut microbiota play a critical role in intestinal homeostasis. Since the gut is open to external environment, the immune system needs to be able to fight pathogenic bacteria that might infect the host while being tolerant to the symbiotic gut microbiota. The presence of trillions of bacteria in the gut poses a threat of inflammatory responses that may damage the tissue and cause disease. Molecules derived from the gut microbiota interact with immune cells in the gut and induce production of inflammatory compounds. However, the presence of overlapping immune-
regulatory mechanisms arising from both the host and the microbiota prevent the undesirable induction of inflammatory responses in the gut (Belkaid & Hand, 2014).

Anomalies in immune responses to these gut microbiota can promote inflammation and damage tissues of the intestinal tract which ultimately cause inflammatory bowel diseases (IBDs) (Belkaid & Hand, 2014, Hooper et al., 2012, Wlodarska et al., 2015). Inflammatory bowel diseases, including Crohn’s disease and ulcerative colitis, are chronic inflammatory disorders of the intestine that are associated with high morbidity. Inflammatory bowel diseases can be caused by an amalgam of factors that interplay such as host genetics, gut microbial composition, and environmental factors such as diet that alter mucosal immune responses, but the precise etiology is not well understood (Kaser et al., 2010). Complex molecular pathways govern the interactions between the mammalian host’s immune system and the symbiotic gut bacteria, which results in tolerating these microbes in healthy conditions, and in inflammation and IBDs when maladaptation occurs in this host-microbe interface. These signaling pathways are usually initiated with microbe-derived molecules and many protein and protein complexes can be involved in a cascade to induce the appropriate immune response.

Microbial derived molecules from both commensals and pathogens are recognized by pattern recognition receptors such as Toll-like receptors (TLRs). The activation of these receptors triggers the production of pro-inflammatory mediators such as IL-6 (Cohen, 2014) (Figure 1). These receptors also activate anti-inflammatory signals, such as IL-10, to prevent chronic inflammation and tissue damage (Wen et al., 2010). The signaling pathways employed by particular gut microbes to actively promote intestinal homeostasis are poorly understood, and so is their interaction with intestinal immune cells such as macrophages.

**Toll-like Receptors (TLRs)**

Toll-like receptors (TLRs) are a class of transmembrane pattern recognition receptors that are on the interface between microbes and host immune cells that have the ability to bind microbial associated molecular patterns. TLRs assist the immune system in discriminating between self and non-self by binding to molecules that are exclusively synthesized by microbes such as lipopolysaccharide, peptidoglycans, and bacterial lipopeptide (Medzhitov & Janeway, 1997, Henderson et al., 1996). In addition, TLRs play an important role in inducing pro-inflammatory and anti-inflammatory cytokines that guide the immune system in response to these recognized microbial compounds based on whether or not these microbes are pathogenic (Figure 1). Two decades ago, differential modulation of TLRs in the intestinal mucosa was first described in inflammatory bowel diseases (Cario & Podolsky, 2000). Since then, many studies have looked at the cell-specific effects and mechanisms through which TLRs recognize and distinguish between diverse molecules derived from luminal microbiota and how TLRs signaling may be important in the etiology of inflammatory bowel diseases. A host with a healthy immune system exhibits TLR signaling that employs immune mechanisms that maintain the host barrier integrity and tolerate commensal and mutualistic microbiota (Cario, 2010). However, within a susceptible individual, dysfunctional TLRs signaling may impair intestinal homeostasis and tolerance to commensal flora, thus contributing to amplifying and sustaining inflammation and tissue damage that could possibly result in inflammatory bowel diseases (Cario, 2010).
Infection of mouse colonies with the mouse pathogen *Helicobacter hepaticus* has improved our understanding of host-microbe interactions in the gut and provided important insights into it. *H. hepaticus* is a common gut pathogen to immunodeficient mice but does not induce immune malfunction in normal mice (Kullberg et al., 2002). *H. hepaticus* have been shown to cause colitis in mouse models through driving the production of pro-inflammatory cytokines in absence of anti-inflammatory signaling (Arnold et al., 2016). *H. hepaticus* can be considered as a patho-symbiont as it can be tolerated by a murine host given an intact anti-inflammatory response, but it is also capable of driving inflammation if anti-inflammatory signals are deficient (Danne et al., 2017). Although direct links between *H. hepaticus* and IBDs in human has not been made, studies have shown their localization in children with Ulcerative Colitis, an IBD that leads to sores called ulcers in the intestinal lining (Casswall et al., 2010).

**Interleukin -10 (IL-10) and Interleukin -6 (IL-6)**

Interleukins (IL) are a group of cytokines, which are signal proteins, that mediate communication between cells and are particularly important in inducing immune responses such as inflammation. IL-10 is the most important and best characterized anti-inflammatory cytokine. It inhibits inflammatory reactions by potently inhibiting the production of proinflammatory cytokines and prevents from tissue damage (Sabat et al., 2010). Recent work has demonstrated that inflammatory bowel disease that starts at infancy can be monogenic. Mutations in IL-10 or its receptor that lead to a loss of IL-10 function causes severe intractable enterocolitis in infants and small children (Glocker et al., 2011, Zhu et al., 2017). In addition, IL-10 polymorphism is linked to inflammatory bowel diseases (IBDs) in humans, suggesting that IL-10 signaling plays an important role in preventing colitis both in humans and in mice (Marrakchi et al., 2009). Mice that are rendered interleukin-10 (IL-10) deficient develop chronic enterocolitis resembling human IBDs when experimentally infected with *H. hepaticus* (Kullberg et al., 2002). This colitis is attributed to subsequent lack of anti-inflammatory interleukins and elevation of pro-inflammatory interleukins. In addition, blocking IL-10 signaling induces colitis in mice colonized with *H. hepaticus* (Kullberg et al., 2006). On the other hand, IL-6 is a pro-inflammatory and immune-regulatory cytokine contributing to host defense against infections, while continuous impaired IL-6 synthesis results in tissue damage and the development of various diseases (Kishimoto & Tanaka, 2015). In infectious inflammation, IL-6 is produced by monocytes and macrophages after the stimulation of TLRs with distinct pathogen-associated molecules (Tanaka et al., 2014).
In a study of the interface between *H. hepaticus* and mouse macrophages in healthy mutualistic states, it was found that a large polysaccharide secreted by *H. hepaticus* secretes a molecule that interacts with the receptor TLR2 on macrophages and activates an anti-inflammatory gene signature such as IL-10 induction and results in lower IL-6 levels (Danne *et al.*, 2017) (Figure 2). Therefore, *H. hepaticus*-induced colitis in mice is likely caused by an immune deficiency, in this case a compromised IL-10, and sustained bacterial triggering to intestinal immune cells that results in chronic inflammation. These *H. hepaticus*-induced colitis resemble human IBDs and therefore they are critical to understanding human IBDs.

**G protein–coupled receptor 109a (Gpr109a)**

Gpr109a is a G protein–coupled receptor that is highly expressed on innate immune cells and adipose tissue (Gille *et al.*, 2008). Gpr109a is activated by niacin, vitamin B3, and butyrate, a short-chain fatty acid and fermentation product of dietary fiber by gut microbiota. It has been previously shown that Gpr109a signaling plays a critical role in the homeostasis in the colon by suppression of colonic inflammation and carcinogenesis (Singh *et al.*, 2014). Gpr109a is thought to interact with gut microbe-derived molecules such as butyrate to induce production of anti-inflammatory cytokines such as IL-10 to suppress colonic inflammation (Singh *et al.*, 2014) (Figure 3). Mice lacking Gpr109a exhibit spontaneous rectal prolapse and colonic inflammation (Bhatt *et al.*, 2018). Depletion of gut microbiota ameliorates spontaneous colonic inflammation in Gpr109a −/− mice (Bhatt *et al.*, 2018). This leads us to believe that Gpr109a is involved in interacting with commensal gut microbes, directly or indirectly, to promote tolerance.

Little research has been done to understand the interface between *H. hepaticus* and innate immune cells. The pathway activated by *H. hepaticus* secreted polysaccharide to prevent inflammation in healthy mice and how this interaction is impaired in mice with colitis is not well understood. Specifically, whether Gpr109a is involved in the TLR2 pathway activated by *H. hepaticus* polysaccharide to induce IL-10 to suppress inflammation and promote a symbiotic relationship is unknown. In this paper, we study the role of Gpr109a in TLR2 pathway activated by *H. hepaticus* secretion. It is hypothesized that *H. hepaticus* induction of IL-10 through TLR2 is mediated by Gpr109a. TLR2 signals though a kinase signal transduction cascade that induces a transcriptional change. ERK1/2 is a kinase protein complex that gets activated when the TLR2 is

![Figure 2. Visual representation of induction of anti-inflammatory signal (IL-10) through stimulation of the TLR2 on macrophages by *H. hepaticus* polysaccharide. The kinase signal transduction pathway that TLR2 activates is illustrated. This interaction suppresses chronic inflammation and promotes intestinal homeostasis which ultimately results in mutualism.](image-url)
stimulated by *H. hepaticus* to induce IL-10 (Danne *et al.*, 2017). It has been shown that *H. hepaticus* supernatant induces a specific CREB-dependent anti-inflammatory and repair gene signature in macrophages via the TLR2 pathway. Following TLR2 stimulation, p38 and ERK1/2 activate MSK1/2 which in turn phosphorylates multiple substrates including CREB to induce mainly anti-inflammatory functions (Reyskens & Arthur, 2016). To test where Gpr109a is involved in the TLR2 pathway to induce IL-10, we chose to test whether Gpr109a is involved in ERK1/2 activation.

**Figure 3. Visual representation of the receptor Gpr109a being activated by one of its ligands which consequently induces a signal transduction pathway to increase anti-inflammatory signal (IL-10) in immune cells.**

**MATERIALS AND METHODS**

**Bacterial Culture**
The Gram-negative mouse pathobiont *Helicobacter hepaticus* strain ATCC51449 was grown in tryptone soya broth (TSB) supplemented with 10% Fetal Calf Serum (FCS) and Skirrow Campylobacter supplements (Oxoid) in microaerophilic conditions (1%–3% oxygen). After 2 weeks at 37°C, the culture broth was centrifuged at 4500 rpm for 50 min and filtrated through a 0.2 µm filter. The supernatants were collected and stored at -20°C.

**Culture of Mouse Bone-Marrow Derived Macrophages**
Wild-type and Gpr109a knockout C57BL/6 mice were originally obtained from Jackson Laboratory (Bar Harbor, ME) and bred and maintained in accredited animal facilities at the Augusta University. Bone-marrow cells were harvested from 54 days old KO, and 50 days old WT male mice, and were cultured for 8 days in complete RPMI with 30% M-CSF and seeded at 400,000 cells on 24-well tissue culture plates overnight before stimulation for ELISA and qPCR analyses, and at 1.5M cells on 35 mm tissue culture plates for p-ERK western blot. The Institutional Animal Care and Use Committee of Augusta University approved all animal procedures.

**Treatment of H. hepaticus Culture Supernatant**
The filtered *H. hepaticus* culture supernatant was treated with 50 mg/ml DNase I and RNase A for 2 hours at 37°C, then with 40 mg/ml proteinase K overnight at 56°C, and finally with heat for 2 h in a water bath at 95°C.
**ELISA Assay**
For measurement of secreted cytokines using Mouse IL-10, IL-6 and TNF-α ELISA kits (R&D Systems), treated BMDM culture supernatants were collected and stored at -20°C.

**RNA Extraction**
BMDMs were lysed in TRK lysing buffer and stored at -80°C and RNA was isolated using MicroElute® Total RNA Kit according to the manufacturer’s instructions.

**Quantitative RT-PCR**
Complementary DNA synthesis was performed using High-Capacity cDNA Reverse Transcriptase (Applied Biosystems). Quantitative PCR reactions for the candidate genes were performed using SYBR®Green supermix gene expression assays. Complementary DNA samples were analyzed in duplicate using Real-Time PCR System, and gene expression levels for each sample were normalized to GAPDH. Mean relative gene expression was determined, and the differences were calculated using the $2^{-\Delta\Delta C(t)}$ method.

**Immunoblot Analysis**
Cells were lysed using sample loading buffer (2% SDS, 10% glycerol, 50 mM DTT, 0.002% bromophenol blue and 62.5 mM Tris HCl, pH 6.8). Equal amounts of proteins were resolved by SDS–PAGE and analyzed with antibodies against total ERK1/2, pY204-ERK1/2 (E-4 from Santa Cruz Biotechnology) followed by detection with horseradish peroxidase (HRP)–conjugated secondary antibodies and the chemiluminescent substrate solution ECL.

**Statistical Analysis**
Statistical tests specified in figure legends were performed using Excel®. Differences were considered to be significant when p < 0.05. All bar charts represent means ± SD.

**RESULTS**
**H. hepaticus induces IL-10 through Gpr109a in Bone Marrow Derived Macrophages (BMDMs)**

It has been previously reported that Gpr109a deficient colonic dendritic cells produce lower amounts of the anti-inflammatory cytokine IL-10 and higher amounts of pro-inflammatory cytokine IL-6 (Singh et al, 2004). In addition, blockade of IL-10 signaling induces colitis in *H. hepaticus* colonized mice (Kullberg et al, 2006). Filtered supernatant of *H. hepaticus* was found to be sufficient to directly induce IL-10, due to *H. hepaticus* secreting a polysaccharide that interacts with TLR2 (Danne et al, 2017). To investigate whether Gpr109a mediates *H. hepaticus* induction of IL-10 in macrophages, we generated M-CSF differentiated bone marrow-derived macrophages (BMDMs) from WT and Gpr109a $^{-/-}$ mice and subjected them to stimulation with a filtered supernatant of *H. hepaticus* culture medium (strain ATCC51449) or the control culture medium (TSB). Measurement of cytokine gene transcription after 1.5 hr stimulation and protein expression after 4 hr stimulation showed that *H. hepaticus* induced significantly higher amounts of IL-10 and lower IL-6 when Gpr109a is present (Figure 4A, B and Figure 5A, B). This suggests that Gpr109a is involved in the macrophages’ response following TLR2 stimulation through *H. hepaticus*’s secretion. IL-10 being induced on the mRNA and protein level indicates that Gpr109a is likely involved in signaling to increase transcription of this anti-inflammatory cytokine in response to stimulation with the bacteria *H. hepaticus*. 
Figure 4. (A, B) mRNA induction of the cytokines IL-10 (A) and IL-6 (B) after stimulating WT and Gpr109a<sup>−/−</sup> BMDMs for 1.5 hr with 50% H. hepaticus supernatant and control medium (TSB) relative to untreated WT BMDMs. Error bars indicate standard deviation for 2 replicates. Statistical significance was calculated using Student t test with two-tailed analysis.

Figure 5. (A, B) Protein induction of the cytokines IL-10 (A) and IL-6 (B) after stimulating WT and Gpr109a<sup>−/−</sup> BMDMs for 4 hr with 50% H. hepaticus supernatant and control medium (TSB). Error bars indicate standard deviation for 2 replicates. Statistical significance was calculated using Student t test with two-tailed analysis.
Gpr109a mediates TLR2 ligands mediated production of IL-10

Toll-like receptors (TLRs) recognize microbial products of commensal and pathogenic microbes and trigger pro-inflammatory mediators when activated (Cohen, 2014). They also activate anti-inflammatory signals such as IL-10 to prevent chronic inflammation and tissue damage (Wen et al, 2010). The active molecule in H. hepaticus supernatant was found to signal through TLR2 to induce IL-10 (Danne et al, 2017). To assess the scope of Gpr109a involvement in IL-10 induction by TLR2, WT and Gpr109a−/− BMDMs were stimulated with TLR2/1 canonical ligand, Pam3CysSerLys4 (Pam3csk), in 75 ng/mL and 1000 ng/mL. Pam3csk is a synthetic bacterial-like lipopeptide that activates TLR2 to induce pro-inflammatory signaling (Aliprantis et al, 1999). Measurement of cytokine protein expression after 4 hr stimulation showed that higher levels of IL-10 are induced by TLR2 when stimulated with Pam3csk in presence of Gpr109a (Figure 6). This suggests that Gpr109a involvement in mediating IL-10 induction by TLR2 is not limited to stimulation with H. hepaticus but rather it is more general to TLR2 ligands mediated IL-10 induction.

Figure 6. Protein induction of the cytokine IL-10 after stimulating WT and Gpr109a−/− BMDMs for 4 hr with 75ng/mL and 1000 ng/mL Pam3csk (TLR2/1 canonical ligand). Error bars indicate standard deviation for 2 replicates. Statistical significance was calculated using Student t test with two-tailed analysis.

ERK1/2 activation following TLR2 stimulation is enhanced in presence of Gpr109a

IL-10 induction by H. hepaticus supernatant was found to involve the ERK pathway (Danne et al, 2017). We investigated whether Gpr109a affects ERK1/2 activation after stimulation of BMDMs with treated H. hepaticus supernatant (SNHht) for 15 and 60 minutes, treated control culture medium (TSBt) for 15 and 60 minutes, or Pam3csk for 30 minutes. Immunoblot analysis showed attenuated phosphorylation of ERK1/2 (pT202/Y204) in Gpr109a−/− BMDMs compared to WT after stimulation with SNHht, with the largest difference being observed after 15 minutes of stimulation (Figure 7A). In addition, phosphorylation pattern induced by Pam3csk were reduced in Gpr109a−/− BMDMs (Figure 7B). These findings show that Gpr109a promotes ERK1/2 activation following stimulation of TLR2 with H. hepaticus supernatant or Pam3csk.
DISCUSSION

The intestine contains billions of bacteria that, along with genetic factors, diet, and the immune system, play a profound role in the host’s health and disease. It has been reported that Gpr109a recognizes bacterial-derived molecules and performs nonredundant functions in the immune cells to mediate suppression of inflammation. The specific interactions between host’s immune cells receptors such as Gpr109a and the gut bacteria that promotes mutualism and tolerance are poorly understood. The current study shows that Gpr109a suppresses inflammation in macrophages when stimulated with \textit{H. hepaticus} secretions. \textit{H. hepaticus} can be tolerated by murine hosts in presence of an intact IL-10 signaling but it also has the potential to cause intestinal inflammation through IL-23 if IL-10 is deficient (Arnold \textit{et al.}, 2016). Our results show that Gpr109a skews the TLR2 mediated production of anti-inflammatory and pro-inflammatory cytokines in favor of the former when stimulated with \textit{H. hepaticus} supernatant or TLR2 ligand Pam3csk.

Previous studies have shown that \textit{H. hepaticus} supernatant was a stronger driver of anti-inflammatory activity in macrophages than canonical TLR2 ligand, Pam3csk (Danne \textit{et al.}, 2017). Our current study does not allow for such comparison because the concentration of the active...
molecule in *H. hepaticus* supernatant is not determined, and IL-10 induction after stimulation with Pam3csk increases with higher dose of Pam3csk, indicating that it is not just a qualitative response, but a quantitative one as well (Figure 5A, 6). In addition, although the concentration of the induced IL-6 through Gpr109a after stimulation with *H. hepaticus* supernatant was greater than controls, it does not mean that Gpr109a is promoting inflammation because interaction with bacterial derived molecules causes an immune response that activates a variety of signals that could be both pro- and anti-inflammatory, and the relative ratio of both determines the final outcome. In untreated controls, IL-10 seems to be differentially induced in with and without presence of Gpr109a (Figure 5A, 6). Although WT showed more significant increase of IL-10 in Figure 5A, IL-10 induction was not significant across WT and Gpr109a−/− in the untreated condition. At low concentration of proteins, the accuracy of measured quantities of proteins are much smaller and the error is large, and therefore no conclusion can be made on Gpr109a mediation induction of IL-10 by unstimulated macrophages based on our data.

It has been shown that *H. hepaticus* supernatant induces a specific CREB-dependent anti-inflammatory and repair gene signature in macrophages via the TLR2 pathway. Following TLR2 stimulation, p38 and ERK1/2 activate MSK1/2 which in turn phosphorylates multiple substrates including CREB to induce mainly anti-inflammatory functions (Reyskens & Arthur, 2016). The findings presented in this study show that Gpr109a promotes ERK1/2 activation when TLR2 is stimulated with *H. hepaticus* supernatant or Pam3csk. Although there is no existing research that studies what happens to Gpr109a in relation to the TLR2 pathway, it was previously shown that TLR4 activation with its ligand, lipopolysaccharide, results in increased expression of Gpr109a (Feingold *et al*, 2014). It is possible that the pathway activated by TLR2 induces Gpr109a as a way to reinforce the anti-inflammatory signature activated. It is also possible that Gpr109a directly interacts with either of TLR2 signaling components that skews the induction of cytokines in favor of the anti-inflammatory. Since ERK1/2 activation is affected by Gpr109a, it is either that Gpr109a is involved ahead of ERK1/2 activation in the pathway, or that it is induced further downstream of TLR2 signaling but it results in positive feedback that promotes continuing signaling (Figure 8).

In addition, no known Gpr109a ligand was added in our study, yet its effect was observed. The findings presented here suggest that either TLR2 or its signaling components directly interact with Gpr109a or that TLR2 signaling generate a Gpr109a ligand. The present study implicates an important anti-inflammatory promoting role to Gpr109a in the TLR2 signaling upon interacting with a bacterial-derived compound as a way for the host and the microbes to communicate and prevent colonic inflammation. Previous studies have shown positive effects in suppressing atherosclerosis when Gpr109a ligand, niacin, is taken pharmacologically (Tavintharan & Kashyap, 2001). Studying more Gpr109a ligands and signaling has promising pharmacological implications for inflammatory bowel diseases. Once adequately studied, administering sufficient doses of the bacterial-derived compound might be effective to activate Gpr109a signaling and protect colon against inflammation and carcinogenesis.
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REFERENCES


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<td>Interleukin 10</td>
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<tr>
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<td>Inflammatory Bowel Diseases</td>
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