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Original Paper

Epigallocatechin-3-Gallate Regulates Anti-Inflammatory Action Through 67-kDa Laminin Receptor-Mediated Tollip Signaling Induction in Lipopolysaccharide-Stimulated Human Intestinal Epithelial Cells

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Key Words
Epigallocatechin-3-gallate • HT-29 cells • Toll-like receptor 4 • Tollip induction • 67-kDa laminin receptor • IL-8 production

Abstract
Background/Aims: Inflammatory bowel disease (IBD) is a condition that involves chronic inflammation in all or part of the digestive tract. Often painful and debilitating, IBD can lead to life-threatening complications and increase the risk for colon cancer. In this study, we investigated the epigallocatechin-3-gallate (EGCG) mediated anti-inflammation response in lipopolysaccharide (LPS)-stimulated human colorectal cells through the negative regulator of Toll-like receptor (TLR) signaling. ***Methods:*** human intestinal epithelial cells (HT-29) were used in all experiments. Cell cytotoxicity and nitric oxide (NO) were evaluated by WST-1 and the Griess reagent. Western blot analysis and ELISA were used to determine inflammatory mediators and 67-kDa laminin receptor (67LR)-mediated Tollip signaling pathways. ***Results:*** Treatment of EGCG and LPS did not affect the cytotoxicity in HT-29 cells. LPS treatment dose-dependently increased the pro-inflammatory cytokine, such as interleukin (IL)-8, whereas EGCG significantly reduced the LPS-stimulated IL-8 production. Additionally, EGCG treatment markedly increased the Toll-interacting protein (Tollip) expression, which negatively regulates the TLR signaling in a dose and time-dependent manner. In particular, in the result from an RNA interference-mediated assay, our finding showed that silencing of Tollip resulted in abrogation of the inhibitory action of EGCG on LPS-induced production of pro-inflammatory mediators (inducible nitric oxide synthase-mediated NO/COX2, and IL-8) and activation of MAPKs and NF-κB signaling pathways. Interestingly, we also found that Tollip expression induced by EGCG

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could be modulated through 67LR expressed on the surface of HT-29 cells. ***Conclusions:*** Our novel finding indicates that 67LR and Tollip signaling activated by EGCG treatment is essential for inhibition of inflammation in human intestinal epithelial cells.

Introduction

Inflammatory bowel disease (IBD) is a typical chronic immune-mediated illness of unknown etiology associated with a dysregulated mucosal immune response to intestinal microorganisms in a genetically susceptible host [1]. IBD is characterized by the destruction of gut tissue, which is initiated from alterations of the intestinal epithelium barrier function involving increased tight junction permeability and maintained by a defective down-regulation of mucosal immunity toward the intestinal microflora [2]. Importantly, this disease is mediated by proinflammatory cytokines and inflammatory mediators [1, 3-5]. Among them, nitric oxide (NO) is a potential biomarker of disease activity in IBD. Excessive formation of NO by inducible NO synthase (iNOS) has been associated with cellular toxicity and tissue damage in experimental models of active intestinal inflammation [3]. Additionally, intestinal inflammation is also deeply mediated to the production of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β [4]. Previous research reported that cytokines, especially IL-8, play a crucial role in the pathogenesis of IBDs, such as Crohn's disease and ulcerative colitis, where they control multiple aspects of the inflammatory response [5]. Thus, understanding the regulating mechanisms of the factors involved in IBD pathogenesis will be important for the effective control of intestinal inflammation.

Green tea contains various catechins, such as (-)-epigallocatechin 3-O-gallate (EGCG), (-)-gallocatechin 3-O-gallate, (-)-epicatechin 3-O-gallate, (-)-epigallocatechin, (+)-gallocatechin, (-)-epicatechin, and (+)-catechin, and it is known to have many physiological functions in the prevention and therapy of disease [6, 7]. Among these, EGCG is a powerful candidate for inhibition of inflammation and cancer [8, 9]. EGCG treatment has been shown to markedly suppress the excessive increase of LPS-stimulated inflammatory cytokine, such as IL-8, which is a multifunctional member of the chemokine family and is also elevated in tissues from IBD patients [10]. In addition, many studies showed that EGCG specifically inhibits the inflammatory mediators and cytokine levels through the 67-kDa laminin receptor (67LR) as a cell-surface EGCG receptor in LPS-stimulated various cells, such as adipocytes, macrophage, and cerebral microvascular endothelial cells [11-13]. Furthermore, 67LR signaling can activate TLR negative regulator, the Toll-interacting protein (Tollip), in macrophages [12]. However, the precise mechanisms for inhibition of excessive inflammatory response induced by EGCG treatment in IBD patients still remain largely unknown.

Thus, the aim of this work was to elucidate the anti-inflammation action of EGCG, including the TLR4 signal transduction in LPS-stimulated human intestinal epithelial cells (HT-29 cells). We hypothesized that 67LR signaling is one of the major anti-inflammatory signals in intestinal epithelial cells. Here, we demonstrate that Tollip induction via 67LR is essential for mediating the anti-inflammatory action of EGCG in LPS-stimulated HT-29 cells.

Materials and Methods

Antibodies and reagents

EGCG was purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-TLR4 polyclonal antibody (Ab), anti-CD14 polyclonal Ab, Anti-phosphorylated extracellular signal-regulated kinase (ERK)1/2 monoclonal Ab, anti-phosphorylated p38 monoclonal (m) Ab, anti-NF-κB (p65) polyclonal (p) Ab, anti-phosphorylated inhibitor of kapper (Iκ)B-α mAb, anti-inducible NO synthase (iNOS) pAb, anti-COX-2 pAb, anti-SOCS1 pAb, anti-IRAK-M mAb, anti-Tollip mAb and anti-Lamin B pAb were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG Ab and HRP-conjugated

anti-rabbit Ab were obtained from Calbiochem (San Diego, CA, USA), and anti-β-actin mAb (AC-15) was purchased from Sigma-Aldrich. IL-8 enzyme-linked immunosorbent assay (ELISA) kits were obtained from BD Biosciences (San Diego, CA, USA).

Cell culture

The human colon cancer cell line, HT-29, was cultured in DMEM (GIBCO, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, GIBCO), 100 U/mL penicillin, and 100 U/mL streptomycin (complete medium) under humidified conditions at 37°C and 5% CO_2 in an incubator until they reached 80% confluence.

Construction of Tollip-suppressed cells

Tollip short hairpin RNA expression vector was purchased from Santa Cruz Biotechnology. shRNA plasmids consist of a pool of three to five antiviral vector plasmids each encoding target-specific 19–25 nt (plus hairpin) shRNAs designed to knockdown gene expression. For each transfection, we added 0.8 mL shRNA plasmid transfection medium to a well and then incubated the cells for 7 h. We further performed the neomycin selection for obtaining stably transfected cells.

Measurement of cell proliferation in HT-29 cells

HT-29 cells were mechanically scraped, seeded in 96-well plates at 5×10^4 cells/mL, and incubated for 24 h. After the incubation, the cells were treated with EGCG for 24 h. The tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophe-nyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, known as WST-1 (Roche, Mannheim, Germany), was used to detect the loss of viability. The supernatant medium was replaced by WST-1 and incubated for 2 h. The colored supernatants without particles were transferred into a clean 96-well plate and measured at 450 nm in a microplate reader. The results are given as the relative percentage to the untreated control.

IL-8 production

Supernatants from experimental HT-29 cells cultures were collected and stored at −70°C until use. The levels of IL-8 in the supernatants were determined using cytokine detection ELISA kits (BD Biosciences, San Diego, CA) according to the manufacturer's instructions, with detection at 450 nm using a microplate reader.

Measurement of NO production

The concentration of NO in a culture supernatant was determined as nitrite, by the Griess reagent (1% sulfanilamide / 0.1% naphtylethyle-nediamine dihydrochloride in 2.5% H_3PO_4) (Sigma, St. Louis, MO,). Cells were seeded into a 6-well plate at 3×10^5 cells/well in 3 mL of complete medium per well and incubated for 24 h at 37°C. The cells were pretreated with 20 μM EGCG for 1 h and stimulated with lipopolysaccharide (LPS; 200 ng/mL) for 24 h. Next, the supernatant of the cell culture medium was collected and assayed for NO production using the Griess reagent. The culture medium (100 μL) was incubated with 100 μL of Griess reagent. The absorbance of the mixture was then measured at 535 nm. The concentration of nitrite was converted into sodium nitrite concentration as a standard.

Immunoblotting analysis.

HT-29 cells were lysed in a 100 μL lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton-X100, 1 mM EDTA, 50 mM NaF, 30 mM Na_4PO_7, 1 mM phenylmethanesulfonyl fluoride (PMSF), 2 μg/mL aprotinin, and 1 mM pervanadate. Whole-cell lysate samples were resolved by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane. The membranes were blocked in 5% skim milk and incubated with the respective Ab for 2 h, followed by incubation with HRP-conjugated secondary Ab for 1 h at room temperature. Epitopes on target proteins recognized specifically by Abs were visualized using an enhanced chemiluminescence advance kit (GE Healthcare, Little Chalfont, UK).

Nuclear extract preparation.

Nuclear extracts from cells were prepared as follows. HT-29 cells were treated with a 100 μL lysis buffer (10 mM HEPES [pH 7.9], 10 mM KCl, 0.1 mM EDTA, 0.5% Nonidet P-40, 1 mM dithiothreitol (DTT), 0.5 mM PMSF) on ice for 10 min. Following centrifugation at 4000 rpm for 5 min, the pellet was re-suspended in a 100 μL extraction buffer (20 mM HEPES [pH 7.9], 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF) and incubated on ice for 30 min. After centrifugation at 12, 000 rpm for 10 min, the supernatant containing nuclear extracts was collected and stored at −80°C until required.

Statistical analysis.

All experiments were repeated at least three times with consistent results. The levels of significance for comparison between samples were determined by Tukey's multiple comparison test distribution using statistical software (GraphPad Prism Software, version 4.03; GraphPad Software, San Diego, CA). The data in the graphs are expressed as mean ± SEM. Each value of $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$ was considered to be statistically significant.

Results

Effect of cell viability and IL-8 production in HT-29 cells

We examined the cell viability in HT-29 cells after EGCG or LPS treatment at various doses to investigate cellular toxicity of EGCG and LPS treatment. As shown in Fig. 1A, up to 20 μM of EGCG displayed no cellular toxicity against HT-29 cells. Similarly, lower doses than 200 ng/mL of LPS also did not affect the cell viability (Fig. 1B). Next, to examine the inhibition of HT-29 cells-induced IL-8 production by EGCG treatment, the cells were pretreated for 1 h with EGCG (5 to 20 μM) prior to exposure to LPS (200 ng/mL) for 24 h. As shown in Fig. 1C and D, LPS treatment markedly enhanced the level of IL-8 production in a concentration-dependent manner (50 to 200 ng/mL), whereas the excessive increased IL-8 level induced by LPS was greatly attenuated by EGCG treatment (Fig. 1D).

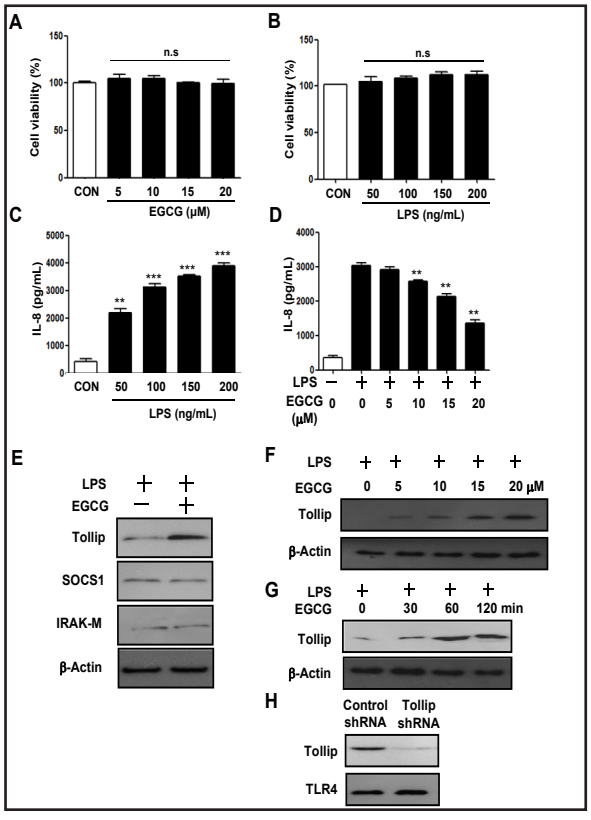

Fig. 1. Inhibitory effect of EGCG on IL-8 production and induction of Tollip protein expression by EGCG treatment in HT-29 cells. (A, B) Cells viability by treatment of EGCG (5, 10, 15, and 20 μM) or LPS (50, 100, 150, and 200 ng/mL) in HT-29 was performed by WST-1 assay. (C) IL-8 production in HT-29 cells stimulated with LPS (50, 100, 150, and 200 ng/mL). (D) HT-29 cells were pretreated with various concentrations of EGCG (5, 10, 15, and 20 μM) for 1 h and stimulated with LPS (200 ng/mL) for 24 h. The concentrations of IL-8 in the culture medium were measured by ELISA. (E) The cells were pretreated with an optimal concentration of EGCG (20 μM) for 1 h and stimulated with LPS (200 ng/mL) for 24 h. Expression levels of SOCS1, IRAK-M, or Tollip were detected using specific Abs. (F, G) HT-29 cells were pretreated with various concentrations of EGCG (5, 10, 15, and 20 μM) for 1 h or various times (30, 60, and 120 min) in an optimal concentration of EGCG (20 μM) and then these cells were stimulated with LPS (200 ng/mL) for 24 h. Expression levels of Tollip were detected using specific Abs. (H) HT-29 cells transfected with the Tollip shRNA vector were pretreated with an optimal concentration of EGCG (20 μM) for 1 h and stimulated with LPS (200 ng/mL) for 24 h, and then protein levels of Tollip were detected by immunoblotting. All data are expressed as the mean ± SD (n = 3) and statistical significance (**p<0.01, ***p<0.001) is shown for the treatments compared to the controls (CON; non-treated cells) and LPS-treated cells. The value of n.s. was defined as no significant effect. β-actin; loading control for cytosolic fractions.

EGCG upregulates Tollip protein expression in HT-29 cells

Recently, we identified Tollip as mediating the anti-inflammatory action of EGCG in macrophages and vascular endothelial cells [14, 15]. However, there has been no observation in human intestinal epithelial cells to determine whether EGCG affects an anti-inflammatory response in inflammatory-induced HT-29 through TLR negative regulators. To elucidate the underlying mechanisms of EGCG-mediated anti-inflammatory action in HT-29 cells, we first examined the expression of TLR negative regulators by EGCG treatment. The results showed that HT-29 cells treated with EGCG and LPS specifically increases the expression of Tollip (Fig. 1E) in a time- (Fig. 1F) or dose dependent manner (Fig. 1G); but other negative regulators, such as SOCS1 and IRAK-M, were not affected (Fig. 1E). These results suggest that EGCG treatment may inhibit the IL-8 production by expression of Tollip in LPS-induced HT-29 cells. Further, we evaluated the amounts of TLR4 expression in Tollip-downregulated cells (Tollip shRNA-treated cells) and control cells (Control shRNA-treated cells). HT-29 cells were stably transfected with a shRNA expression vector to reduce the Tollip expression. As shown in Fig. 1H, the total cellular Tollip expression was lower in HT-29 cells transfected with shRNA for Tollip, whereas the expression of TLR4 in the Tollip-downregulated cells was not altered compared with control cells. This result suggested that the silencing of Tollip did not affect the expression of TLR4, and that the effect of LPS to TLR4 may be equal in both the Tollip-down-regulated and control cells.

EGCG suppresses LPS-induced IL-8 and NO production through Tollip in HT-29 cells

To determine whether the effects of EGCG on LPS-induced IL-8 and NO production could be mediated through Tollip signaling, Tollip-downregulated HT-29 cells and control cells were stimulated with EGCG for 1 h and then the cells were treated with LPS for 24 h. We found that the levels of IL-8 and NO production significantly increased with treatment with LPS (200 ng/mL), and these inflammatory mediators, IL-8 and NO, were inhibited by EGCG treatment in the control cells. However, in case of Tollip-downregulated cells, no inhibition effects of inflammatory mediators were observed (Fig. 2A and B). Therefore, these results powerfully suggest that Tollip signaling may lead to the anti-inflammatory action induced by EGCG in HT-29 cells.

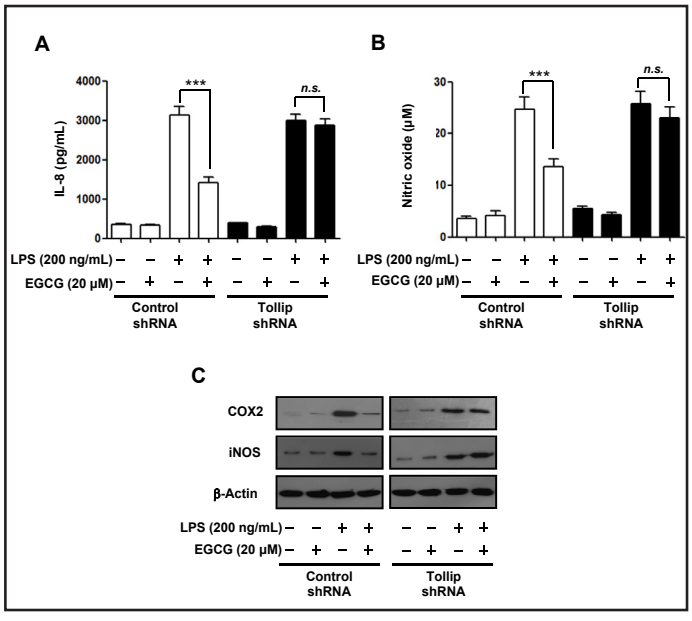

Fig. 2. Inhibitory effect of IL-8 and NO production in control shRNA- and Tollip shRNA-transfected HT-29 cells. Control shRNA- and Tollip shRNA-transfected HT-29 cells were pretreated with 20 μM EGCG for 1 h and stimulated with LPS (200 ng/mL) for 24 h. (A) IL-8 production in the culture medium was measured by ELISA. (B) NO production in the culture medium was measured by Griess reagent using assay. The data are shown as means ± SD (n = 3 samples) from one representative plot out of three independent experiments; **p<0.001. The value of n.s. was defined as no significant effect. (C) For measurement COX-2 and iNOS expression in control shRNA- and Tollip shRNA-transfected HT-29 cells, cells were treated with EGCG (20 μM) for 1 h and stimulated with LPS (200 ng/mL) for 24 h and total cellular proteins were resolved by SDS-PAGE, and the separated proteins were transferred to nitrocellulose membranes and detected using specific Abs (COX-2 and iNOS). One representative plot out of three independent experiments is shown.

Effect of Tollip-downregulation on EGCG-induced inactivation of iNOS and COX-2 proteins

Inducible NOS (iNOS) or COX-2 are major mediators involved in acute and chronic inflammation, and these mediators were induced prior to the secretion of inflammatory mediators [16]. Therefore, we examined whether EGCG suppresses LPS-induced iNOS or COX-2 expressions through Tollip. As shown in Fig. 2C, iNOS and COX-2 expressions were significantly inhibited by EGCG treatment; however, this inhibitory effect was not observed in Tollip-downregulated cells, suggesting that Tollip mediates the suppressive effect of EGCG on the production of LPS-induced inflammatory mediators.

EGCG inhibits LPS-induced NF-κB and MAPKs activation through Tollip

Next, we examined whether EGCG suppresses LPS-induced NF-κB and MAPKs activation through Tollip. HT-29 cells were pretreated with EGCG for 1 h before exposure to LPS for 30 min, and then the phosphorylation of MAPKs (including ERK1/2, and p38), the phosphorylation/degradation of IκB-α and the nuclear translocation of p65 were measured through an immunoblot analysis. As shown in Fig. 3, LPS-induced phosphorylation of ERK1/2 and p38 was inhibited by treatment with EGCG (Fig. 3A), and EGCG also resulted in a significant suppression of both nuclear translocation of p65 and phosphorylation of IκB-α in LPS-treated control cells (Fig. 3B). However, in the case of Tollip-downregulated cells, the inhibitory effect of EGCG on LPS-induced up-regulation of NF-κB and MAPKs activation was attenuated when compared to the control cells (Fig. 3A and B). These results indicate that EGCG inhibits LPS induced NF-κB and MAPKs activation through activation of Tollip.

EGCG up-regulates Tollip protein expression through 67-kDa laminin receptor (67LR) in HT-29 cells

TLR4 has been shown to play a crucial role in LPS-induced inflammatory responses, and CD14 is required as a co-receptor for TLR4 to recognize LPS. In addition, it has been reported that LPS induces the IL-8 secretion through TLR4 in HT-29 cells [17]. Therefore, we

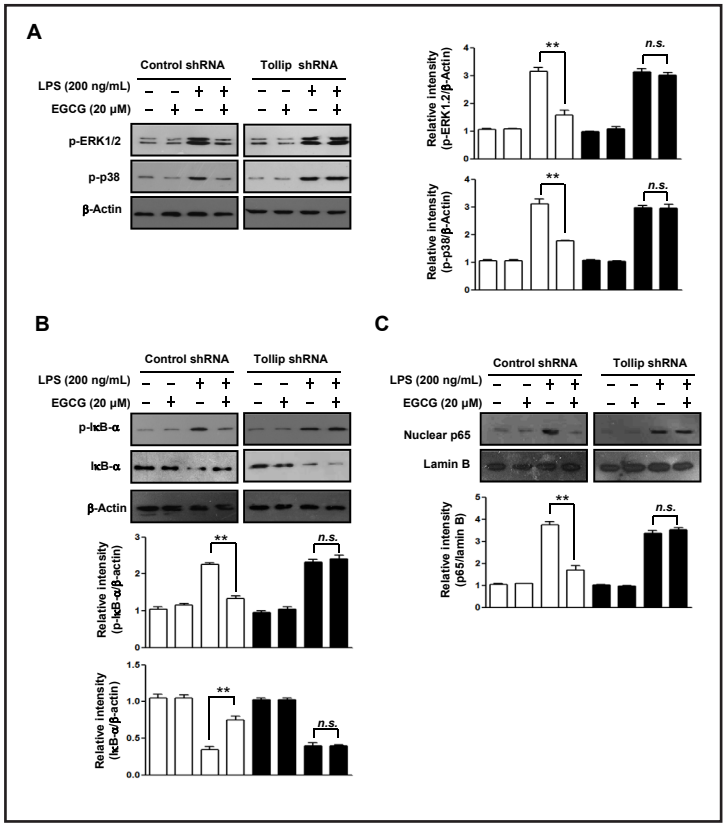

Fig. 3. Inhibitory effect of the MAPK and NF-κB signaling pathway in control shRNA- and Tollip shRNA-transfected HT-29 cells. HT-29 cells were incubated with 20 μM EGCG for 1 h and then stimulated with 200 ng/mL LPS for 45 min. Cell lysates were subjected to SDS-PAGE, and an immunoblot analysis was performed using specific Abs to (A) phosphor-ERK1/2 (p-ERK1/2), phosphor-p38 (p-p38), (B) phosphor-IκB-α (p-IκB-α), IκB-α and (C) p65 NF-κB. β-Actin and Lamin B were used as loading controls for cytosolic and nuclear fractions, respectively. The relative band intensity of each protein is expressed as a percentage. The data are shown as means ± SD (n = 3 samples) from one representative plot out of three independent experiments; **p<0.001. The value of n.s. was defined as no significant effect.

first evaluated the expression of TLR4 and CD14 in anti-67LR-treated cells and control cells (Isotype-treated cells). As a result, the expression of TLR4 and CD14 in anti-67LR-treated cells by EGCG treatment was not altered compared with control cells (Fig. 4A). Furthermore, the protein levels of 67LR and TLR4 were not affected (Fig. 4B). Thus, these results suggest that EGCG treatment did not affect the expression of 67LR and TLR4. We next investigated whether EGCG upregulates Tollip protein expression through 67LR. After anti-67LR treatment, HT-29 cells were incubated with EGCG for 1 h, and then the cells were treated with LPS for 24 h. HT-29 cells treated with anti-67LR, EGCG and LPS abrogated Tollop expression compared to cells treated with isotype Ab, EGCG and LPS (Fig. 4C). Finally, to examine the role of 67LR in cytokine production of LPS-induced HT-29 cells, anti-67LR Ab-treated HT-29 cells were pretreated with EGCG for 1 h prior to treatment with LPS for 24 h. As shown in Fig. 4D, in isotype-treated cells, HT-29 cells treated with LPS and EGCG inhibited LPS-induced IL-8 production, which was different from the LPS-treated HT-29 cells. However, in anti-67LR-treated cells, the inhibition of LPS-induced IL-8 production was not changed by EGCG treatment (Fig. 4D). These results indicate that the ability of EGCG to reduce the IL-8 secretion is mediated through its binding to the 67LR of HT-29 cells.

Discussion

EGCG as a bioactive polyphenol in green tea is well known for anti-inflammatory action by the inhibition of LPS-stimulated pro-inflammatory mediators through TLR4-triggered

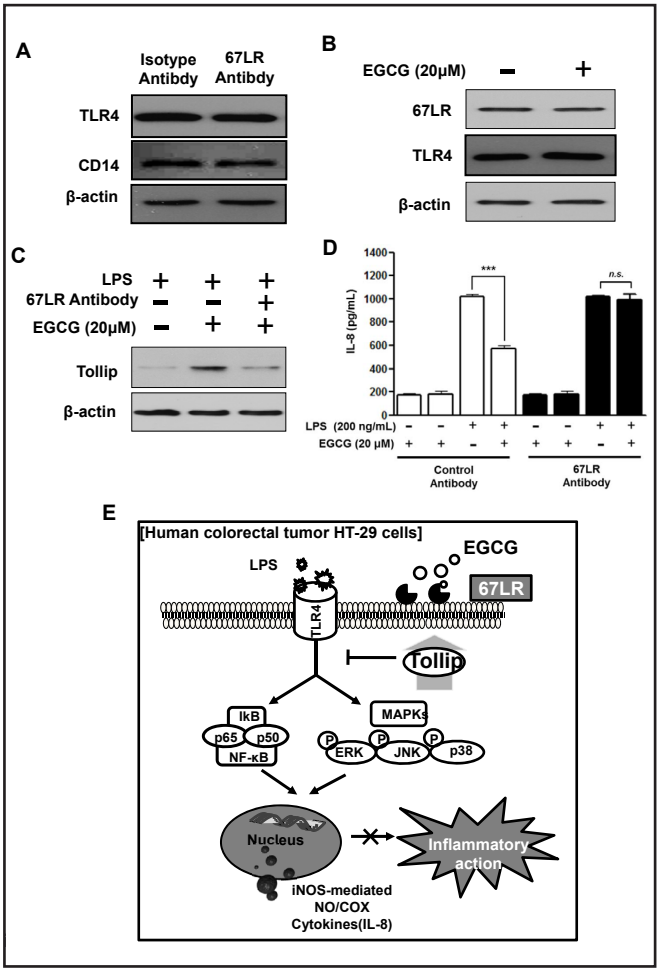

Fig. 4. Anti-inflammatory action of EGCG-treated HT-29 cells by 67LR. (A) HT-29 cells were incubated with either anti-67LR Ab or isotype Ab for 24 h. The expression levels of TLR4 and CD14 in isotype- and anti-67LR Ab-treated cells were measured by immunoblot analysis using specific Abs. (B) HT-29 cells were treated with EGCG (20 μM) for 24 h, and protein expression of 67LR and TLR4 was determined by immunoblot analysis using specific Abs. (C) HT-29 cells were treated with EGCG (20 μM) for 1 h. Total cellular proteins were resolved by immunoblot analysis using specific Tollip Ab. (D) HT-29 cells were incubated with either an anti-67LR Ab or isotype Ab for 1 h. The cells were then pretreated with EGCG (20 μM) for 1 h before exposure to LPS (200 ng/mL) for 24 h. The concentrations of IL-8 in the culture medium were measured by ELISA. All data are shown as means ± SD (n = 3 samples) from one representative plot out of three independent experiments; **p<0.001. The value of n.s. was defined as no significant effect. (E) The proposed mechanism of the anti-inflammatory action induced by EGCG in HT-29 cells.

NF-κB and MAPK signaling pathways [18]. Our previous study showed that EGCG treatment significantly reduced the inflammatory mediator, such as inflammatory cytokine, cell surface molecules and MAPK signals in LPS-treated macrophages, and here we first demonstrated that this anti-inflammation action by EGCG was mediated by the activation of Tollip protein, the negative regulator of TLR [14]. However, there have been no studies about gut inflammatory action of EGCG containing the detailed mechanisms which involved a negative regulator of TLR in intestinal cells. Here, the results of this study demonstrated that EGCG, which suppressed the excessive inflammatory factors, such as IL-8 and NO, in LPS-induced inflammatory intestinal epithelial cells (HT-29 cells).

Recently, negative TLR regulators have become a focus area in research on various inflammatory systems [19]. Several studies have reported that TLR-mediated over-activation of the host immune response is regulated by multiple intracellular negative regulators of TLRs, including the suppressor of cytokine signaling 1 (SOCS1), Tollip, and IL-1 receptor-associated kinase (IRAK)-M [20, 21]. Among them, the deletion of Tollip promoted excessive inflammation associated with increased susceptibility to DSS-induced colitis, and Tollip expression is significantly decreased in IBD patients [22, 23]; thus, Tollip plays critical roles in the inflammatory process of IBD because Tollip has been implicated as a negative regulator of inflammatory signals, such as MAPK and NF-κB signals [24]. A recent study demonstrated that both MAPK and NF-κB signals, which are key regulators in intestinal inflammation, have immunostimulatory properties; they induce the activation and cytokine secretion in various immune cells, thereby driving intestinal tissue damage via inflammatory cytokines [25, 26]. Furthermore, the NF-κB signal does not trigger excessive inflammation unless it is accompanied by MAPK signaling activation [27]. Thus, effective regulation of Tollip, MAPK and NF-κB signals are one important step for new drug development for IBD. Interestingly, this study also showed that EGCG suppressed the excessive increase of IL-8 and NO production via Tollip expression in LPS-treated HT-29 cells; it also induced the suppression of LPS-induced MAPKs and NF-κB signals via Tollip expression in HT-29 cells.

As mentioned above, EGCG regulates the inflammation via Tollip signaling in intestinal epithelial cells. However, the mechanism of EGCG-induced Tollip expression in intestinal epithelial cells has not been understood. Recently, 67LR, a non integrin cell surface receptor for the extracellular matrix, has been identified as a cell surface receptor for EGCG that mediates the anti-inflammatory activity of EGCG [28]. Interestingly, EGCG induces a strong Tollip expression via 67LR in LPS-treated HT-29 cells and shows anti-inflammatory activity via 67LR.

In conclusion, our results provide a new insight into the immune-pharmacological role of EGCG in the inflammatory signaling negatively regulated by Tollip induction through 67LR in LPS-stimulated HT-29 cells, and these findings strongly suggest a novel approach for the manipulation of intestinal epithelial cells in the development and progression of the IBD.

Acknowledgements

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Eui-Baek Byun and Woo Sik Kim: methodology, data analysis, editing and writing original draft; Nak-Yun Sung: methodology and data analysis; Eui-Hong Byun: conceptualization, supervision, review, and editing.

Disclosure Statement

No conflict of interests exists.

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