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The green tea polyphenol (—)-epigallocatechin-3-gallate inhibits leukocyte activation by bacterial formylpeptide through the receptor FPR

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Abstract

Although green tea polyphenol catechin is considered as a potential anti-inflammatory agent, its effect on bacterial component-induced inflammation has been poorly investigated. We examined the capacity of epigallocatechin gallate (EGCG) to regulate leukocyte responses to bacterial chemotactic peptide N-formylmethionyl-leucyl-phenylalanine (fMLF), which is recognized by a human G protein-coupled receptor FPR on phagocytic leukocytes. Pretreatment of human monocytic cells or FPR-transfected rat basophilic leukemia cells (ETFR cells) with EGCG significantly inhibited fMLF-induced chemotaxis. Intraperitoneal administration of EGCG in mice suppressed fMLF-induced leukocyte infiltration into the air pouch created in the skin. Mechanistic studies revealed that EGCG dose-dependently suppressed fMLF-induced calcium flux in monocytic cells and ETFR cells. fMLF-induced ETFR cell migration was significantly inhibited by a specific MEK1/2 inhibitor, PD98059, which was associated with reduction in fMLF-induced ERK1/2 phosphorylation. These results suggest that EGCG inhibits FPR-mediated leukocyte activation thus is a promising anti-inflammatory compound.

Keywords

EGCG; Leukocyte; fMLF; Inflammation; Calcium mobilization; MAP kinase

1. Introduction

Leukocyte recruitment to sites of inflammation and infection is dependent upon the presence of a gradient of locally produced chemoattractants. By binding to their receptors,

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Conflict of interest

The authors state no conflict of interest.

chemoattractants direct the trafficking of phagocytic leukocytes and activate leukocytes to generate superoxide anions and release granule contents that enable leukocytes to serve as the first-line host defense against invading microorganisms [1]. However, excessive activation of phagocytes by chemoattractants is often a major cause of tissue damage. In addition, chemoattractants are involved in a number of diseases such as HIV-1 infection [2], tumor growth [3], acute and chronic inflammation [4]. Therefore, development of chemoattractant inhibitors may yield drugs with significant therapeutic benefit [5,6].

(—)-Epigallocatechin gallate (EGCG) is a major catechin compound in tea extracts and is also the most biologically active form among tea catechins. For instance, EGCG has anticarcinogenic, antioxidant, cardiovascular protective and neuroprotective activities [7,8]. Furthermore, EGCG has been reported to inhibit inflammatory response mediated by leukocytes. EGCG induces apoptosis of human monocytes [9], inhibits chemokine-induced leukocyte chemotaxis [10–12], release of reactive oxygen species, nitric oxide and cytokine production [10,13–16], neutrophil and basophil degranulation [17,18], lymphocyte proliferation [16], inflammation and allergy in animal models [10,11,19,20].

N-formyl-methionyl-phenylalanine (fMLF) is the prototypic peptide of a series of chemoattractants isolated from bacterial culture suspensions and may also be released by disrupted mammalian cells [21,22]. fMLF binds with high affinity to a specific G protein-coupled formylpeptide receptor, FPR, originally identified on phagocytic leukocytes but later also found on other cell types [22]. Activation of FPR on leukocytes by fMLF results in increased chemotaxis, phagocytosis, and production of reactive oxygen and inflammatory inter-mediate [1,21,22]. Although EGCG has been shown to inhibit leukocyte migration induced by several chemokines [10–12], its effect on fMLF-induced leukocyte migration and activation is unclear. In this study, we report the inhibitory effect of EGCG on fMLF-induced chemotaxis of human monocytic cell line THP-1 and FPR transfected rat basophil leukemia cells (ETFR cells), as well as leukocyte infiltration into the air pouch in mice. We further report that the effect of EGCG is likely due to its inhibition on FPR-mediated Ca^{2+} and ERL1/2 pathway.

2. Materials and methods

2.1. Materials

EGCG with purity N99% was purchased from Sigma-Aldrich (St. Louis, MO) or Zhejiang Yixin Pharmaceutical Co., Ltd (China) and dissolved in PBS before use. fMLF and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma-Aldrich (St. Louis, MO). Fluo-3 AM and Pluronic F-127 were from Molecular Probes. Collagen type I was from Collaborative Biomedical Products (Bedford, MA). Antibodies against both phosphorylated and unphosphorylated ERK1/2 were obtained from Cell Signaling Technology (Beverly, MA). DMEM and RPMI 1640 were purchased from Gibco BRL (Burlington, Ontario, Canada). FBS was from Hyclone (Logan, UT). All other reagents were obtained from Sigma-Aldrich unless otherwise described.

2.2. Animals and treatment

Six-week old KM mice were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China) and maintained in a temperature controlled room (22 ± 2 °C) with a 12-h light/dark cycle. Mouse air pouches were raised on the dorsum by subcutaneous injection of 3 ml sterile air on days 0 and 3. On day 5, mice with well-formed air pouches were treated with EGCG (20 mg/kg body weight/day, i.p.) or vehicle (identical volume of normal saline) for 2 d. On day 6, 4 h after EGCG injection, 1 ml of endotoxin-free normal saline alone or containing 1 μ M fMLF was injected into the air pouch. 4 h after the injection, mice were sacrificed by CO₂ asphyxiation. The air pouches were washed with PBS containing 5 mM EDTA and 20 U/ml heparin, and leukocytes recovered from the air pouches were counted by Wright's staining. The present animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Biological Research Ethics Committee of Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

2.3. Cell cultures

THP-1 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 units/ml penicillin and 100 μ g/ml streptomycin in a humidified atmosphere at 37 °C with 5% CO₂. FPR transfected rat basophil leukemia cells (ETFR cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, 800 μ g/ml G418, 100 units/ml penicillin and 100 μ g/ml streptomycin in a humidified atmosphere at 37 °C with 5% CO₂.

2.4. Chemotaxis assay

Migration of THP-1 cells and ETFR cells in response to fMLF was assessed using a 48-well microchemotaxis chamber technique as previously described [23]. Briefly, different concentrations of fMLF were placed in the wells of the lower compartment of the chamber (Neuro Probe, Cabin John, MA). THP-1 cells (4×10^6 cells/ml) or ETFR cells (10^6 cells/ml) were placed in the wells of the upper compartment. The two compartments were separated by a polycarbonate filter (GE Osmonics, Minnetonka, MN; 5 μ m pore-size for THP-1 cells and 10 μ m for ETFR cells. For migration of ETFR cells, the filters were pre-coated with 50 μ g/ml collagen type I to favor the attachment of the cells). After incubation at 37 °C (2 h for THP-1 cells, 4.5 h for ETFR cells), the filters were removed, stained, and the cells migrated across the filters were counted under light microscope after coding the samples. The results are presented as the number of cells per high power field.

2.5. Calcium (Ca²⁺) flux assay

Cells were incubated with 1 μ g/ml Fura-3 AM in loading medium (RPMI 1640 without L-glutamine, 10% FCS, 10 mM HEPES, and 0.005% Pluronic F-127) at 37 °C for 30 min in dark, washed, and re-suspended in Emission buffer (phosphate buffered solution containing 1 mM CaCl₂, 0.75 mM MgCl₂ and 5 mM glucose) with vehicle or different concentrations of EGCG at a density of 4×10^6 cells/ml for 15 min. The cell suspension was immediately transferred to black-walled, clear-base 96-well plate. The cell plate was then placed into the FlexStation II³⁸⁴ system (Molecular Devices Sunnyvale, CA) to monitor fluorescence before

and after the addition of different concentrations of fMLF prepared in Emission buffer containing 0.025% Pluronic F-127. The fluorescence was measured with the excitation wavelength at 485 nm and the emission wavelength at 525 nm, and presented as RFU (relative fluorescence units).

2.6. Western blot analysis

The cells were lysed with cold lysis buffer containing 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 100 mM Tris (pH 8.0), 10 mM NaF, 1% deoxycholic acid, 1 mM PMSF, 1 mM sodium vanadate, 1 mM DTT, 10 μ M aprotinin and 4 μ M leupeptin. The cell lysate was centrifuged at 14,000 rpm and 4 °C for 20 min to remove insoluble materials, and the concentration of protein was determined by Bradford assay. Western blotting of phosphorylated ERK1/2 was performed according to the manufacturers' instruction using phospho-specific antibodies. Briefly, proteins were electrophoresed on a 10% SDS-PAGE gel, and transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Bedford, MA). The membranes were blocked with 5% nonfat milk, and then were incubated with primary anti-phospho-ERK1/2 antibody overnight at 4 °C. After incubation with an HRP-conjugated secondary antibody, the protein bands were detected with a Supersignal West Pico chemiluminescent substrate (Pierce, Rockford, IL) and X-Omat BT film (Eastman Kodak Company, Rochester, New York). For detection of total ERK1/2, the membranes were stripped with Restore Western Blot Stripping Buffer (Pierce), followed by incubation with specific antibody against ERK1/2.

2.7. Statistical analysis

Data are presented as means $\pm$ SD. Statistical significance of differences between groups was analyzed by unpaired Student's *t* test.

3. Results

3.1. EGCG inhibits fMLF-induced cell chemotaxis

The effect of EGCG on fMLF-induced cell chemotaxis was studied by using multiwell-type Boyden chambers in vitro. Treatment of THP-1 or ETFR cells with EGCG at concentrations between 5 and 500 μ M for 24 h had no effect on cell viability as examined by MTT assay (data not shown). THP-1 cells (Fig. 1A) and FPR-expressing ETFR cells (Fig. 1B) migrated in response to a wide concentration range of fMLF. Pretreatment of THP-1 cells with 300 μ M EGCG for 30 min significantly inhibited 0.1–1 nM fMLF-induced THP-1 cell migration (Fig. 1A). EGCG also significantly inhibited ETFR chemotaxis in response to 1–100 nM fMLF (Fig. 1B). EGCG inhibited fMLF-induced ETFR cell migration at concentration as low as 10 μ M and the inhibitory effect was dose-dependent (Fig. 1C). These results suggest that EGCG is an effective inhibitor of leukocyte chemotaxis mediated by the receptor FPR.

3.2. EGCG reduces fMLF-induced leukocyte infiltration in mice

We next examine the capacity of EGCG to inhibit leukocyte infiltration in vivo. As shown in Fig. 2, injection of fMLF into air pouches in mice significantly induced leukocyte infiltration. When EGCG was administered to mice by intraperitoneal injection (20 mg/kg

body weight/day) for 2 d before fMLF challenge, the infiltration of leukocytes into the air pouch induced by fMLF was significantly inhibited.

3.3. Suppression of fMLF-induced calcium flux and ERK phosphorylation by EGCG

Having observed that EGCG inhibited fMLF-induced leukocyte migration both in vitro and in vivo, we then explored the potential mechanisms. As shown in Fig. 3, Fura-3 AM-loaded THP-1 and ETFR cells demonstrated a robust and transient calcium flux after stimulation with fMLF, which was suppressed when the cells were pretreated with EGCG. PD98059, an inhibitor of MEK1/2, which did not affect cell viability, dose-dependently inhibited ETFR cell migration in response to fMLF (Fig. 4A and data not shown), suggesting that ERL1/2 activation is involved in FPR-mediated leukocyte migration. To support this notion, fMLF-stimulated phosphorylation of ERK1/2 in ETFR cells was inhibited by EGCG (Fig. 4B). These results suggest that EGCG is capable of interrupting FPR signaling cascade in monocytic cell line and FPR transfected cells, leading to the inhibition of fMLF-induced leukocyte activation.

4. Discussion

In the present study, we have demonstrated that EGCG inhibited fMLF-induced chemotaxis of monocytic cells and FPR transfected cells (ETFR cells). As EGCG has been reported to suppress leukocyte chemotaxis induced by other chemoattractants, including chemokines [10–12], we hypothesize that the effect of EGCG is not specific on FPR, but rather, it disrupts signal transduction pathways shared by FPR and other chemoattractant receptors. Intracellular Ca^{2+} release and extracellular Ca^{2+} flux, as well as MAP kinase ERK1/2 activation are involved in fMLF-induced neutrophil migration [24,25]. Our present study showed that PD98059, an inhibitor of MEK1/2, inhibited ETFR cell migration in response to fMLF, indicating that ERK1/2 is indeed utilized in FPR signaling pathway leading to cell migration. We further found that EGCG inhibited fMLF-stimulated Ca^{2+} flux and ERK1/2 phosphorylation, indicating that EGCG may inhibit fMLF-stimulated leukocyte migration through Ca^{2+} and ERK1/2 related pathways.

The presence of viable bacteria in infected tissues has long been observed to attract leukocytes. fMLF is a potent chemoattractant derived from bacteria. FPR was defined as a high affinity receptor for the bacterial fMLF and analogues. The primary function of FPR has been suggested to promote the recruitment of innate immune cells to the sites of bacterial infection and tissue damage, where chemotactic formylpeptides are generated [21,22]. However, persistent leukocyte accumulation in tissue could nevertheless be the cause of persistent injury of normal architecture and functions, mediated by the local release of proteases and inflammatory mediators, and could eventually lead to abnormal repair. Our results showed that EGCG not only inhibited leukocyte activation by fMLF in vitro, but also inhibited leukocyte infiltration in an air-pouch type inflammation model. Therefore, the inhibitory effect of EGCG on fMLF-stimulated leukocyte activation may be beneficial for bacterial infection-related inflammation. In addition, FPR has been reported to be aberrantly expressed on highly malignant human glioblastoma cells and promotes tumor cell migration, proliferation and production of vascular endothelial growth factor in response to agonists

released by necrotic tumor cells [26]. It is therefore plausible that EGCG may form a basis for the development of new therapeutic agents to circumvent the undesirable consequences of FPR activation in inflammatory diseases and malignant glioblastoma.

In summary, we have demonstrated that tea polyphenol EGCG inhibited leukocyte activation by bacterial chemotactic peptide fMLF through the receptor FPR. Because the compound clearly decreased leukocyte infiltration into the sites of inflammation in mice, it could be considered as a promising anti-inflammatory agent.

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References

- [1]. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* 1994;12:593–633. [PubMed: 8011292]
- [2]. Le Y, Cui Y, Ying G, Iribarren P, Wang JM. The role of chemokine receptors in the promotion of viral infections. *Contrib Microbiol* 2003;10:210–31. [PubMed: 12530328]
- [3]. Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. *Cancer Lett* 2007;256:137–65. [PubMed: 17629396]
- [4]. Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000;95:3032–43. [PubMed: 10807766]
- [5]. Proudfoot AE, Power CA, Rommel C, Wells TN. Strategies for chemokine antagonists as therapeutics. *Semin Immunol* 2003;15:57–65. [PubMed: 12495641]
- [6]. Johnson Z, Schwarz M, Power CA, Wells TN, Proudfoot AE. Multi-faceted strategies to combat disease by interference with the chemokine system. *Trends Immunol* 2005;26:268–74. [PubMed: 15866240]
- [7]. Dufresne CJ, Farnworth ER. A review of latest research findings on the health promotion properties of tea. *J Nutr Biochem* 2001;12:404–21. [PubMed: 11448616]
- [8]. Mandel SA, Avramovich-Tirosh Y, Reznichenko L, Zheng H, Weinreb O, Amit T, et al. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. *Neurosignals* 2005;14:46–60. [PubMed: 15956814]
- [9]. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, et al. Epigallocatechin gallate induces apoptosis of monocytes. *J Allergy Clin Immunol* 2005;115:186–91. [PubMed: 15637567]
- [10]. Donà M, Dell'Aica I, Calabrese F, Benelli R, Morini M, Albini A, et al. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. *J Immunol* 2003;170:4335–41. [PubMed: 12682270]
- [11]. Takano K, Nakaima K, Nitta M, Shibata F, Nakagawa H. Inhibitory effect of (—)-epigallocatechin-3-gallate, a polyphenol of green tea, on neutrophil chemotaxis in vitro and in vivo. *J Agric Food Chem* 2004;52:4571–6. [PubMed: 15237969]
- [12]. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, et al. Epigallocatechin gallate attenuates adhesion and migration of CD8+ T cells by binding to CD11b. *J Allergy Clin Immunol* 2004;113:1211–7. [PubMed: 15208607]
- [13]. Lin YL, Lin JK. (—)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor- κ B. *Mol Pharmacol* 1997;52:465–72. [PubMed: 9281609]

- [14]. Yang F, de Villiers WJ, McClain CJ, Varilek GW. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. *J Nutr* 1998;128:2334–40. [PubMed: 9868178]
- [15]. Ichikawa D, Matsui A, Imai M, Sonoda Y, Kasahara T. Effect of various catechins on the IL-12p40 production by murine peritoneal macrophages and a macrophage cell line, J774.1. *Biol Pharm Bull* 2004;27:1353–8. [PubMed: 15340218]
- [16]. Watson JL, Vicario M, Wang A, Moreto M, McKay DM. Immune cell activation and subsequent epithelial dysfunction by *Staphylococcus enterotoxin B* is attenuated by the green tea polyphenol (—)-epigallocatechin gallate. *Cell Immunol* 2005;237:7–16. [PubMed: 16213476]
- [17]. Tou JS. Differential regulation of neutrophil phospholipase d activity and degranulation. *Biochem Biophys Res Commun* 2002;292:951–6. [PubMed: 11944907]
- [18]. Tachibana H, Sunada Y, Miyase T, Sano M, Maeda-Yamamoto M, Yamada K. Identification of a methylated tea catechin as an inhibitor of degranulation in human basophilic KU812 cells. *Biosci Biotechnol Biochem* 2000;64:452–4. [PubMed: 10737211]
- [19]. Kim SH, Park HJ, Lee CM, Choi IW, Moon DO, Roh HJ, et al. Epigallocatechin-3-gallate protects toluene diisocyanate-induced airway inflammation in a murine model of asthma. *FEBS Lett* 2006;580:1883–90. [PubMed: 16516891]
- [20]. Suzuki M, Yoshino K, Maeda-Yamamoto M, Miyase T, Sano M. Inhibitory effects of tea catechins and O-methylated derivatives of (—)-epigallocatechin-3-O-gallate on mouse type IV allergy. *J Agric Food Chem* 2000;48:5649–53. [PubMed: 11087533]
- [21]. Prossnitz ER, Ye RD. The N-formyl peptide receptor: a model for the study of chemoattractant receptor structure and function. *Pharmacol Ther* 1997;74:73–102. [PubMed: 9336017]
- [22]. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. *Trends Immunol* 2002;23:541–8. [PubMed: 12401407]
- [23]. Le Y, Ye RD, Gong W, Li J, Iribarren P, Wang JM. Identification of functional domains in the formyl peptide receptor-like 1 for agonist-induced cell chemotaxis. *FEBS J* 2005;272:769–78. [PubMed: 15670157]
- [24]. Partida-Sánchez S, Cockayne DA, Monard S, Jacobson EL, Oppenheimer N, Garvy B, et al. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat Med* 2001;7:1209–16. [PubMed: 11689885]
- [25]. Hii CS, Anson DS, Costabile M, Mukaro V, Dunning K, Ferrante A. Characterization of the MEK5-ERK5 module in human neutrophils and its relationship to ERK1/ERK2 in the chemotactic response. *J Biol Chem* 2004;279:49825–34. [PubMed: 15381709]
- [26]. Zhou Y, Bian X, Le Y, Gong W, Hu J, Zhang X, et al. Formylpeptide receptor FPR and the rapid growth of malignant human gliomas. *J Natl Cancer Inst* 2005;97:823–35. [PubMed: 15928303]

Fig. 1.

Effect of EGCG on cell migration induced by fMLF. Cells pretreated with 300 μM (A: THP-1 cells, B: ETFR cells), or different concentrations of EGCG (C: ETFR cells) at 37 °C for 30 min were examined for chemotactic response to fMLF. Data were expressed as means ±SD and represent one of three separate experiments with similar results. ** $P < 0.01$ compared with cells treated with control medium. ## $P < 0.01$ compared with cells treated with fMLF alone.

Fig. 2.

Inhibitory effect of EGCG on fMLF-induced leukocyte infiltration in mice. In KM mice with well-formed air pouch, EGCG (20 mg/kg body weight/day, i.p.) or control vehicle was administered for 2 d. The air pouch was injected with 1 ml 1 μ M fMLF or vehicle. After 4 h, leukocytes were recovered from the air pouches and counted. Data are expressed as means $\pm$ SE ($n=4-5$) and represent one of three separate experiments. ** $P<0.001$ compared with control group. ## $P<0.01$ compared with mice treated with fMLF alone.

Fig. 3.

Inhibitory effect of EGCG on calcium mobilization induced by fMLF. THP-1 cells (A) or ETFR cells (B) were loaded with Fura-3 AM and then were treated with EGCG at indicated concentrations for 15 min, followed by stimulation with 1 μM fMLF. The fluorescence was measured and presented as RFU (relative fluorescence units). Data are representative of three independent experiments.

Fig. 4.

EGCG inhibits ETFR cell migration in response to fMLF through suppression of ERK1/2 phosphorylation. A. Pretreatment of ETFR cells with different concentrations of PD98059 at 37 °C for 1 h inhibited chemotactic response to 10 nM fMLF. DMSO at various concentrations was used as control dissolvent of PD98059. The results were expressed as the chemotaxis index representing the fold increase in cell migration over control medium. Data are presented as means±SD and representative of three separate experiments. ** $P < 0.001$ compared with cell response to control medium. ## $P < 0.001$ compared with cell response to fMLF. B. ETFR cells were pretreated with or without different concentrations of EGCG for 30 min followed by 0.1 μM fMLF for 5 min. The cells were then lysed and measured for ERK1/2 phosphorylation by Western blotting using an anti-phosph-ERK1/2 antibody. Total

ERK1/2 was examined as a loading control. Data were representative of three separate experiments.

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