Epigallocatechin Gallate, a Potential Immunomodulatory Agent of Tea Components, Diminishes Cigarette Smoke Condensate-Induced Suppression of Anti-Legionella pneumophila Activity and Cytokine Responses of Alveolar Macrophages

Kazuto Matsunaga, Thomas W. Klein, Herman Friedman, and Yoshimasa Yamamoto*
Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, Florida

Received 17 January 2002/Returned for modification 11 March 2002/Accepted 3 April 2002

Even though cigarette smoking has been shown to suppress immune responses in the lungs, little is known about the effect of cigarette smoke components on respiratory infections. In the present study, the effects of cigarette smoke condensate (CSC) on bacterial replication in alveolar macrophages and the immune responses of macrophages to infection were examined. Furthermore, a possible immunotherapeutic effect of epigallocatechin gallate (EGCg), a major form of tea catechins, on the CSC-induced suppression of antimicrobial activity and immune responses of alveolar macrophages was also determined. The treatment of murine alveolar macrophage cell line (MH-S) cells with CSC significantly enhanced the replication of Legionella pneumophila in macrophages and selectively down-regulated the production of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) induced by bacterial infection. The treatment of macrophages with EGCg not only overcame the CSC-induced suppression of antimicrobial activity but also strengthened the resistance of macrophages to infection. EGCg also markedly up-regulated the CSC-suppressed IL-6 and TNF-α production by macrophages in response to infection. The results of exogenous TNF-α treatment and neutralization treatment with anti-TNF-α and anti-gamma-interferon (IFN-γ) antibodies and the determination of IFN-γ mRNA levels indicate that CSC-suppressed macrophages can be activated by EGCg to inhibit L. pneumophila growth by up-regulation of TNF-α and IFN-γ production. Thus, this study revealed that CSC selectively alters the immune responses of macrophages to L. pneumophila infection and leads to an enhancement of bacterial replication in macrophages. In addition, the tea catechin EGCg can diminish such suppressive effects of CSC on alveolar macrophages.

The development of cellular immunity is essential in the host defense to respiratory infection caused by intracellular pneumonia-causing bacteria, such as Legionella pneumophila. It is widely accepted that the activation of macrophages to suppress intracellular bacterial growth is an essential effector mechanism for the resolution of infection (10) and that the T helper 1 cell (Th1) cytokine gamma interferon (IFN-γ) can activate macrophages and monocytes to inhibit L. pneumophila growth (2, 26). Besides the direct effect of the Th1 cytokine IFN-γ in activating macrophages, Th1 cells play an essential role in the development of cell-mediated immunity to pathogens (11). Both the Th1 cytokine interleukin-12 (IL-12), which has a major role in the differentiation of T helper cell phenotypes, and IFN-γ are now recognized to be produced by macrophages (4, 7, 24, 30). It is also known that the inflammatory cytokine tumor necrosis factor alpha (TNF-α) is required for the prompt resolution of pneumonic legionellosis, which points to a direct role for TNF-α in the activation of phagocytes (37). Other inflammatory cytokines, such as IL-6, are also known to control infections (5, 15). In contrast, Th2 cytokines, particularly IL-10, may facilitate the growth of L. pneumophila in permissive mononuclear phagocytes due in part to IL-10-mediated inhibition of TNF-α secretion and IFN-γ-mediated mononuclear phagocyte activation (28). Nevertheless, all of these cytokines, IL-6, IL-10, IL-12, TNF-α, and even IFN-γ, are known to be produced by macrophages in response to bacterial infections and may be involved in the regulation of infection. Therefore, the modulation of the production of such key cytokines by macrophages may eventually affect the outcome of the infection.

Although cigarette smoking is known to be linked to serious illness and disruption of normal physiological function, this habit continues to be a relatively common practice in our society (31). Since cigarette smoking has been shown to suppress the immune responses in the lungs, it has been recognized as one of the risk factors for respiratory infections, such as pneumonic legionellosis (8, 32, 39). Previous studies have demonstrated that cigarette smoking suppresses the production of IL-1β, IL-6, and TNF-α by alveolar macrophages obtained from the bronchoalveolar lavage fluids of smokers (20, 38, 44). Furthermore, it has also been shown that the production of IL-1β, IL-2, IFN-γ, and TNF-α in human peripheral blood is suppressed by cigarette smoke extracts (27). Since cigarette smoke is composed of over 4,000 chemicals (36), the harmful effects of cigarette smoke components on the immune responses of alveolar cells are not well understood. In addition, little is known about the effect of cigarette smoke components on bacterial replication in alveolar macrophages, which are a major defense against invading pathogens in the lungs.

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612-4799. Phone: (813) 974-2332. Fax: (813) 974-4151. E-mail: yyamamoto@hsc.usf.edu.
Epigallocatechin gallate (EGCg) is a major form of tea catechins and has a variety of physiological activities, including antioxidant and immunomodulatory activities as well as activities against some pathogens (9, 12, 13, 33, 41, 43). In addition, our recent study showed that EGCg enhances the activity of alveolar macrophages against L. pneumophila through an activation of selective cytokine production (19). Therefore, in the present study, the cigarette smoking condensate (CSC)-induced suppression of the antimicrobial activity and immune responses of alveolar macrophages and a possible immunotherapeutic effect of EGCg on the CSC-induced suppression of macrophages were examined by utilizing a newly established in vitro L. pneumophila infection model with MH-S murine alveolar macrophage cells (17).

MATERIALS AND METHODS

CSC and EGCg. CSC (1R3F) (27) and EGCg were purchased from Murty Pharmaceuticals, Lexington, Ky., and Sigma Chemical Co., St. Louis, Mo., respectively. The stock solution of CSC was prepared in dimethyl sulfoxide (DMSO) at a concentration of 40 mg/ml and stored in a small aliquot at −20°C until used. EGCg was dissolved in pyrogen-free water at a concentration of 5 mg/ml, sterilized through a membrane filter, and stored at −20°C in a tube covered with foil to protect it from light. Both CSC and EGCg were diluted with the medium to prepare solutions for the experiments.

Bacteria. L. pneumophila M124 serogroup 1 was originally obtained from a case of fatal legionellosis (6). The bacteria were cultured on buffered charcoal-yeast extract (BCYE) medium (Becton Dickinson, Sparks, Md.) for 3 days at 37°C with foils to protect it from light. Both CSC and EGCg were diluted to prepare solutions for the experiments. The resulting cell monolayers were washed with HBSS, supplied with 10% FCS. The cultures were then incubated for up to 48 h at 37°C in 5% CO2.

Macrophage treatment. The macrophage cultures infected with bacteria were treated with various concentrations (1 to 3 µg/ml) of CSC for up to 48 h at 37°C in 5% CO2. The resulting cell monolayers were washed with Hanks' balanced salt solution (HBSS), supplied with 10% FCS-RPMI 1640 medium without antibiotics, and then used for experiments.

Macrophage viability. The monolayer macrophage cells were infected with L. pneumophila (infectivity ratio, 10 bacteria per cell) for 30 min, washed to remove nonphagocytosed bacteria, and incubated in RPMI 1640 medium containing 10% FCS. The cultures were then incubated for up to 48 h at 37°C in 5% CO2.

Macrophage viability. The macrophage cultures infected with bacteria were treated with various concentrations (1 to 3 µg/ml) of CSC for up to 48 h at 37°C in 5% CO2. As a vehicle control, DMSO at concentrations equivalent to those of CSC and EGCg were similarly incubated with bacteria and treated with CSC and/or EGCg for 24 h and then the viability of the macrophages was determined by the trypan blue dye exclusion method. Data represent the mean values ± standard deviations (SD) from three independent experiments. *P < 0.05 (significantly different from the values obtained with the nontreated control group).

Measurement of cell viability. The protocol was followed exactly as described in the manufacturer's manual.

Viable bacteria in cell cultures (CFU assay). The number of CFU in cell lysates was determined by standard plate counts on BCYE medium, as described previously (46). In brief, after incubation the cell monolayers were lysed with 0.1% saponin. The cell lysates were appropriately diluted with HBSS and plated on BCYE agar plates. The plates were incubated at 37°C for 72 h. The resulting bacterial colonies on the plates were counted as CFU.

RT-PCR. Total RNA was extracted from cells by the microspin technique with an RNeasy Mini kit (Qiagen, Valencia, Calif.) in accordance with the manufacturer's instructions. Reverse transcription (RT) of total RNA (1 µg) was performed with avian myeloblastosis virus transcriptase in a commercial reaction mixture (Reverse Transcription System; Promega). The resulting cDNA was subjected to PCR with primers for β2-microglobulin (BMG) and IFN-γ. The primer sequences for BMG were 5'-GCC TCG CTC GGT GCT CCT CTT T-3' (sense) and 5'-TCT GCA GGA GTA TGT ATG CTC A3'- (antisense) (47). The primer sequences for IFN-γ were 5'-CAT TGA AAG CCT AGA AAG TCT-3' (sense) and 5'-CTC ATG GAA TGC ATC CTT TTT CG-3' (antisense). The PCR was performed in a Minicycler (MJ Research, Watertown, Mass.) for either 25 cycles at an annealing temperature of 60°C (BMG) or 40 cycles at an annealing temperature of 55°C (IFN-γ). PCR products were analyzed on an ethidium bromide-stained 2% agarose gel.

Statistical analysis. Statistical analysis was performed using a repeated-measure analysis of variance.

RESULTS

Effects of CSC and EGCg on macrophage viability. In order to determine the toxicities of both CSC and EGCg to macrophages, the cells were treated with various concentrations of either CSC or EGCg for 24 h and then the viability of the macrophages was assessed by the trypan blue exclusion method. As shown in Fig. 1, CSC did not show any significant cytotoxicity at concentrations up to 10 µg/ml. However, CSC treatment at 30 µg/ml significantly reduced the macrophage viability. In contrast, EGCg treatment did not affect the cell viability at any of the concentrations tested. The results ob-

![FIG. 1. Effects of CSC and EGCg on macrophage viability. Macrophage monolayers were treated with either CSC or EGCg at various concentrations for 24 h, and the viability of the macrophages was determined by the trypan blue dye exclusion method. Data represent the mean values ± standard deviations (SD) from three independent experiments. *P < 0.05 (significantly different from the values obtained with the nontreated control group).](http://cvi.asm.org/)

Vol. 9, 2002 TOBACCO-INDUCED SUPPRESSION AND RECOVERY BY EGCg 865

Downloaded from http://cvi.asm.org on March 19, 2020 by guest
tained by the cell count method were also confirmed by a colorimetric method using a tetrazolium compound (data not shown).

**Effect of CSC on *L. pneumophila* growth in macrophages.** Since it is known that the growth of *L. pneumophila* in macrophages is dependent on the activation of host macrophages (45), treatment of macrophages with CSC may alter the growth of *L. pneumophila* in cells, if CSC has any immunosuppressive activity on macrophages. Based on preliminary experiments and the above-mentioned results, which indicate that CSC at more than 10 μg/ml showed cytotoxicity to MH-S cells, we selected nontoxic concentrations, such as 1 to 3 μg of CSC/ml, to evaluate the effect of CSC on *L. pneumophila* growth in macrophages. The treatment of macrophages with CSC after infection with bacteria induced an enhancement of the growth of *L. pneumophila* in the cells in a dose-dependent manner (Fig. 2A). A significant effect of CSC on *L. pneumophila* growth was observed at a concentration as low as 1 μg/ml at 24 h after infection. The DMSO treatment (vehicle control) did not show any alteration of the bacterial growth in the macrophages.

**Effect of CSC on cytokine production by macrophages.** From the above-mentioned results, it can be conjectured that macrophage inactivation by CSC may be a reason why *L. pneumophila* growth was enhanced. In order to investigate such a possibility, the effect of CSC on cytokine production by macrophages was examined. As shown in Fig. 3, the treatment of macrophages with CSC alone did not show any significant induction of the cytokines tested, such as IL-6, IL-10, IL-12, and TNF-α. However, CSC markedly down-regulated the IL-6 and TNF-α production by macrophages in response to *L. pneumophila* infection in a dose-dependent manner. The other cytokines, such as IL-10 and IL-12, were not affected by CSC treatment. IFN-γ was not detected by ELISA (lower detection limit, 32 pg/ml) in the culture supernatants of macrophages infected with *L. pneumophila* or left untreated. The DMSO treatment (vehicle control) did not show any alteration of macrophage cytokine production.

**Effect of EGCg on CSC-suppressed anti-*L. pneumophila* activity of macrophages.** In order to determine a possible therapeutic activity of EGCg on CSC-suppressed antimicrobial activity, macrophages were infected, treated with CSC, and then incubated with various concentrations of EGCg, which has been shown to activate macrophages (19). As shown in Fig. 2B, the treatment of macrophages with EGCg at a concentration of 50 μg/ml induced a marked inhibition of *L. pneumophila* growth in cells. Furthermore, when CSC-suppressed macrophages were treated with EGCg at a concentration as low as 0.5 μg/ml, their antimicrobial activity readily recovered to the control level. Higher concentrations of EGCg, such as 5 and 50 μg/ml, even strengthened the antimicrobial activity of CSC-suppressed macrophages in a dose-dependent manner.

**Effect of EGCg on cytokine production by CSC-suppressed macrophages in response to *L. pneumophila* infection.** In order to analyze cytokine involvement in the recovery of the antimicrobial activity of macrophages induced by EGCg, cytokine production by macrophages in culture supernatants infected with bacteria and treated with CSC and EGCg was assessed. As shown in Fig. 4, treatment with only EGCg markedly up-regulated the IL-12 and TNF-α production and down-regulated the IL-10 production by macrophages induced by bacterial infection, as was observed in a previous study (19). The CSC-suppressed production of IL-6 and TNF-α by macrophages in response to infection was completely restored by EGCg treatment; furthermore, high EGCg concentrations, such as 50 μg/ml, induced even greater production of TNF-α. However, EGCg did not induce any detectable IFN-γ production, as determined by ELISA, in the culture supernatants of macrophages infected with bacteria and treated with CSC or left untreated.

**Effect of exogenous TNF-α on *L. pneumophila* growth in CSC-suppressed macrophages.** The above-mentioned results indicate that the CSC suppression of TNF-α production may be involved in the enhancement of *L. pneumophila* growth in macrophages. Furthermore, the EGCg up-regulation of TNF-α production by CSC-suppressed macrophages may be related to the recovering antimicrobial activity. Therefore, if TNF-α is added exogenously to the cultures of CSC-suppressed macrophages, recovery of antimicrobial activity may be predicted. In fact, when CSC-suppressed macrophages were treated with exogenous TNF-α after infection, inhibition of the bacterial growth in the cells was evident (Fig. 2C). The treatment of macrophages with 1 ng of exogenous TNF-α per ml resulted in almost complete recovery of the antimicrobial activity suppressed by CSC. A higher concentration of TNF-α, such as 5 ng/ml, caused even greater inhibition of bacterial growth even in CSC-suppressed macrophages.

**Involvement of enhanced cytokine production in the inhibition of *L. pneumophila* growth by EGCg.** Since both endogenous and exogenous TNF-α and IFN-γ are known to activate macrophages to inhibit *L. pneumophila* growth (14, 16, 22, 26), the involvement of enhanced TNF-α and IFN-γ production, which was not detected in the culture supernatants by ELISA, in the EGCg-induced inhibition of *L. pneumophila* growth was examined by neutralization with specific antibodies to these cytokines. The macrophages infected and treated with CSC and EGCg were incubated with anti-TNF-α antibody, anti-IFN-γ antibody, both anti-TNF-α and anti-IFN-γ antibodies, or control IgG. The numbers of viable bacteria in the cultures were then determined at 24 and 48 h after infection. As shown in Fig. 2D, treatment of macrophages with anti-TNF-α antibody significantly abolished the EGCg-induced antimicrobial activity of CSC-suppressed macrophages, but this effect was partial. Anti-IFN-γ antibody treatment of macrophages also showed a partial abolishment of the EGCg-induced antimicrobial activity of CSC-suppressed macrophages. However, when the macrophages were treated with both anti-TNF-α and anti-IFN-γ antibodies, the EGCg-induced antimicrobial activity was almost completely abolished. The control IgG-treated macrophages did not show any alteration in the EGCg-induced antimicrobial activity of CSC-suppressed macrophages. The levels of IL-10, IL-12, and TNF-α produced by macrophages were not affected by neutralization with anti-TNF-α antibody, anti-IFN-γ antibody, or both antibodies (Fig. 5).

**Effect of EGCg on IFN-γ mRNA expression by CSC-suppressed macrophages in response to *L. pneumophila* infection.** Since the anti-IFN-γ antibody treatment showed the abolishment of antimicrobial activity induced by EGCg, the steady-state levels of IFN-γ mRNA in macrophages were analyzed by RT-PCR. The ratios of the expression levels of IFN-γ mes-
sages to the levels of housekeeping-gene BMG mRNA were compared among experimental groups (Fig. 6). Noninfected control macrophages showed a faint level of message expression, but the infection induced a marked elevation of the expression levels. The CSC treatment significantly depressed the induction of IFN-γ messages. In contrast, EGCg treatment of CSC-suppressed macrophages clearly enhanced the expression levels of IFN-γ mRNA. EGCg alone did not induce any IFN-γ
mRNA expression in macrophages at either 6 or 24 h after treatment (data not shown).

**DISCUSSION**

It is known that smoking enhances the binding of bacteria to pharyngeal cells (29) and damages local defenses by impairing mucociliary flow, increasing the permeability of the respiratory epithelium, reducing humoral responses to inhaled antigens, and increasing the susceptibility of the host to respiratory pathogens (35). The in vitro activity of rat alveolar macrophages obtained from animals exposed to tobacco smoke against *Staphylococcus epidermidis* is also known to be impaired (34). However, how smoking directly alters the host defense of the lungs to infections, particularly in its effect on the immune responses of alveolar macrophages, which are critical effector cells in lung defense, is not well understood. In this regard, the present study concerning the effect of CSC on the susceptibility of alveolar macrophages to *L. pneumophila* infection revealed that CSC causes a suppression of the immune responses of macrophages to *L. pneumophila* infection and leads to an enhancement of bacterial replication in macrophages. The precise mechanism of the CSC-induced suppression of the antimicrobial activity of macrophages is still unclear. However, from the results showing the selective inhibition of cytokine production by CSC and the recovery of cytokine production as well as antimicrobial activity by EGCG treatment, it seems likely that impaired TNF-α and IFN-γ production may be a major mechanism responsible for the CSC-induced impairment of activity against *L. pneumophila* infection. This hypothesis can be supported by previous reports showing that both TNF-α and IFN-γ are strong activators of the induction of anti-*L. pneumophila* activity by macrophages (2, 14, 16, 19, 22, 26). The results of the exogenous TNF-α treatment experiments were also consistent with this hypothesis. The complete recovery of the antimicrobial activity of CSC-suppressed macrophages by exogenous TNF-α may not rule out IFN-γ involvement in the antimicrobial activity, since exogenous TNF-α is believed to collaborate with endogenous IFN-γ. On the other hand, the role of reduced IL-6 production in CSC-induced impairment of antimicrobial activity is not clear, but it may not have a direct role due to the absence of IL-6 modulatory effects on macrophages with regard to anti-*L. pneumophila* activity (21).

The production of IFN-γ in the culture supernatants of CSC-treated macrophages infected with *L. pneumophila* and stimulated with or without EGCG could not be detected by ELISA. However, reduced expression levels of IFN-γ mRNA in CSC-treated macrophages and recovery of the expression levels by EGCG were observed. In addition, the treatment of
macrophages with anti-IFN-γ antibody inhibited the EGCg-induced anti-
*L. pneumophila* activity. From these results, it seems likely that even the small amount of IFN-γ, which could not be detected by ELISA, in culture supernatants of macrophages stimulated by EGCg in response to *L. pneumophila* infection may be involved in the EGCg-induced anti-*L. pneumophila* activity in CSC-treated macrophages. This conclusion is consistent with reports in the literature which indicate the production of IFN-γ by macrophages (4, 7, 24, 30), even though it is generally considered that NK cells and activated T lymphocytes are the major sources for IFN-γ.

The cytokine IL-10 has been shown to exhibit important deactivating effects on macrophages in murine models of *Legionella* (28), leishmanial (1), and mycobacterial (3) infections. Moreover, it is known that IL-10 is secreted by *L. pneumophila*-infected monocytes and alveolar macrophages, enhances bacterial growth, reverses the protective effect of IFN-γ, and blocks the secretion of TNF-α by infected cells (28). However, modulation of IL-10 may not be directly involved in the EGCg-induced anti-*L. pneumophila* activity of macrophages in vitro, since the diminution of anti-*L. pneumophila* activity was not associated with changes in IL-10 production.

Since it is known that a smoker is exposed to, on average, roughly 18 mg of CSC per cigarette (1R3F) and approximately 1.16 mg of nicotine (6% of CSC) when a cigarette is smoked to a 30.0-mm butt length (23), the CSC concentrations (3 mg/ml) used in this study seem likely to be comparable to those in the lungs of smokers. CSC is suspected of containing several thousand substances, because cigarette smoke is known to be composed of over 4,000 chemicals (36). Therefore, it is not clear what substance responsible for the CSC-induced immunosuppression of macrophages. However, our recent study of the effect of nicotine on alveolar macrophage functions indicates that nicotine, which showed an inhibitory activity to TNF-α production by macrophages at 0.1 mg/ml (18), may be one of the chemicals in CSC responsible for this immunosuppression. Nevertheless, it is obvious that cigarette smoke components can impair the immune function of alveolar macrophages, leading to greater susceptibility to bacterial infections.

The recovery of the CSC-impaired antimicrobial activity of macrophages by the tea catechin EGCg is notable. Even though many studies, including our recent study (19), have been conducted to investigate the effects of EGCg on antimicrobial as well as immunomodulatory activities (9, 12, 13, 33, 41, 43), there have been no studies concerned with the recovery of impaired cell functions, particularly those induced by tobacco, by this natural substance. As little as 0.5 mg of EGCg/ml significantly restored CSC-suppressed antimicrobial activity to the control level. Furthermore, a relatively high concentration of EGCg, such as 50 mg/ml, strengthened the antimicrobial activity of CSC-suppressed macrophages to a level greater than that of the non-CSC-treated control cells. The bioavailability of tea catechins after oral administration of tea extracts is high due to their excellent absorption charac-

![FIG. 4. Effect of EGCg on cytokine production of CSC (3 mg/ml)-treated (closed columns) or CSC-untreated (open columns) macrophages in response to *L. pneumophila* infection. The results are expressed as mean values plus SD from three independent experiments. ∗, P < 0.05 (significantly different from the values obtained with the bacterially infected control group without CSC); ∗∗, P < 0.05 (significantly different from the values obtained with the CSC-treated and bacterially infected control group).](http://cvi.asm.org/Downloaded from)
characteristics. For example, consumption of 5 capsules of green tea extracts (approximately 10 cups of green tea) results in a plasma level of approximately 2 g/ml of EGCg/ml 90 min after ingestion (25, 48). Furthermore, it is evident that radiolabeled EGCg orally administered to mice is distributed to various organs of the body, including the skin (40). In fact, it has been shown that the oral administration of tea extracts induces protection against UV-induced photocarcinogenesis of skin in mice (42). These observations indicate that the bioavailability of tea catechins in tissues after oral administration, including daily consumption of tea beverages, can be predicted at certain levels. In addition, inhalation treatment with tea extracts for intractable respiratory infections results in clinical and microbiological improvements (43). Therefore, even a high EGCg concentration, such as 50 g/ml, can be applied to lung cells in vivo by an inhalation treatment.

The timing of the CSC and EGCg treatment of alveolar macrophages may be an important factor in consideration of the inhibitory effect of tobacco smoking and the therapeutic effect of tea catechins on the host defense to infections. In this study, only simultaneous treatment of macrophages with CSC, EGCg, and bacterial infection was examined. Since pretreatment of the cells with CSC and EGCg prior to infection may alter the entire process of infection, including the uptake of bacteria, resulting in a more complex situation, a simplified experimental condition was utilized for the initial study. Even though the study reported here was conducted under such limited experimental conditions, CSC clearly showed an inhibitory effect on alveolar macrophages and EGCg diminished such inhibition of antimicrobial as well as immunological activity. It is obvious that EGCg treatment results in not only inhibition of the CSC effect but also activation of macrophage resistance to bacterial infection and production of certain cytokines critical in the host defense to infections. These results are important since the development of stringent in vitro studies is critical for the establishment of parameters for future in vivo investigations. Bacterial pneumonia in immunocompromised patients, as well as in individuals who smoke tobacco heavily, often becomes a life-threatening disease despite the extensive use of antibiotics. In addition, the prevalence of antibiotic-resistant bacteria in both ambulatory and hospitalized patients requires additional types of therapeutic approaches to supplant the traditional treatment with antibiotics. Thus, development of a new approach to treat or care for patients who are susceptible to respiratory infections is an urgent clinical matter. In this regard, the tea catechin EGCg may be one of the novel agents for such a purpose due to its potential immunomodulatory as well as antimicrobial activity.

ACKNOWLEDGMENTS

We thank Susan Pross and Geoffrey Patton for helpful suggestions regarding CSC experiments.
