Beneficial effects of green tea catechins on alveolar macrophages infected with pneumonia causing bacteria.

Kazuto Matsunaga, Thomas W. Klein, Herman Friedman, and Yoshimasa Yamamoto
Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, FL 33612, USA

Summary

Bacterial pneumonia in immunocompromised patients as well as elderly persons often becomes a life threatening disease, even when effective antibiotics are used extensively. In addition, the appearance of antibiotic-resistant bacteria in medical facilities as well as in patients requires another approach to treat such patients besides treatment with antibiotics. In this regard, green tea catechins, such as epigallocatechin gallate (EGCg), may be one of the potential agents for such purpose due to its possible potential immunomodulatory as well as antimicrobial activity. Current studies in this laboratory showed that EGCg enhanced the in vitro resistance of alveolar macrophages to *Legionella pneumophila* infection by selective immunomodulatory effects on cytokine formation. Furthermore, the tobacco smoking-induced impairment of alveolar macrophages regarding antibacterial as well as immune activity was also restored by EGCg treatment. These results indicate that EGCg may be a possible potential immunotherapeutic agent against respiratory infections in immunocompromised patients, such as heavy smokers.

Keywords
Pneumonia, *Legionella pneumophila*, epigallocatechin gallate, alveolar macrophages, infection

Introduction

Alveolar macrophages are the preferential site for growth of pneumonia causing intracellular bacteria, such as *Legionella pneumophila* which is a causative agent of legionnaires disease. It is widely accepted that the activation of macrophages is an essential effector mechanism for the resolution of infection with intracellular pathogens. Therefore, the modulation of immune functions of macrophages may eventually affect the outcome of the infection. Epigallocatechin gallate (EGCg) is a major form of green tea catechins and has a variety of biological activities including antioxidant as well as anti-microbial activities against some pathogens (3, 15). Although the biological activities of EGCg have extensively been studied, its immunological effects are not well understood, particularly the beneficial effect on immune cells regarding anti-microbial activity of cells. The studies conducted in this laboratory revealed a possible immunotherapeutic activity of EGCg on *L. pneumophila* infection of alveolar macrophages (8). The results may introduce a possible new alternative immunotherapeutic agent to the treatment of respiratory infections in patients who do not have a strong immune defense against pathogens.

Materials and Methods

A mouse alveolar macrophage cell line MH-S was infected with *L. pneumophila*, as described previously (7). The cells infected with bacteria were also treated with nicotine hydrogen tartrate salt (Sigma, St. Louis, MO), cigarette smoking condensate (CSC, Murty Pharmaceuticals, Lexington, KY) and/or EGCg (Sigma). In some experiments, macrophage cultures infected
with bacteria and treated with EGCg were incubated with anti-TNFα antibody. The cells infected with bacteria and treated with CSC or nicotine were also incubated with mouse recombinant TNFα (R & D Systems, Minneapolis, MN). The number of viable bacteria (CFU) in the cultures as well as protein levels for cytokines in the culture supernatants were measured, as described previously (9, 16).

Results and Discussion

**EGCg inhibits the growth of L. pneumophila in alveolar macrophages without any direct effect on bacteria.** *L. pneumophila*, a gram-negative facultative intracellular pathogen, is the causative agent of pneumonia in immunocompromised patients (2). The activation of macrophages to suppress intracellular bacterial growth is an essential effector mechanism of the resolution of legionellosis. The treatment of alveolar macrophage cell line (MH-S) cells with EGCg (0.5 – 50 μg/ml) after infection with *L. pneumophila* induced a marked inhibition of the growth of bacteria in the cells in a dose dependent manner. Even as little as a 0.5 μg/ml of EGCg induced a significant inhibition of bacterial growth in macrophages. A study of a possible direct anti-bacterial activity of EGCg on *L. pneumophila* showed that EGCg did not possess any direct anti-*L. pneumophila* activity at up to the concentration of 500 μg/ml in bacterial culture broth.

**EGCg modulates production of certain cytokines induced by L. pneumophila infection.** From the previous results, it seemed likely that EGCg might inhibit bacterial growth due to macrophage activation. In order to determine such a possibility, the effect of EGCg on the production of macrophage cytokines was examined. The treatment of macrophages with EGCg alone slightly induced production of some cytokines at the high EGCg concentration, such as 50 μg/ml. However, EGCg markedly up-regulated the production of TNFα and IL-12 induced by *L. pneumophila* infection, even at a concentration as little as 0.5 μg/ml in the case of TNFα. In contrast, the IL-10 production induced by bacterial infection was strongly down-regulated by EGCg (0.5 – 50 μg/ml) in a dose dependent manner. On the other hand, the production of IL-6 induced by bacterial infection was not affected by EGCg.

**Enhanced cytokine production is involved in the inhibition of bacterial growth by EGCg.** Since both endogenous and exogenous TNFα and IFNγ are known to activate macrophages to inhibit *L. pneumophila* growth (4, 6, 9), involvement of enhanced TNFα and IFNγ in the EGCg-induced bacterial growth inhibition was examined by neutralization with specific antibodies to these cytokines. The treatment of macrophages with either anti-TNFα antibody or anti-IFNγ antibody almost completely abolished the EGCg (50 μg/ml)-induced bacterial growth inhibition. These results clearly indicate that both TNFα and IFNγ are involved in the anti-*L. pneumophila* activity of macrophages induced by EGCg treatment.

**EGCg treatment of macrophages infected with L. pneumophila enhances the level of IFNγ message.** Since the treatment with anti-IFNγ antibody induced a marked abolishment of the EGCg induced-bacterial growth inhibition, levels of IFNγ mRNA in macrophages infected with bacteria and stimulated with or without EGCg were analyzed. The results showed that messages for IFNγ were induced in bacteria infected cells at 24 hrs after infection. Moreover, when the macrophages were infected and treated with EGCg, a remarkable enhancement of IFNγ mRNA accumulation was observed. However, EGCg (50 μg/ml) alone did not induce any IFNγ mRNA in macrophages.

**EGCg restores the anti-L. pneumophila activity of alveolar macrophages suppressed by treatment with nicotine or CSC.** Tobacco smoking is known to cause deleterious effects on immune cells followed by susceptibility to respiratory infections. For example, it is shown that
smoking accelerates pneumonia caused by *S. pneumoniae* (12). Pneumonia caused by other bacteria, such as Chlamydia, Legionella and Mycobacteria, is also known to frequently occur in smokers (1, 5, 13, 14). However, the specific biological mechanisms by which exposure to tobacco smoke increases the risk of bacterial pneumonia are poorly understood. In this regard, our studies showed that both nicotine (1 μg/ml) and CSC (2 μg/ml) induced a significant increase of bacterial growth in alveolar macrophages associated with down-regulation of production of certain cytokines. The EGCg treatment (0.5 – 50 μg/ml) restored and even strengthened the anti-L. pneumophila activity of macrophages suppressed by nicotine or CSC treatment. Furthermore, EGCg (50 μg/ml) diminished the nicotine or CSC-induced inhibition of cytokine productions.

The results obtained in this study clearly showed that EGCg inhibited the growth of *L. pneumophila*, which is an important pneumonia causing pathogen in immunocompromised patients, in alveolar macrophages at a concentration as low as 0.5 μg/ml, which can be reached in plasma by ingestion of capsules of tea extracts (10, 11, 17, 18). The EGCg selectively up-regulated the production of IL-12 and TNFα and down-regulated IL-10 production of macrophages induced by bacterial infection, but did not alter IL-6 production. The up-regulation of the message levels of IFNγ by EGCg was also demonstrated. Treatment of macrophages with anti-TNFα and anti-IFNγ antibodies abolished the anti-bacterial activity of macrophages induced by EGCg treatment. These results indicate that EGCg selectively alters the immune responses of macrophages to pathogens and leads to enhanced anti-bacterial activity of macrophages mediated by enhanced production of both TNFα and IFNγ. Furthermore, EGCg treatment restores the anti-bacterial activity of macrophages suppressed by nicotine or CSC treatment, which is a mimic of alveolar cells in tobacco smokers and reversed by EGCg. Thus, these results revealed the selective immunomodulatory effect of EGCg on alveolar macrophages, which have a critical role in respiratory infections. The study warrants a further study of EGCg as a potential alternative immunotherapeutic agent in respiratory infections.

**Acknowledgement**

This work was supported by grants AI45169 from the National Institute of Allergy and Infectious Diseases and the American Lung Association of Florida.

**References**