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Green tea polyphenol inhibits *Mycobacterium tuberculosis* survival within human macrophages

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Abstract

Lack of maturation of phagosomes containing pathogenic *Mycobacterium tuberculosis* within macrophages has been widely recognized as a crucial factor for the persistence of mycobacterial pathogen. Host molecule tryptophan-aspartate containing coat protein (TACO) has been shown to play a crucial role in the arrest of such a maturation process. The present study was addressed to understand whether or not polyphenols derived from green tea could down-regulate TACO gene transcription. And if yes, what impact TACO gene down-regulation has on the uptake/survival of *M. tuberculosis* within macrophages. The reverse-transcriptase polymerase chain reaction and reporter assay technology, employed in this study, revealed that the major component of green tea polyphenols, epigallocatechin-3-gallate had the inherent capacity to down-regulate TACO gene transcription within human macrophages through its ability to inhibit Sp1 transcription factor. We also found out that TACO gene promoter does contain Sp1 binding sequence using bioinformatics tools. The down-regulation of TACO gene expression by epigallocatechin-3-gallate was accompanied by inhibition of mycobacterium survival within macrophages as assessed through flow cytometry and colony counts. Based on these results, we propose that epigallocatechin-3-gallate may be of importance in the prevention of tuberculosis infection.

Keywords: Green tea polyphenols; Epigallocatechin-3-gallate; TACO gene; Transcription; Phagosome; Tuberculosis

1. Introduction

The resurgence of concern about tuberculosis has resulted in the molecular dissection of the membrane trafficking pathway to study the travel route of *Mycobacterium tuberculosis* within mycobacterial phagosome. Intracellular pathogens like *M. tuberculosis* have evolved novel mechanisms to inhibit their fusion with the lysosomes (Meresse et al., 1999). Specifically, mycobacteria containing phagosomes, also known as 'mycobacterial phagosome', inhibits its lysosomal delivery because of a block in the endosomal trafficking that prevents phagosome maturation (Vergne, Chua, Singh, & Deretic, 2004). Phagosomal maturation involves a series of sequential fusion events with various vesicles from the endocytic pathway, by which nascent phagosomes attain microbicidal properties (Desjardins, Huber, Parton, & Griffiths, 1994; Koul, Hergert, Klebl, & Ullrich, 2004). During this process, the phagosomes lose early endosomal markers such as Rab5 and acquire markers of the late endosomal/lysosomal pathway such as Rab7 (associated with vesicular fusion) and cathepsin D (involved in acidification of the phagosome) and thus become a 'phagolysosome' (Koul et al., 2004; Kusner, 2005; Vergne et al., 2004). However, the shuttling between

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these Rab markers is altered in phagosomes carrying pathogenic mycobacteria (Via et al., 1997). Similarly, reduced or altered recruitment was observed for another Rab5 effector, early endosome antigen 1 (EEA1), that serves as an organelle tethering molecule between membranes destined for fusion (Christoforidis et al., 1999; Fratti, Backer, Gruenberg, Corvera, & Deretic, 2001; Simonsen et al., 1998). EEA1 interacts with Rab5 as well as FYVE domain of phosphatidylinositol-3-phosphate (PI3P), generated on endosomal membranes by hVPS34, a type III phosphatidylinositol-3-kinase (Christoforidis et al., 1999; Simonsen et al., 1998). Consequently, phosphatidylinositides have also become a focus of recent studies aimed at identifying the molecular events causing *M. tuberculosis* phagosome maturation arrest (Chua & Deretic, 2004; Vergne et al., 2005).

Another molecule that has been shown to inhibit endosomal/lysosomal fusion and therefore acidification, is host protein TACO (tryptophan-aspartate containing coat protein), also known as coronin-1, which imparts non-fusogenic property to specifically mycobacteria containing phagosomes (Ferrari, Langen, Naito, & Pieters, 1999). TACO/coronin-1 protein is acquired from the plasma membrane at the time of phagocytosis and persists around phagosomes carrying pathogenic mycobacteria *Mycobacterium bovis* BCG (Ferrari et al., 1999; Gatfield & Pieters, 2000). The active retention of TACO correlates well with the mycobacterial evasion of the endocytic pathway and thus the intracellular survival of the pathogen (Pieters, 2001, 2002). Increased TACO recruitment and retention has also been observed in phagosomes carrying pathogenic *Helicobacter pylori* strain in comparison to phagosomes carrying strains that lack virulent factors (Zheng & Jones, 2003). This indicates that TACO may be of importance in survival of other intracellular microbes as well. In contrast to this, one study indicated that although TACO is involved in *M. bovis* BCG uptake, persistence of this protein was not observed in phagosomes containing less than five bacteria although phagosomes containing more than five bacteria conspicuously retained TACO (Schuller, Neefjes, Ottenhoff, Thole, & Young, 2001). TACO protein in association with cholesterol within plasma membrane also facilitates entry of mycobacteria within macrophages (Gatfield & Pieters, 2000). We have proposed that a cholesterol specific receptor-C_k can in part contribute to the entry of mycobacteria (Kaul, Anand, & Verma, 2004a). Therefore, mycobacteria that enter macrophages at cholesterol rich plasma membrane domains are subsequently sequestered within TACO coated phagosomes, preventing lysosomal delivery and ensuring their intracellular survival. This indicates that TACO helps both in entry as well as intracellular survival of *M. tuberculosis*.

Considerable interest has been expressed in the biological activity of green tea (*Camellia sinensis*) and its impact to human health (Cos et al., 2004). Green tea (GT) is well known because of its antioxidant and free radical scavenging abilities associated with its active components polyphenols (Higdon & Frei, 2003). The polyphenol fraction of GT includes epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) of which EGCG generally accounts for greater than 40% of the total and is considered the most potent component of the green tea polyphenols (Orzechowski, Ostaszewski, Jank, & Berwid, 2002). EGCG in particular has been shown to modulate different signaling pathways that prevent/control various pathological processes (Kaul, Sikand, & Shukla, 2004b; Park & Dong, 2003). EGCG has been shown to modulate MAP-kinase pathway and cytokine signaling through various transcription factors like Sp1, NF-κB and AP-1 (Manson et al., 2000). Sp1, a DNA-binding protein, is known to enhance the transcription by binding to Sp1 response elements in the promoter region of target genes (Boisclair, Browns, Casola, & Rechler, 1993; Kadonaga, Jones, & Tjian, 1986). Specifically, EGCG has been shown to block this activity thus acting as a negative regulator of Sp1-dependent genes (Ren, Zhang, Mitchell, Butler, & Young, 2000; Yeh, Chen, Chiang, Lin-Shiau, & Lin, 2003).

Keeping in view the above-mentioned findings, an attempt has been made to understand the effect of GT-derived polyphenols (GTPs), especially EGCG, on TACO gene transcription. Such a study revealed that EGCG has the inherent ability to down-regulate TACO gene transcription in a dose-dependent manner. Further, the study has been extended to find out the mechanism of this down-regulation. Since TACO helps in entry and intracellular survival of mycobacteria, we also investigated the effect of this EGCG down-regulation of TACO gene transcription on *M. tuberculosis* entry and survival.

2. Materials and methods

2.1. Cells and materials

THP-1 and Jurkat cell lines were obtained from National Centre for Cell Science (NCCS), Pune. Human monocytes were isolated from blood of healthy donors who were not on any kind of medication, including herbal medicines for 2 weeks prior to blood donation. Purified epicatechin (EC), epigallocatechin-3-gallate (EGCG), culture media and fetal bovine serum (FBS)