Enhancement of phagocytic activity of macrophage-like cells by pyrogallol-type green tea polyphenols through caspase signaling pathways

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Abstract We investigated the phagocytosis-enhancing activity of green tea polyphenols, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) catechin (+C) and strictinin, using VD3-differentiated HL60 cells. EGCG, EGC, ECG and strictinin, but not EC and +C, increased the phagocytic activity of macrophage-like cells, and a caspase inhibitor significantly inhibited phagocytic activities. These results suggest that the pyrogallol-type structure in green tea polyphenols may be important for enhancement of the phagocytic activity through caspase signaling pathways.

Keywords Pyrogallol-type polyphenols · Phagocytic activity · Caspase signaling pathways · Green tea

Introduction

Phagocytosis by macrophages is an important non-specific host-defense mechanism (innate immune

system) immediately or within several hours after exposure to antigen, and is also used as an important indicator of the activation of immune function (Oberley et al. 2004; Lee et al. 2001). Phagocytosis requires repeated triggering of phagocytic receptors (Shaw and Griffin 1981), which are categorized into six different structural classes, C-type lectin receptors (mannose receptor), integrins, Ig superfamily members (FcγR), leucine-rich repeat receptors (CD14), receptor tyrosine kinases (Mer), and scavenger receptors (Peiser and Gordon 2001); moreover, Toll-like receptors (TLRs) modulate phagocytic signaling (Underhill and Gantner 2004). Lipopolysaccharide (LPS), β -glucan, peptidoglycan, lipopeptide, and nucleic acid are well known ligands binding to these innate immune receptors (Peiser and Gordon 2001; Underhill and Gantner 2004; Rock et al. 1998; Dunne and O'Neill 2003). Recently, it was reported that caspase signaling also regulates phagocytic activity in macrophages, such as dendritic cells (Ho et al. 2009). It is known that green tea polyphenols induce caspase activation (Saeki et al. 2000; Hayakawa et al. 2001; Vergote et al. 2002; Hsu et al. 2007), but phagocytosis induction by green tea polyphenols through caspase signaling has not been investigated. Therefore, in this study, we investigated the phagocytosis-enhancing activity of green tea polyphenols using VD3-differentiated HL60 cells in which phagocytosis is activated by various immune-enhancing substances.

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Materials and methods

Phagocytosis assay

The phagocytosis assay was performed as previously described (Monobe et al. 2007). Briefly, to differentiate cells along the monocytic pathway, HL60 cells (a human acute promyelocytic cell line; American-Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 medium (Gibco-Invitrogen, Burlington, ON, Canada) supplemented with 120 nM 1,25-dihydroxyvitamin D3 (VD3; Wako Pure Chemical Industries, Ltd.). Differentiated HL60 cells were seeded in 48-well plates (BD Biosciences, Franklin Lakes, NJ). Cells were treated with a 25 μ L test sample. Next, 25 μ L of a 1% suspension of YG-labeled microspheres (Polysciences, Inc., Warrington, PA) was added, and the solution was incubated at 37 °C in 5% CO₂ for 16 h. The cells were fixed with 2% formaldehyde and resuspended in phosphate-buffered saline. The rate of phagocytosis was measured with an EPICSXL flow cytofluorometer (Beckman Coulter, Fullerton, CA).

Treatment of caspase inhibitor

VD3-differentiated HL60 cells were incubated in the presence of test polyphenols with a caspase inhibitor Z-Asp-CH-DCB (200 μ M; Enzo Life Sciences, Inc.) at 37 °C in 5% CO₂ for 16 h, and the rate of phagocytosis was measured.

Results and discussion

The chemical structures of green tea polyphenols are shown in Fig. 1. We investigated the phagocytosis-enhancing activity of green tea polyphenols using VD3-differentiated HL60 cells in which phagocytosis is activated by various immune-enhancing substances. As shown in Fig. 2, polyphenols with a tri-hydroxylated (pyrogallol type) B-ring or with a galloyl group increased the phagocytic activity of VD3-differentiated HL60 cells. In contrast, polyphenols with a di-hydroxylated (catechol type) B-ring or without a galloyl group did not stimulate VD3-differentiated HL60 cells. The pyrogallol-type polyphenol showed immunostimulating activity in the order of EGC > strictinin > EGCG > ECG. These results suggest that

Fig. 1 Chemical structures of green tea polyphenols

Fig. 2 Phagocytic activities of EGCG, EGC, EC, ECG, +C and strictinin. VD3-differentiated HL60 cells were incubated with beads in the presence of polyphenols. Phagocytosis activity in the absence of polyphenols (control) is normalized to 100%. Values are the means, $n = 3$. ** $p < 0.01$ and *** $p < 0.001$ versus the control

the pyrogallol-type structure, such as the pyrogallol-type B-ring or galloyl group, is an important factor in the phagocytic activity of VD3-differentiated HL60 cells. This is similar to the results of Yamada et al. (1997), who showed that pyrogallol-type polyphenols, such as EGCG, gallic acid and pyrogallol, enhanced

Fig. 3 Change in phagocytic activities by treatment with caspase inhibitor. VD3-differentiated HL60 cells were incubated with polyphenols in 0.1% DMSO or caspase inhibitor Z-Asp-CH-DCB (200 μ M). Phagocytosis activity in the absence of polyphenols (control) is normalized to 100%. Values are the means, $n = 3$. ** $p < 0.01$ and *** $p < 0.001$ versus the control (0.1% DMSO). + $p < 0.05$ and ++ $p < 0.01$ versus the absence of caspase inhibitor

immunoglobulin production in mesenteric lymph node lymphocytes of Sprague–Dawley rats.

Polyphenols with a pyrogallol-type structure induce the apoptosis of malignant cells through a caspase signaling pathway (Saeki et al. 2000; Hayakawa et al. 2001; Vergote et al. 2002); however, caspase is a protease playing an important role not only in apoptosis but also in innate immunity (Martinon and Tschopp 2004; Winoto 2004), and regulates phagocytic activity in macrophages, such as dendritic cells (Ho et al. 2009); therefore, we investigated the involvement of the caspase pathway in phagocytic activity. As shown in Fig. 3, the increase of phagocytic activity by EGCG, EGC, ECG and strictinin was significantly inhibited by a caspase family protease inhibitor (Z-Asp-CH2-DCB).

These results suggest that the pyrogallol-type structure in green tea polyphenols is important for enhancement of the phagocytic activity through caspase signaling pathways. Further studies are needed to clarify the molecular mechanisms of the phagocytic activity induced by pyrogallol-type green tea polyphenols; however, these findings may be a fundamental clue to the activation of innate immunity by pyrogallol-type green tea polyphenols.

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