Note

Enhancement of the Phagocytic Activity of Macrophage-Like Cells from a Crude Polysaccharide Derived from Green Tea (Camellia sinensis) Extract

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To identify the immunostimulants included in green tea extract, we investigated a crude tea polysaccharide related to increases in phagocytic activity using macrophage-like cells, and found that the crude tea polysaccharide from immature tea leaves included many RNAs as compared with that from mature tea leaves. Furthermore, the crude tea polysaccharide increased phagocytosis through toll-like receptor 7.

Key words: green tea extract; crude tea polysaccharide; phagocytic activity; RNA; Toll-like receptor

Tea from the plant Camellia sinensis L. is one of the most popular beverages worldwide in its green, black, and oolong forms. Tea polysaccharides, one of the main components of tea extracts, are known to have immunomodulating activities.1–3) Phagocytosis by macrophages is an important nonspecific host-defense mechanism, and is used as an important indicator of activation of immune function.4,5) Phagocytosis requires repeated triggering of phagocytic receptors.6,7) Toll-like receptors (TLRs) also modulate phagocytic signaling,8) and lipopolysaccharide, β-glucan, lipopeptide, and nucleic acid are well known ligands binding to these innate immune receptors.7–12) The components of a crude tea polysaccharide (TPS), an ethanol precipitation of tea extract, are polysaccharides, protein, and nucleic acid. Tea polysaccharide has been reported to have immunomodulating activities and is a complex of proteins or polyphenols,1,2) but there has been no report on its activity in a complex of nucleic acids and polysaccharides in tea extract. Hence, in this study we investigated the involvement of nucleic acid as a component of TPS that increases phagocytic activity.

Preparation of TPS was performed as previously described.13) Briefly, dried tea leaves were boiled in distilled water (DW). The extract was precipitated using ethanol (final concentration 70%). The sediment was washed alternately with ethanol and acetone, and dried. Ethanol (final concentration 70%). The sediment was washed alternately with ethanol and acetone, and dried.

TPS was analyzed by HPLC with an inserted comb. Five ml of 10 mg/ml TPS solution was loaded into the wells. Electrophoresis was carried out in TBE buffer using a Mupid-21 electrophoresis system (Advance, Tokyo), and allowing it to solidify at room temperature with an inserted comb. Five ml of 10 mg/ml TPS solution was loaded into the wells. Electrophoresis was carried out in TBE buffer using a Mupid-21 electrophoresis system (Advance, Tokyo), and allowing it to solidify at room temperature.

HPLC analysis of TPS was performed as previously described.13) Briefly, TPSs were analyzed by HPLC equipped with a refractive index (RI) detector (RID-10A; Shimadzu, Kyoto, Japan), a UV detector (SPD-M; Shimadzu), and a Shodex OHpak SB-806 M HQ column (8.0 mm × 300 mm). Elutions of the standard molecular weights of dextran and pullulans were carried out in the same manner.

First we measured the phagocytic activity of TPS using VD3-differentiated HL60 cells in which phagocytosis was activated by various immunostimulants. As shown in Fig. 1, TPS from immature leaves (mixture of bud and first, second, and third leaves) had very high activity compared with the TPS from mature leaves (mixture of fourth, fifth, and sixth leaves). Next we measured the absorption spectrum of TPS. The absorption spectrum of an immature TPS solution in DW, observed between 220 nm and 330 nm, exhibited one maximum at about 260 nm, not 280 nm (data not shown). Figure 2A and B shows electrophoresis profiles of immature, mature, and stem TPSs. The polysaccharides and nucleic acids in TPS were determined with an RI detector (Fig. 2A) and a UV detector (Fig. 2B) respectively. All the TPSs had a peak of more than 10⁵ M.W., which was the active fraction.13) However, the nucleic acid was not identified.
acid profiles, and not the polysaccharide profiles, showed large differences in peak area size among the three TPSs. The peak area sizes in these TPSs were in the order of immature TPS > mature TPS > stem TPS.

Next, the nucleic acids in the immature TPS, the mature TPS and the stem TPS were analyzed by agarose gel electrophoresis in the presence of ethidium bromide, a nucleic acid intercalator. As shown in Fig. 2C, the TPSs included nucleic acid fragments, and the content of nucleic acid was in the order of immature TPS > mature TPS > stem TPS, in agreement with the results shown in Fig. 2B. To identify the nucleic acid fragments, we treated the immature TPS with deoxyribonuclease (DNase I; Takara Shuzo, Kyoto, Japan) or ribonuclease (RNase A; Wako) for 12 h at 37°C. As shown in Fig. 2D, the fragments were digested with RNase A (a single-strand RNA hydrolase). The high-activity TPS treated the immature TPS with deoxyribonucleic acid (DNase I; Takara Shuzo, Kyoto, Japan) or ribonucleic acid (RNase A; Wako) for 12 h at 37°C. As shown in Fig. 2D, the fragments were digested with RNase A (a single-strand RNA hydrolase). The high-activity TPS

Fig. 1. Phagocytic Activities of Immature TPS, Mature TPS, and Stem.
VD3-differentiated HL60 cells were incubated with beads in the presence of TPS (final conc. 100 μg/ml). Phagocytosis activity in the absence of TPS (control) was normalized to 100%. Values are the means, n = 3. **p < 0.001 versus the control; ***p < 0.001 (paired t-test).

Fig. 2. Characterization of TPSs.
A. Profile measured with a refractive index detector. B. Profile measured with a UV detector at 260 nm. I, Immature TPS; II, Mature TPS; III, Stem TPS. Ten μl of 10-mg/ml TPS solution was injected into the HPLC system. C. Agarose gel electrophoresis of TPS. Lane 1, DNA molecular marker; lane 2, immature leaves TPS; lane 3, mature leaves TPS; lane 4, stem TPS. Five μl of 10-mg/ml TPS solution was loaded into the wells. D. Agarose gel electrophoresis of immature TPS treated with DNase or RNase. Lane 1, non-treated TPS (containing DNase solution buffer); lane 2, DNase-treated TPS; lane 3, non-treated TPS (containing RNase solution buffer); lane 4, RNase-treated TPS.
derived from immature leaves included many ssRNA fragments. The difference in RNA contents in the immature, mature, and stem TPSs was considered to reflect differences in cell multiplication activity in the immature leaves, mature leaves, and stem.

It is well known that TLRs play an important role in innate immunity, and that TLR7 and TLR8 are receptors for ssRNA and play important roles, especially in antiviral immunity.\(^1\) Moreover, TLR7 induces a phagocytic gene program.\(^2\) Here we investigated the involvement of TLR7 in the induction of phagocytosis by immature TPS. Activation of TLR7 is inhibited by immunoregulatory DNA sequences (IRS)\(^6\) or immature TPS. That is, immature TPS activated phagocytosis through TLR7.

In this study, the TPS (70% ethanol sediment) in green tea extract (2.5 g of first-crop leaves/100 ml) constituted 10–40 mg, and the total RNA content in immature TPS constituted 10–40 mg, and the total RNA content in immature TPS was considered to reflect differences in cell multiplication activity in the immature leaves, mature leaves, and stem.

References