A Phagocytic Inducer from Herbal Constituent, Pentagalloylgucose Enhances Lipoplex-Mediated Gene Transfection in Dendritic Cells

Shinichiro KATO, Keiko KOIZUMI, Miyuki YAMADA, Akiko INUIJIMA, Nobuhiro TAKENO, Tsuyoshi NAKANISHI, Hiroaki SAKURAI, Shinsaku NAKAGAWA, and Ikuo SAIKIA

Division of Pathogenic Biochemistry, Institute of Natural Medicine, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan; Laboratory of Hygienics, Gifu Pharmaceutical University; 5–6–1 Mitahora-higashi, Gifu 502–8585, Japan; and Department of Biotechnology and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University; Suta, Osaka 565–0871, Japan. Received May 6, 2010; accepted August 19, 2010

Antigen-presenting cells are key vehicles for delivering antigens in tumor immunotherapy, and the most potent of them are dendritic cells (DCs). Recent studies have demonstrated the usefulness of DCs genetically modified by lipofection in tumor immune therapy, although sufficient gene transduction into DCs is quite difficult. Here, we show that Paeoniae radix, herbal medicine, and the constituent, 1,2,3,4,6-penta-O-galloyl-ß-D-glucose (PGG), have an attractive function to enhance phagocytosis in murine dendritic cell lines, DC2.4 cells. In particular, PGG in combination with lipofectin (LPF) enhanced phagocytic activity. Furthermore, PGG enhanced lipofection efficacy in DC2.4 cells, but not in colorectal carcinoma cell lines, Colon26. In other words, PGG synergistically enhanced the effect of lipofectin-dependent phagocytosis on phagocytic cells. Hence, according to our data, PGG could be an effective aid in lipofection using dendritic cells. Furthermore, these findings provide an expectation that constituents from herbal plant enhance lipofection efficacy.

Key words dendritic cell; lipofection; 1,2,3,4,6-penta-O-galloyl-ß-D-glucose; phagocytosis

Dendritic cells (DCs) play a pivotal role in initiating and controlling the T cell-dependent immune response.1) Immature DCs, localized in non-lymphoid tissues, have optimal capabilities for antigen uptake, processing and the formation of peptide-major histocompatibility (MHC) complexes. Antigen uptake and some cytokines, for example, in the inflammatory environment, promote their maturation and migration to T cell areas of regional lymphoid tissues, where mature DCs strongly present MHC class I and II restricted peptides to naive T cells, inducing an immune response and differentiation.3,4) Because of these properties, DCs have been considered quite attractive immune cells to achieve gene transduction for DNA-based immunization in tumor immunotherapy4–7) and many gene delivery methods have attempted to optimize transduction and transfection to human and murine dendritic cells.8–10) However, despite advances in the understanding of DC biology, the development of genetic immunization strategies using DC-transfected plasmid DNA has been limited by their low transfection efficiencies.11) Currently, the most efficient method for DC transduction is infection using a viral vector based on poxvirus, lentivirus, and adenovirus,1,8,12,13) but viral vectors may be associated with safety concerns and generally require DNA codon optimization to overcome poor gene expression.14,15) An attractive alternative to vector-mediated delivery into DCs is lipofection, non-viral gene transduction. The main advantages of lipofection are its ability to transfect all types of nucleic acids in a wide range of cell types, its ease of use, reproducibility and low toxicity.16) Furthermore, recent studies have demonstrated the usefulness of DCs genetically modified by lipofection in tumor immunotherapy,17) while sufficient gene transduction to DCs is quite difficult.18) Therefore, it is easily thought that more efficient (DNA-based) DC vaccine therapy could be developed by not only understanding DC immune biology but also finding methods or substances which enhance lipofection efficiency in DCs.

We have sought out immunomodulating compounds derived from herbal medicine or Kampo preparations (formulation) for cancer therapy, especially metastasis.19,20) In this study, we found that 1,2,3,4,6-penta-O-galloyl-ß-D-glucose (PGG), which is contained in Paeoniae radix (roots of Paeonia lactiflora),21) enhanced phagocytosis in murine dendritic cell line, DC2.4, especially in combination with lipofectin (LPF). Lipofectin reagent (LPF; Invitrogen, CA, U.S.A.) is a cationic liposome, mixture of N-[1-(2,3-dioleoyl-oxypolypropy]n,n,n-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanol amine (DOPE) at 1:1 (w/w), and is widely used as a device for transfection of RNA, DNA, oligonucleotide and protein.22–26)

These results motivated us to explore the effect of PGG on DC2.4 cells. PGG is a naturally occurring polyphenolic compound contained in many medical plants27,28) and a number of studies have reported that PGG exhibits diverse bioactivity, for example, anti-tumor, anti-oxidant, and anti-inflammatory effects.29) However, the effect of PGG on the phagocytosis of dendritic cells (DCs) has not been investigated, while the effect of polyphenol and tannins (a polyphenol) on phagocytosis and dendritic cells have been investigated well.30–32) Phagocytosed exogenous antigens complexed with LPF induce high-antigen presentation via MHC class I and II and cytotoxic T lymphocytes (CTLs).33,34) Hence, PGG may be a powerful candidate for tumor immunotherapy because it enhances the phagocytic effect of DC2.4 cells.

On the other hand, we hypothesized that PGG could enhance lipofection efficacy and have a new application for lipofection on DCs because lipofection largely depends on the phagocytic effect.35) therefore, the present study investigated the effect of PGG on lipofection efficacy in DC2.4 and bone marrow-derived dendritic cells (BMDCs). Moreover, to demonstrate whether the effect of PGG depends on phagocytosis, we also tested the effect on Colon 26, murine colorectal carcinoma cell line. According to our data, the enhancement of lipofection efficacy by PGG was specific for dendritic cells in combination with lipofectin, but not in colorectal carcinoma cell lines. Therefore, it is easily thought that more efficient (DNA-based) DC vaccine therapy could be developed by not only understanding DC immune biology but also finding methods or substances which enhance lipofection efficiency in DCs.
for lipofection.

MATERIALS AND METHODS

Reagents  AIM-V and Opti-MEM were purchased from Invitrogen (Carlsbad, CA, U.S.A.)/GIBCO BRL (Grand Island, New York, U.S.A.). The aqueous extraction from Paeoniae radix was performed, as previously mentioned. Briefly, about 45 g dried and cut roots were brewed with 900 ml water. Then the filtrate was collected after filtration. The residue was boiled with 800 ml water again and added in the filtrate after filtration. The filtrate was lyophilized. The powder was stored at 4°C. The concentration used in the experiment was based on the dry weight of the extract (mg/ml). 1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG) was purchased from Toronto Research Chemicals Inc. PGG stock solution, originally dissolved to a concentration of 5 mM in 100% dimethyl sulfoxide (DMSO). Paeoniflorin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) Paeoniflorin and Gallic acid (GA) stock solution originally dissolved to a concentration of 10 mM in distilled water. 1,3,6-tri-O-galloyl-β-D-glucose (TGG) stock solution originally dissolved to a concentration of 10 mM in 100% DMSO. These all chemical compounds were diluted to the desired concentration in AIM-V or Opti-MEM just before using.

Cell Culture  DC2.4 cell, derived from a c57BL/6 immature dendritic cell line, was maintained in RPMI1640 supplemented with 50 μM β-melcaptoethanol. Colon26, derived from BALB/c colorectal cancer, was maintained with RPMI1640 supplemented with 2 mM l-glutamine. All media contained 10% fetal calf serum (FCS). Colon26, derived from BALB/c colorectal cancer, was maintained in RPMI1640 supplemented with 2 mM l-glutamine. All media contained 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin, and cultures were kept at 37 °C in a humidified atmosphere of 5% CO₂/95% air.

Phagocytosis in DC2.4  Fluorescence isothiocyanate (FITC) conjugated-dextran (average molecular weight 40 kDa) was purchased from SIGMA-ALDRICH (St. Louis, MO, U.S.A.) and originally dissolved to a concentration of 10 mg/ml in balanced salt solution (BSS). Phagocytosis in DC2.4 was performed by modification of a previously reported method. In this assay, AIM-V media was used instead of growth media. Briefly, 1×10⁶ cells/well were seeded in a 24-well plate (Corning) and pre-incubated with PGG for 1.5 h at 37 °C. Pre-treated DC2.4 was phagocytosed in the presence of 10−500 μg/ml FITC-dextran or lipopolysaccharide for 1 h at 37 °C, which was made of FITC-dextran and 20 μl Lipofectin (Invitrogen) by co-incubation for 35 min. To inhibit phagocytosis of DC2.4 cells, DC2.4 cells were pre-incubated with wortmannin (final concentration; 5 μM) for 20 min before addition of FITC-dextran. FITC-positive cells were detected by fluorescence microscopy using a Keyence fluorescence microscope. For quantitative determinations of transfection efficiency, fluorescent cells were assessed by fluorescence-activated cell sorting (FACS) using a FACSCalibur flow cytometer (Becton-Dickinson, Mountain View, CA, U.S.A.) and CellQuest software.

Differentiation of Bone Marrow-Derived Dendritic Cells  Bone marrow-derived dendritic cells (BMDCs) were differentiated from c57BL/6NCrSlc (9−10-weeks-old specific pathogen free female, Japan SLC (Hamamatsu, Japan)) as reported previously. Differentiated BMDCs were qualified by immunophenotypes and phagocytic activity using FITC-dextran and we used defined BMDCs which express both CD11c and MHC class II more than 70% of total population.

Plasmids  The vectors encoding green fluorescent protein (GFP) mutant, pEGFP-N1 and pEGFP-C1, were purchased from Clontech (Palo Alto, CA, U.S.A.). These vectors encode a mutant GFP that contains more than 190 silent nucleotide changes to optimize the coding sequence based on human codon-usage preferences, and mutations at residue 64 (Phe→Leu) and 65 (Ser→Thr), which results in enhanced fluorescence and a single excitation peak at 488 nm.

Lipofection and Transgenes Expression  Lipofection using a lipofectin reagent was performed by following the modified instructions of the manufacture. One day before transfection, 2×10⁵ cells were seeded in growth media, and nearly 60% confluent cells were used for lipofection. Four microliters of lipofectin reagent was diluted in 100 μl Opti-MEM in one tube and incubated for 30 min at room temperature. Meanwhile, 2−4 μl pEGFP-N1 (1 μg/μl) and pEGFP-C1 (1 μg/μl) (Clontech) were diluted in 100 μl Opti-MEM in another tube separately for 15 min at room temperature. The transfection reagents and plasmid solution were then mixed and incubated at room temperature based on the manufacturer’s instructions. Cells were familiarized with Opti-MEM for 30 min before lipofection. Eight-hundred microliters of Opti-MEM were added to the mixed solution (finally 200 μl + 800 μl), then after removal of the conditioned Opti-MEM, cells were added to transfection solutions (1 ml) and incubated for 5 h. After lipofection for 5 h, transfection solution was replaced with growth media. In PGG-assisted lipofection, PGG was used for incubation of lipoplex throughout lipofection. Twenty-four hours following lipofection, enhanced green fluorescent protein (EGFP)-positive cells were detected and quantified by the same methods as for the phagocytosis of DC2.4.

Flow Cytometric Analysis and Immunophenotypic Analysis  After removal of the supernatant, cells were split off using 0.13% trypsin and ethylenediaminetetraacetic acid (EDTA), pelleted by centrifugation, resuspended in phosphate buffered saline (PBS) containing 2% FCS to a final density of ~5×10⁶ cells/ml, and filtered through a nylon membrane to remove cell aggregates. Flow cytometry for EGFP and propidium iodide (PI) fluorescence were performed using a FACSCalibur (BD Biosciences). For immunophenotypic analysis of DC2.4 cells, split cells were suspended in FACS buffer (0.5−1×10⁶ cells/50 μl), containing PBS in 0.02% FCS to a final density of ~5×10⁶ cells/ml, and filtered through a nylon membrane to remove cell aggregates. Flow cytometry for EGFP and propidium iodide (PI) fluorescence were performed using a FACSCalibur (BD Biosciences). For immunophenotypic analysis of DC2.4 cells, split cells were suspended in FACS buffer (0.5−1×10⁶ cells/50 μl), containing PBS in 0.02% NaCl (w/v) and 2% FCS (v/v). Cells were first incubated with an antibody against FcRg (clone 2.4G2) for 5 min and then labeled with antibodies against CD80 (clone 16-10A1), CD86 (clone GL1), MHC class I (clone 28-14-8) and MHC class II (clone M5/114.15.2) for 30 min. All polyethylene (PE)-conjugated mAb were acquired from BD Biosciences.

Statistical Analysis  Three means (±1 S.D.) were composed using analysis of variance (ANOVA) (Figs. 1, 3). Two means (±1 S.D.) were composed using the unpaired Student’s t-test (two tailed) (Figs. 2, 4). A p value of less than 0.05 was considered significant.
RESULTS AND DISCUSSION

Phagocytosis in DC2.4 Cells Was Enhanced by 1,2,3,4,6-Penta-O-galloyl-β-D-glucose (PGG) Recently, we found that the ability of *Paeonia radix* (aqueous extractions) to engulf FITC-dextran into DC2.4 cells occurred in an FITC-dextran and *Paeonia radix* (PR) (Figs. 1A, C). To identify which chemical compounds enhanced engulfment in DC2.4 cells from *Paeonia radix*, we tested the effect of paoniflorin (PF) and PGG, contained in PR, on incorporation of FITC-dextran in DC2.4 cells. Of these compounds, PGG enhanced engulfment in DC2.4 cells at the same level as PR, while PF, which is the major compound contained in PR, hardly enhanced engulfment (Fig. 1A). PGG had little effect on engulfment with FITC-dextran alone but engulfment was apparently enhanced with the FITC-dextran/lipofectin (LPF) complex (Fig. 1C). The effect was cancelled by wortmannin, a phagocytosis inhibitor, suggesting that the increase of intracellular FITC-dextran depended on the enhancement of phagocytic activity (Figs. 1A, C). This means that PGG synergistically enhances phagocytosis in combination with LPF in DC2.4 cells. In this property, PGG is utilized for lipofection using LPF. Furthermore, we also found the importance of the chemical structure of PGG in the enhancement of phagocytosis in DC2.4 cells (Figs. 1B, C). PGG has five ester bonds formed between the hydroxyl group of the glucose backbone and the carboxyl group of gallic acid (GA). 1,3,6-Tri-O-galloyl-β-D-glucose (TGG), which has three ester bonds, enhanced the phagocytosis of DC2.4 in the absence of LPF and had a tendency to enhance

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**Fig. 1.** Ability of 1,2,3,4,6-Penta-O-galloyl-β-D-glucose (PGG) to Phagocytose into DC2.4

After pre-incubation with (A) 1 mg/ml *Paeoniae radix* (PR), 10 mM Paoniflorin (PF) or 10 mM PGG and (B) 10 mM gallic acid (GA), 10 mM 1,3,6-tri-O-galloyl-β-D-glucose (TGG) or 10 mM PGG for 1.5 h, DC2.4 phagocytes for 1 h at 37 °C in the presence of 250 mg/ml FITC-dextran combined without (left) or with (right) Lipofectin (LPF). Phagocytosed DC2.4 were analyzed by flow cytometer. Filled profile, non-treated cells as control; gray dotted line, FITC-dextran without preincubation with indicated compounds; black line, FITC-dextran with preincubation with indicated compounds. FITC-dextran with preincubation with PGG and 5 μM wortmannin (abbreviation: W) to inhibit phagocytosis. Data are representative of at least two independent experiments. *p < 0.05, **p < 0.01, vs. LPF (-) vehicle group, ††p < 0.01, vs. LPF (+) vehicle group by analysis of variance (ANOVA) with Bonferroni correction.
phagocytosis in the presence of LPF but the enhancement was lower than that of PGG. Additionally, GA also had a tendency to enhance phagocytosis, and the enhancement of GA was lower than that of TGG, suggesting that the ester binding mode between the glucose core and gallic acid rather than gallic acid itself is strongly involved in the enhancement of phagocytosis in DC2.4 cells (Fig. 1B).

Enhanced the Efficacy of Lipofection Using PGG in Mouse Dendritic Cells  Gene transfer into DCs is critical for potential therapeutic applications as well as for study of the genetic basis of DC-mediated immunological development and immune regulation, however, transfection into DCs is difficult.\(^\text{11}\) In particular, it is difficult to transduce naked or plasmid DNA on DCs.\(^\text{9}\) The main advantages of lipofection are its ability to transfect all types of nucleic acids in a wide range of cell types, its ease of use, reproducibility and low toxicity.\(^\text{16}\) therefore, development of more efficient lipofection must enable DC vaccine therapy, and knowledge of DCs biology should be disseminated.

Thus, we tested the effect of PGG on lipofection in DC2.4 cells because PGG enhanced phagocytic activity. In pilot studies of our lipofection, we determined the optimal concentrations of the transfection reagent, plasmid vector and the length of incubation required for the best expression of EGFP in DC2.4 cells. We basically performed lipofection according to the instructions of the manufactures using a GFP

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**Fig. 2. Lipofection with Lipofectin (LPF) Combined with PGG in DC2.4 and Bone Marrow Derived Dendritic Cells**

DC2.4 bone marrow derived dendritic cells were transfected with LPF and PGG together for 5h. After 24h of culture with growth medium at 37 °C, EGFP expression was obtained by fluorescence microscopy and flow cytometry. (A) Transfected DC2.4 cells with 2 μg pEGFP-N1 vector with 200 nM PGG (without Lipofection) (upper left), vector + LPF (lipoplex) (upper right), vector + LPF + PGG indicated concentration (lower panel). Up and downward square observe pictures under phase-contact microscopy and fluorescence image. Scale bar=100 μm. EGFP gene transduction of DC2.4 lipofected with indicated PGG concentration was expressed as EGFP expression (relative EGFP-positive cell proportion (fold)). (B) Histogram image shows EGFP intensity on DC2.4 cells transfected respectively. EGFP gene transduction of DC2.4 lipofected with indicated combination was expressed as EGFP expression (relative EGFP-positive cell proportion (fold)). (C) EGFP gene transduction of BMDCs lipofected with 4 μg pEGFP vector and indicated combination was expressed as EGFP expression (relative EGFP-positive cell proportion vs. conventional lipofection (fold)). Data are presented as the means±S.D. of (B) five or (C) three independent experiments. *p<0.05, **p<0.01, ***p<0.005, vs. respective LPF-untreated group. †p<0.01, vs. PGG-untreated group by two-tail unpaired Student’s t-test.
reporter construct, pEGFP-N1 and pEGFP-C1 vector to determine lipofection efficacy at 24 h posttransfection. For lipofection, we used 2 μg pEGFP-N1 or pEGFP-C1 vector as cytotoxicity (i.e., cell detachment) was noted at higher amounts (data not shown) and 4 μl LPF. Lipofectin required an incubation period of >1 h with DC2.4 cells, whereas optimal results were obtained with about 5-h incubation in serum-free medium (EGFP-positive (EGFP⁺) cells; 2.7±0.9%, n=5). Our lipofection efficacy corresponds to previous studies using lipofection in dendritic cells (lipofection efficacy is less than 2% or not detectable).9, 41)

To evaluate the effect of PGG on lipofection, we used PGG in the preceding lipofection during incubation to prepare the pEGFP vector/LPF complex (lipoplex) for lipofection, and determined the efficacy of EGFP for lipofection efficacy by flow cytometry and fluorescent microscopy. PGG unexpectedly enhanced the expression of EGFP (Fig. 2) and enhancement was achieved in a concentration-dependent manner, but more than 2 μM PGG did not enhance the expression of EGFP (Fig. 2A). Furthermore, DC2.4 cells transfected with pEGFP-N1 vector in combination with 200 nM PGG (without LPF) produced no detectable EGFP fluorescence. A similar phenomenon was obtained using another vector, pEGFP-C1 (Fig. 2B). Thus, transfection activity in an immature dendritic cell line DC2.4 cells was highly enhanced by PGG.

To sophisticate and assess the function of PGG as an inducer of lipofection, we used bone marrow derived dendritic cells (BMDCs) instead of DC2.4 cells. BMDCs were evaluated by flowcytometer and phagocytic activity using FITC-dextran. BMDCs express both CD11c and MHC class II more than 70% of total population were used in our experiments and lipofection procedure on DC2.4 was also applied to BMDCs except for amount of plasmid vector, 2 μg plasmid vector changed to 4 μg. As a result, although the effect of PGG on lipofection efficacy in BMDCs did not seem to be strong, it was significantly enhanced (Fig. 2C). Therefore, PGG could enhance the lipofection efficacy in dendritic cell or phagocytic cell-specific manner.

Effect of Lipofection with PGG on Dendritic Cells Via-

bility In addition to measuring EGFP expression at 24 h posttransfection, we assessed the effect of lipofection combined with PGG on cell viability by staining with propium iodide (PI) and using a flow cytometer. In Fig. 3, DC2.4 and BMDCs were not damaged by lipofectin in combination with PGG, and 200 nM PGG by itself also did not exhibit cytotoxicity on these cells (determined using WST-1: cell proliferation assay; data not shown). PGG might not exhibit remarkable cytotoxicity because of the low concentration. Usually, non-viral transfections are harmful to DCs9,17) and lipoplex can increase cytotoxicity along with lipofection efficacy in a dose-dependent manner.42) Clinically, all vaccines and DNA-based DC vaccine therapies must be safe, therefore, PGG would be a useful aid for lipofection.

Does Lipofection in Combination with PGG Depend on Phagocytosis? We have suggested that PGG enhanced the effect of lipofection on DC2.4 cells and BMDCs, however, the action mechanism of PGG is still unclear. Although one possibility was shown that PGG enhanced phagocytic activity in DC2.4 cells (Fig. 1), it remained to be confirmed. PGG may also enhance lipofection efficacy by increasing phagocytic activity. In the next study, to determine whether the effect of PGG on lipofection depends on phagocytosis, we tested its effect using colon 26 cells, a murine colon carcinoma cell line. The endocytic liposome uptake pathway is further separated into phagocytosis (phagocytic cells) and pinocytosis (all cells).43) As shown in Fig. 4, PGG slightly enhanced lipofection efficacy on colon 26 but not significantly, therefore, the enhancement of lipofection efficacy on DC2.4 and BMDCs by PGG greatly depend on intensified phagocytosis and is not implicated in the fusogenic effect. Namely, the effect of PGG would be restricted to phagocytic cells, like DCs.

The internalization mechanism of lipoplex is not well understood. An early report suggested that the fusogenic effect between the positively charged lipoplex and the plasma membrane is responsible for transducing DNA into cytosol,22) however, subsequent studies recently have shown the involvement of phagocytosis in delivering DNA.44,45) Hence, it is currently believed along with this historical background that membrane fusion is important in lipofection but uptake of lipoplex largely depends on phagocytosis and endocytosis. According to our data, PGG specifically enhanced lipofection efficacy in DC2.4 cells and BMDCs. In other words, PGG enhanced some functions specifically found in DCs. Additionally, it was thought that phagocytosis facilitated by PGG enhanced lipofection efficacy.

A remaining question for our further study is which site of action of PGG is responsible for phagocytosis. In our lipofection protocol, PGG was added during the generation of lipoplex and transfection; namely, PGG affects lipoplex, DC2.4 or both to enhance lipofection efficacy.

If PGG modified lipoplex more effectively by making a lipoplex/PGG complex, PGG must enhance lipofection efficacy on colon 26 cells. It is unknown why they make a complex with each other, but the lipoplex/PGG complex must be phagocytosed through a DC-specific receptor. The recent identification of surface receptors expressed on DCs allow for a specific target for effective transfection.46) Receptors such as FcγRI47,48) and mannose49,50) are attractive candidates for target cell surface molecules. If PGG has an affinity with
these cell surface receptors, the lipoplex/PGG complex may be easily phagocytosed. In previous studies, Li et al. reported that PGG binds to cell surface insulin receptor \(^{51}\) and a number of researchers have studied the binding activity to biomolecules of PGG \(^{29}\); however it remains to be determined whether PGG binds to a DC-specific cell surface receptor. Furthermore, we have already suggested the importance of lipoplex incubation in combination with PGG for effective lipofection (data not shown). Consequently, we need to investigate the binding activity to cell surface receptor on DC2.4 cells and BMDCs in our further studies.

Secondly, we should focus on the DC maturation state if the action mechanism depends on the enhancement of phagocytosis. DCs have high phagocytic properties in the immature state, while few phagocytic properties in the mature state. Although DCs can phagocytose, they can be transformed by lipofection only with very low efficacy, perhaps due to their high nuclease content or easy of maturity by transfection. In this study, we used an immortalized murine immature DC line, DC2.4 cells \(^{21}\) and primary immature BMDCs. These cells may be matured by lipofection and lose phagocytic activity. As one possibility, PGG could maintain the phagocytic activity by inhibiting their maturation. DC maturation and differentiation are regulated by nuclear factor \(\kappa\)B 2 (NF-\(\kappa\)B2) and RelB, and these proteins are involved in vesicular transport. \(^{52-54}\) Because previous reports have shown the inhibitory effect of PGG on NF-\(\kappa\)B activity, \(^{29,55}\) PGG probably maintains an immature state by inhibiting a transcriptional factor, such as NF-\(\kappa\)B. However, it has been reported that lipofectin alone or lipoplex could not induce maturation on DCs related to the cell surface expression of major histocompatibility (MHC) class I or II, CD40,

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**Fig. 4. Lipofection with Lipofectin (LPF) Combined with PGG in Colon 26**

Colon 26 cells were transfected with LPF and PGG together. Lipofection and detection of EGFP used the same methods as for DC2.4 cells (cf. Fig. 2). (A) Transfected colon 26 with 2 \(\mu\)g pEGFP-N1 vector only (without Lipofection) (upper left), vector + 200 nM PGG (upper right), vector + LPF (lower left), vector + LPF + 200 nM PGG (lower right). Small squares show images under phase-contact microscopy. Scale bar = 100 \(\mu\)m. (B) EGFP gene transduction of colon 26 lipofected with the indicated combination was expressed as EGFP expression (EGFP-positive cell proportion (%)). Data are presented as the means \(\pm\) S.D. of two or three independent experiments. **\(p<0.005\), vs. respective LPF-untreated group by two-tail unpaired Student’s t-test. n.s. = no significant difference.
CD80, CD86, ICAM-I and IL-12 p40 expression, and an inflammatory signal, LPS, was needed to change immature DCs into mature DCs. Our phenotypical analysis of DC2.4 cells correspondingly demonstrated that cell surface expressions of MHC class II and CD80 were hardly increased by lipofection but were not inhibited by PGG (data not shown). Thus, we considered that PGG did not suppress DC maturation to maintain phagocytic activity.

Our data showed that PGG affected phagocytosis and lipofection efficacy in DC2.4 cells but the action mechanism has not been clarified. Hereafter, we must elucidate the mechanism in detail to understand DC biology and structure for a more highly effective lipofection method. If these challenges are achieved, DC vaccine therapy will develop markedly. Conventional DC vaccine therapies have already succeeded in grapes and the red wine prepared from them. But, these preparations showed only biological activities of medicinal plant extracts but also the pharmaceutical technological effectiveness as an application. Finally, our present findings provide an expectation that constituents from herbal plant enhance lipofection efficacy.

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