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ORIGINAL ARTICLE

Traditional Chinese Medicine Ingredients *Rosa damascena* and *Poria cocos* Promote Phagocytosis and a Dendritic Cell Phenotype in THP-1 Cells

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ABSTRACT

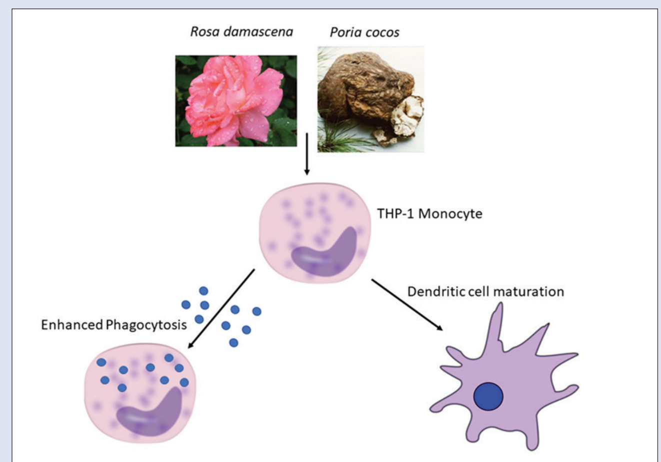
Background: *Rosa damascena* and *Poria cocos* are ingredients commonly used in Traditional Chinese Medicine. *R. damascena* is used to promote blood circulation as well as liver and stomach function, while *P. cocos* is used to eliminate dampness and enhance spleen function. **Objective:** The objective of the study is to investigate possible mechanisms by which *R. damascena* and *P. cocos* may promote immune function. **Materials and Methods:** Phagocytosis and dendritic cell (DC) surface marker expression assays were used to evaluate the effect of *R. damascena* and *P. cocos* extracts on human THP-1 monocytic leukemia cell biology. **Results:** *R. damascena* and *P. cocos* extracts both enhanced phagocytosis of latex beads by THP-1 cells, and when combined, phagocytosis was enhanced to a level greater than what might be expected by adding the individual phagocytosis responses together. In addition, both extracts enhanced maturation of THP-1 cells into a DC phenotype as measured by increased surface expression of the costimulatory molecules CD14, CD40, CD80, and CD86. **Conclusion:** These results suggest that *Rosa damascena* and *P. cocos* may promote monocyte phagocytosis and then stimulate differentiation of the cells into DCs thereby bridging innate and adaptive immune responses.

Key words: Dendritic, immune, phagocytosis, Traditional Chinese Medicine, THP-1

SUMMARY

- The aim of this study was to study potential immune function enhancing properties of Traditional Chinese Medicine ingredient. Results. *Rosa damascena* and *Poria cocos* extracts enhanced phagocytosis and a dendritic cell phenotype in THP-1 cells.

Abbreviations used: AKT: Protein kinase B; AP-1: Activator protein-1; Bcl2: B cell lymphoma-2; CD: Cluster of differentiation; COX-2: Cyclooxygenase-2; DC: Dendritic cell; EGFR: Epidermal growth factor receptor; FOXO1: Forkhead box protein-1; GM-CSF: Granulocyte/macrophage colony stimulating factor; HLA: Human leukocyte antigen; HPLC: High-performance liquid chromatography; IL-1β: Interleukin-1β; IL-4: Interleukin-4; M1: Classically activated macrophage; M2: Alternatively activated macrophage; MAPK: Mitogen-activated protein kinase; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3: NLR Family Pyrin Domain Containing 3; ϕ: Phagocytic index; p53: Tumor protein p53; PARP: Poly (ADP-ribose) polymerase; PMA: Phorbol 12-myristate 13-acetate; PRR: Pattern recognition receptor; STAT: Signal transducer and activator of transcription SD: Standard deviation; Syk: Spleen tyrosine kinase; TCM: Traditional Chinese Medicine.



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INTRODUCTION

Monocytes are peripheral blood mononuclear cells that make up approximately 10% of circulating leukocytes in humans. They are a heterogeneous cell population with the ability to differentiate into macrophages, dendritic cells (DCs), and osteoclasts. Monocytes are an important part of the innate immune system as they recognize potential pathogens due to their expression of pattern recognition receptors (PRRs), and on PRR engagement, produce proinflammatory cytokines and chemokines that serve to recruit other leukocytes to an infection site and amplify the resulting inflammatory response. They are also able to phagocytose microbes, and following phagocytosis may link innate immunity to adaptive immunity by migrating to draining lymph nodes where they differentiate into DC to present antigen to T lymphocytes with increased expression of costimulatory receptors such as CD40, CD80, and CD86.[1] DCs are the primary type of antigen-presenting cells

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and therefore play a primary role in the initiation of adaptive immune responses.[2,3]

Traditional Chinese Medicine (TCM) is an ancient form of medicine based on the theory that the body is healed by returning to a state of homeostasis through the use of herbal remedies.[4] Herbs are chosen based on observations of their clinical use, along with a number of different characteristics including the herb's temperature property, taste, channel tropism, and toxicity.[5] Two such TCM herbs are *Poria cocos* and *Rosa* sp. *P. cocos,* also known as Fu-Ling, is a fungus that is usually found at the base of trees of the genus *Pinus*. It develops a large sclerotium that is widely used in TCM as it is considered one of the nine magical herbs. It has been used for thousands of years as a diuretic, sedative, and tonic to treat chronic gastritis, edema, nephrosis, dizziness, nausea, and inflammation.[6-8] *Rosa* sp., also known as Mei Gui Hua, has also been used in TCM for hundreds of years to regulate liver function, improve blood circulation and treat stomach aches, diarrhea, menoxenia, diabetes mellitus, pain, and chronic inflammatory disease.[9,10]

In the present study, we investigated whether *P. cocos* and *R. damascena* extracts may modulate monocyte function as both are traditionally used to treat forms of inflammation as mentioned above. Specifically, we tested whether the extracts promoted phagocytosis by monocytes, and in addition, as monocytes can migrate to draining lymph nodes, whether monocytes can mature into DC as measured by surface expression of the costimulatory molecules.

MATERIALS AND METHODS

Reagents

Unless otherwise specified, all cell culture media components were purchased from Mediatech (Manassas, VA). Human recombinant interleukin-4 (IL-4) and granulocyte/macrophage colony stimulating factor (GM-CSF) were purchased from Peprotech (Rocky Hill, NJ, USA). Phorbol 12-myristate 13-acetate (PMA), FITC-dextran (40,000 avg. MW), cytochalasin D, and piceatannol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amino 3-micron latex beads were purchased from Polysciences (Warrington, PA, USA). CypHer5e mono NHS ester was purchased from GE Healthcare (Pittsburgh, PA, USA). Phycoerythrin-labeled anti-human CD14, Alexa fluor 488-labeled anti-human CD40, PerCP/Cy5.5-labeled anti-human CD80, and Alexa fluor 488 labeled anti-human CD86 antibodies were purchased from Biolegend (San Diego, CA, USA).

Plant extracts

R. damascena and *P. cocos* extracts were purchased from Sinphar Tianli Pharmaceutical Co, Ltd. (Zhejiang, China). Both were generated by ethanol extraction and then spray dried. *R. damascena* extract is standardized to polyphenols (>30% by UV-VIS) and procyanidin (≥2.0% by high-performance liquid chromatography). An herbarium voucher verifying its identity was prepared by the Institute of Medicinal Plant Development at the Chinese Academy of Sciences. *P. cocos* extract is standardized to triterpenes (>15% by UV-VIS). An herbarium voucher verifying its identity was prepared by the Kunming Institute of Botany of the Chinese Academy of Sciences.

CypHer5e conjugation of latex beads

Latex beads were labeled as described by Beletskii *et al.*[11] Briefly, 1 mL of 3 μamino latex particles were washed three times with 0.1 M carbonate buffer (pH 9.0). The washed beads were suspended in 0.5 mL of carbonate buffer and were incubated at room temperature for 2 h with constant agitation with 0.3 mg of CypHer5e mono NHS ester that had been dissolved in 0.1 M carbonate buffer. Unreacted dye was removed by washing the beads three times with carbonate buffer. Following washing, the beads were resuspended in 12 mL of 0.1 M carbonate buffer and were stored at 4°C.

Phagocytosis assay

Human monocytic THP-1 cells (ATCC# TIB-202) were purchased from ATCC (Manassas, VA, USA) and were maintained in RPMI 1640 media with 10% fetal bovine serum (HyClone, Logan, UT, USA), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin B. For the phagocytosis assay, THP-1 cells were plated in 6 well plates at 1×10^6 cells/well in a volume of 2 mL. The cells were treated with samples and PMA at 1 nM for 20 h at 37°C in a humidified, 5% CO_2 atmosphere. During the past 30 min of the incubation period, the viability dye Cell Tracker green (Invitrogen, Carlsbad, CA, USA) was added to the cells at a concentration of 1 μM. Following the incubation period, the cells were collected by repeated pipetting of the media in the wells to dislodge the cells from the well surface. The cells were transferred to 5 mL polypropylene tubes and pelleted by centrifugation. The cells were resuspended in 2 mL of RPMI-10%, and 100 μL of the CypHer5e-labeled beads were added to the tubes. When phagocytosis inhibitors were used, they were added for 15 min at 37°C before the addition of the beads to the tubes. The tubes were loosely capped and incubated an additional 18 h in the humidified 5% CO_2 atmosphere at 37°C. Following incubation with beads, the THP-1 cells were washed once and resuspended in ice cold phosphate buffered saline (PBS). The cells were analyzed by flow cytometry on a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA) using FL1 (Cell Tracker green) to measure viable cells and FL4 to measure phagocytosed beads. Cells that were double positive therefore were viable cells that had taken up the CypHer5e labeled beads. Data were calculated as the ratio of double positive cells to single positive (FL1) cells. A phagocytic index was then calculated by comparing this ratio from each experimental condition to PMA-treated cells.

Costimulatory molecule expression

THP-1 cells were plated at 2×10^6/well in 6 well plates in a volume of 2 mL for 120 h. The cells were exposed to *R. damascena* and *P. cocos* extracts at 100 and 50 μg/mL respectively at time 0 and 48 h. One half of the media was removed at 48 h. and replaced with an equal volume of fresh media and additional sample. A combination of IL-4 and GM-CSF (5 ng/mL each) were used as positive control with additions at 0 and 48 h. Cells were collected using PBS-ethylenediaminetetraacetic acid (2 mM) with gentle repeated pipetting to dislodge the cells from the culture well surface. The cells were stained for 1 h at 4°C with anti-human-CD14-PE,-CD40-Alexa Fluor 488,-CD80-PerCP/Cy5.5 or-CD86-Alexa Fluor 488 antibody according to the manufacturer's specifications and were then washed three times with cold PBS. Fluorescence was assessed by flow cytometry on a Becton Dickinson FACSCaliber.

Statistical analysis

The results of most experiments described here are expressed as mean ± standard deviation (SD) values and are representative of three independent experiments. Statistical analysis was carried out by Student's *t*-test using PRISM version 6.01 statistical analysis software (GraphPad Software, Inc., San Diego, CA, USA). Levels of statistical significance between data sets were significant if the $P < 0.05$ (**), and highly significant if $P < 0.01$ (***).

RESULTS

Effect of Rosa damascena and Poria cocos extracts on phagocytosis

During the screening of several botanical TCM samples, *R. damascena* and *P. cocos* were found to enhance phagocytosis (data not shown). To

confirm and further characterize this activity, the effect of a dose-response of *R. damascena* se and *P. cocos* extracts was conducted. The data in Figure 1a show that both *R. damascena* and *P. cocos* extracts induced a dose-dependent enhancement of phagocytosis. The phagocytic (ϕ) index for *R. damascena* peaked at ~30 while the ϕ index for *P. cocos* peaked at ~60. As the phytochemical content of the *R. damascena* extract is primarily of flavonoids and the *P. cocos* extract is primarily triterpenoids, it was possible that these extracts enhance phagocytosis by different mechanisms of action. As such, these different mechanisms might result in a synergistic response if the samples were combined to treat the cells. To test this possibility, THP-1 cells were treated with a combination of *R. damascena* and *P. cocos* extracts at a ratio of 5:1 with *R. damascena* extract at 50 μg/mL and *P. cocos* extract at 10 μg/mL. These suboptimal concentrations were selected from the dose-response results to improve the likelihood of seeing an additive or synergistic response. The data in Figure 1b show that the combination of *R. damascena* and *P. cocos* stimulated phagocytosis at a level higher than would be expected from a simple additive effect. The hypothetical additive effect on phagocytosis would have resulted in a ϕ index of 17 whereas the actual ϕ index was 27. These results suggest that *R. damascena* and *P. cocos* extracts work by different mechanisms to augment phagocytosis and that when combined, may synergize for enhanced phagocytic activity.

Effect of inhibitors on *Rosa damascena* and *Poria cocos*-enhanced phagocytosis

To confirm these results, two known inhibitors of phagocytosis were tested for their effect on *R. damascena* and *P. cocos*-enhanced phagocytosis. The mycotoxin cytochalasin D is a potent inhibitor of actin polymerization,[12] while the resveratrol metabolite piceatannol is known to inhibit Syk-kinase mediated actin polymerization.[13] The data in Figure 1b and c show that both compounds totally inhibited phagocytosis stimulated by *R. damascena* and *P. cocos* extracts. These results provide supporting evidence that *R. damascena* and *P. cocos* extracts stimulate phagocytosis.

Effect of *Rosa damascena* and *Poria cocos* extracts on dendritic cell maturation

Once monocytes have ingested pathogens, they might migrate to local lymph nodes and differentiate into DC for efficient antigen presentation to initiate adaptive immune responses. To do so, DC must express costimulatory receptors on their surface for efficient antigen presentation to lymphocytes. To test whether *R. damascena* and *P. cocos* extracts might also promote monocyte differentiation into DC, THP-1 cells were incubated with *R. damascena* and *P. cocos* extracts and then tested for expression of CD14, CD40, CD80, and CD86. The data in Figure 2a show that treatment of the cells with *R. damascena* and *P. cocos* extract promoted an increase in CD14 expression equal to the effect induced by the IL-4/GM-CSF control. Figure 2b shows that both extracts had a slight impact of CD40 expression. Figure 2c shows that both *R. damascena* and *P. cocos* extracts had a significant effect on expression CD80 while the IL-4/GM-CSF had a minimal effect. Finally, the data in Figure 2d similarly show a strong effect of both extracts, especially *R. damascena* extract, on CD86 expression which was also greater than the effect of the IL-4/GM-CSF control.

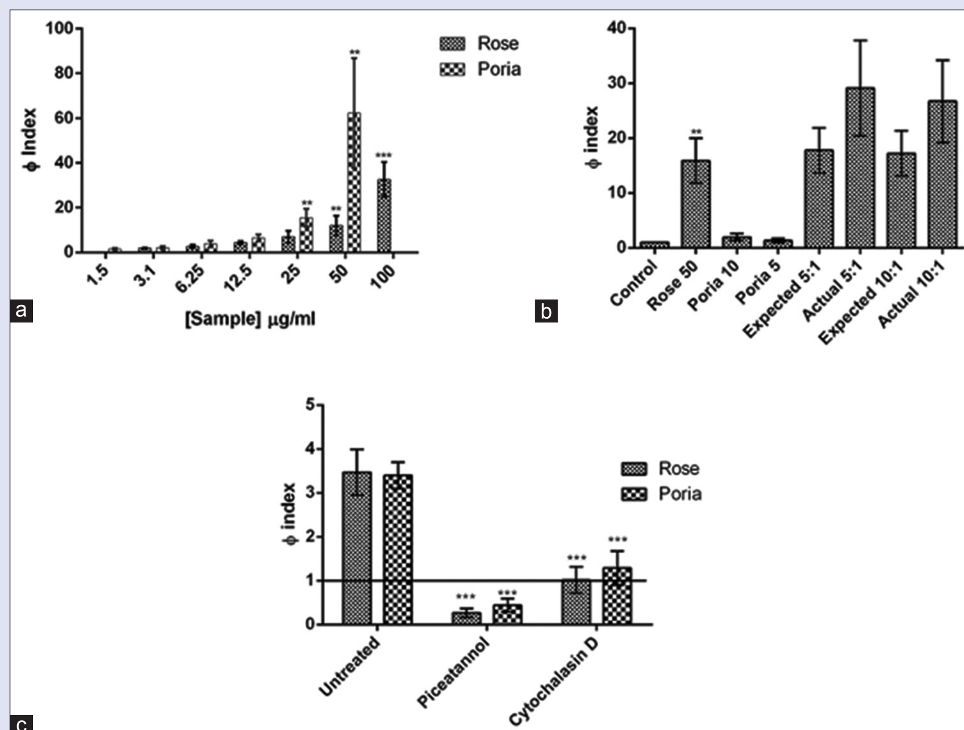

Figure 1: Effect of *Rosa damascena* and *Poria cocos* extracts on monocyte phagocytosis. (a) Dose response effect of *Rosa damascena* and *Poria cocos* extracts on phagocytosis. Phagocytosis was assessed as mentioned above. Data are expressed as the mean + standard deviation three replicates from a representative experiment. (b) Effect of combinations of *Rosa damascena* and *Poria cocos* extracts on phagocytosis. THP-1 cells were treated with PMA, *Rosa damascena* extract (50 μg/mL) alone or combined with *Poria cocos* extract (5 or 10 μg/mL) for 20 h. Phagocytosis was assessed as described above. Data are expressed as the mean + standard deviation three replicates from a representative experiment. (c) Effect of Cytochalasin D[12] and piceatannol[13] on phagocytosis. Cells were treated as above but with 10 μg/mL of both extracts. Cytochalasin D and piceatannol were added to the cells at 100 and 125 μM respectively and the cells were incubated for 5 min at 37°C before addition of the CypHer5e labeled latex beads. Phagocytosis was assessed as described above. Data are expressed as the mean + standard deviation three replicates from a representative experiment

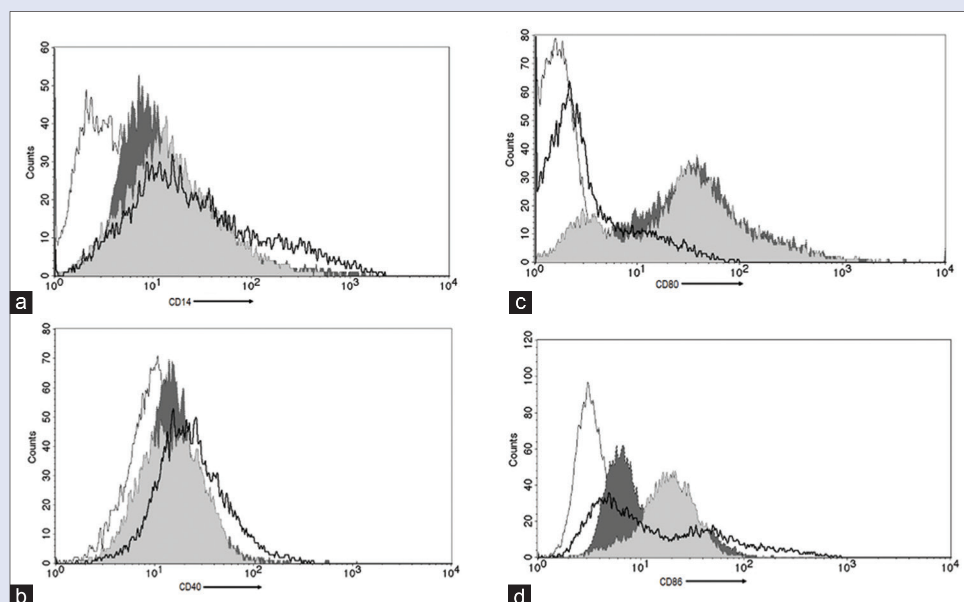

Figure 2: Effect of *Rosa damascena* and *Poria cocos* extracts on CD expression. Histograms showing distribution of fluorescent staining from a representative of three experiments and show shift in expression of each of the CD molecules tested in response to IL-4/GM-CSF (each 5 ng/mL), *Rosa damascena* (100 µg/mL), or *Poria cocos* (50 µg/mL) treatment compared to untreated cells (a) CD14 expression. (b) CD40 expression. (c) CD80 expression. (d) CD86 expression

DISCUSSION

We have shown in the work presented here that extracts of two ingredients from TCM, *R. damascena,* and *P. cocos*, enhance the ability of monocytes to phagocytose latex beads and to differentiate into cells with properties of DC. For these experiments, we used the THP-1 cell line which was derived from the blood of a 1-year-old male with acute monocytic leukemia.[14] These cells have been widely studied as their biology is very similar to peripheral monocytes.[15,16] One of the advantages of using THP-1 cells is that their plasticity is similar to peripheral blood monocytes. They have been studied for their ability to differentiate into macrophages in response to PMA[17] and Vitamin D[18] and can also be polarized into M1 and M2 phenotypes. Treatment with Interferon-γ-causes them to polarize into pro-inflammatory M1 or classically activated macrophages, whereas IL-4, IL-13, and IL-10 treated THP-1 cells express an anti-inflammatory M2 or an alternatively activated phenotype.[19-21] THP-1 cells can also differentiate into DC in the presence of IL-4 and GM-CSF based on expression of costimulatory molecules, the ability to endocytose, and to present antigen to HLA matched lymphocytes.[22,23] Due to this wide range of phenotypes that THP-1 cells are capable of expressing, they make an attractive model for studying how natural products might influence the differentiation of monocytes and their involvement in the interface between innate and adaptive immune function.

We modified the method of Beletskii *et al*. to assess the ability of *R. damascena* and *P. cocos* extracts to enhance phagocytosis.[11] In this assay, latex beads are conjugated CypHer5e, a dye that is nonfluorescent at neutral pH, but in maximally fluorescent at pH <5.5. Thus, the beads only fluoresce in the acidic environment of the phagosome. Our results demonstrate that both *R. damascena* and *P. cocos* extracts enhanced the ability of THP-1 cells to phagocytose latex beads in a dose-dependent manner [Figure 1]. In addition, when the cells were treated with a combination of *R. damascena* and *P. cocos* extracts, there was an unexpected increase in the level of phagocytosis above what would be expected adding the responses to either extract alone [Figure 2].

Following phagocytosis, monocytes may migrate to local draining lymph nodes to initiate an adaptive immune response. Phagocytosis of subcutaneously injected latex beads has been demonstrated *in vivo* in mice, and approximately 25% of the beads were cleared from the skin.[1] The authors attributed this to phagocytosis of beads by monocytes which subsequently migrated to the T cell area of draining lymph nodes where they acquired DC-restricted markers and high expression of CD86.[1]

Monocytes can be directed *in vitro* to differentiate into DCs when they are cultured in the presence of IL-4 and GM-CSF,[24-26] and THP-1 cells too can be differentiated using this method.[16,22] Under these conditions, THP-1 cells upregulate expression of a number of surface markers, the ability to endocytose and produce cytokines consistent with a DC phenotype.[22] We found that incubation of THP-1 cells with *R. damascena* and *P. cocos* PMAextracts, in the absence of IL-4 and GM-CSF, induced increased expression of several surface proteins (CD14, CD40, CD80, and CD86) associated with a DC phenotype. The greatest effect was on the expression of CD80 and CD86 which suggests that both *R. damascena* and *P. cocos* may enhance costimulation during T-cell activation.[27] Upregulation of CD40 suggests that *R. damascena* and *P. cocos* may also promote immunoglobulin class switching during B-cell stimulation.[28]

The mechanism of action of *R. damascena* and *P. cocos* extracts in our model is unknown. As mentioned above, *R. damascena* extract is standardized to >30% polyphenols while *P. cocos* extract is standardized to >15% triterpenoids, and our results are consistent with many other reports on the effects of polyphenols and triterpenoids on monocytes and macrophages. Representatives from each of these phytochemical classes have been shown to augment macrophage phagocytosis.[29-31] Likewise, both have also been shown to augment expression of CD14, CD80, and CD86.[32,33] Polyphenols have been shown to act on many molecular targets including COX-2, AP-1, STAT, EGFR, AKT, Bcl2, NF-κB, Bcl-xL, p53, FOXO1, PARP, and MAPK.[34,35] Triterpenoids have been shown to activate immune cells by several mechanisms. The saponin QS-21 is used as a vaccine adjuvant[36] and may work through activation of the inflammasome NLRP3.[37] Aggregated ursolic acid stimulates IL-1β release by a CD36-dependent mechanism.[38] CD36 is known to promote phagocytosis,[39] and engagement of CD36 is known to activate a number

of downstream signaling events including nonreceptor tyrosine kinases, MAPKs, and the Vav family of guanine nucleotide exchange factors.[40]

CONCLUSION

The results presented here suggest that the TCM ingredients *R. damascena* and *P. cocos* may enhance immune function in several ways. First, these samples both enhance phagocytosis of particulates by monocytes. Second, following phagocytosis and migration to lymph nodes, the samples may promote differentiation of the same monocytes into DC. The resulting DC may then participate adaptive immunity through T and/or B cell activation.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo*. Immunity 1999;11:753-61.
2. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al.* Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767-811.
3. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245-52.
4. Stone JA, Yoder KK, Case EA. Delivery of a full-term pregnancy after TCM treatment in a previously infertile patient diagnosed with polycystic ovary syndrome. Altern Ther Health Med 2009;15:50-2.
5. Zhu YP. Chinese *Materia Medica*: Chemistry, Pharmacology and Applications. Amsterdam, Harwood Academic Press; 1998.
6. Giner-Larza EM, Máñez S, Giner-Pons RM, Carmen Recio M, Ríos JL. On the anti-inflammatory and anti-phospholipase A (2) activity of extracts from lanostane-rich species. J Ethnopharmacol 2000;73:61-9.
7. Sun Y. Biological activities and potential health benefits of polysaccharides from *Poria cocos* and their derivatives. Int J Biol Macromol 2014;68:131-4.
8. Lee KY, Jeon YJ. Polysaccharide isolated from *Poria cocos* sclerotium induces NF-kappaB/Rel activation and iNOS expression in murine macrophages. Int Immunopharmacol 2003;3:1353-62.
9. Xie Y, Zhang W. Antihypertensive activity of *Rosa rugosa* thunb. flowers: Angiotensin I converting enzyme inhibitor. J Ethnopharmacol 2012;144:562-6.
10. Gu D, Yang Y, Bakri M, Chen Q, Xin X, Aisa HA. A LC/QTOF-MS/MS application to investigate chemical compositions in a fraction with protein tyrosine phosphatase 1B inhibitory activity from *Rosa rugosa* flowers. Phytochem Anal 2013;24:661-70.
11. Beletskii A, Cooper M, Sriraman P, Chiriac C, Zhao L, Abbot S, *et al.* High-throughput phagocytosis assay utilizing a pH-sensitive fluorescent dye. Biotechniques 2005;39:894-7.
12. Sampath P, Pollard TD. Effects of cytochalasin, phalloidin, and pH on the elongation of actin filaments. Biochemistry 1991;30:1973-80.
13. Oliver JM, Burg DL, Wilson BS, McLaughlin JL, Geahlen RL. Inhibition of mast cell fc epsilon R1-mediated signaling and effector function by the syk-selective inhibitor, piceatannol. J Biol Chem 1994;269:29697-703.
14. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer 1980;26:171-6.
15. Qin Z. The use of THP-1 cells as a model for mimicking the function and regulation of monocytes and macrophages in the vasculature. Atherosclerosis 2012;221:2-11.
16. Chanput W, Mes JJ, Wichers HJ. THP-1 cell line: An *in vitro* cell model for immune modulation approach. Int Immunopharmacol 2014;23:37-45.
17. Chanput W, Mes JJ, Savelkoul HF, Wichers HJ. Characterization of polarized THP-1 macrophages and polarizing ability of LPS and food compounds. Food Funct 2013;4:266-76.
18. Hmama Z, Nandan D, Sly L, Knutson KL, Herrera-Velit P, Reiner NE. 1alpha, 25-dihydroxyvitamin D (3)-induced myeloid cell differentiation is regulated by a Vitamin D receptor-phosphatidylinositol 3-kinase signaling complex. J Exp Med 1999;190:1583-94.
19. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3:23-35.
20. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003;73:209-12.
21. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549-55.
22. Berges C, Naujokat C, Tinapp S, Wieczorek H, Höh A, Sadeghi M, *et al.* A cell line model for the differentiation of human dendritic cells. Biochem Biophys Res Commun 2005;333:896-907.
23. Ogasawara N, Kojima T, Go M, Fuchimoto J, Kamekura R, Koizumi J, *et al.* Induction of JAM-A during differentiation of human THP-1 dendritic cells. Biochem Biophys Res Commun 2009;389:543-9.
24. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179:1109-18.
25. Ardavín C, Martínez del Hoyo G, Martín P, Anjuère F, Arias CF, Marín AR, *et al.* Origin and differentiation of dendritic cells. Trends Immunol 2001;22:691-700.
26. Chan WK, Cheung CC, Law HK, Lau YL, Chan GC. *Ganoderma lucidum* polysaccharides can induce human monocytic leukemia cells into dendritic cells with immuno-stimulatory function. J Hematol Oncol 2008;1:9.
27. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol 2013;13:227-42.
28. Ma DY, Clark EA. The role of CD40 and CD154/CD40L in dendritic cells. Semin Immunol 2009;21:265-72.
29. Shah V, Bayeta E, Lau BH. Pycnogenol® augments macrophage phagocytosis and cytokine secretion. Pak J Nutr 2002;1:196-201.
30. Wu TF, Hsu CY, Huang HS, Chou SP, Wu H. Proteomic analysis of pycnogenol effects in RAW 264.7 macrophage reveals induction of cathepsin D expression and enhancement of phagocytosis. J Agric Food Chem 2007;55:9784-91.
31. Tong H, Jiang G, Guan X, Wu H, Song K, Cheng K, *et al.* Characterization of a polysaccharide from *Rosa davurica* and inhibitory activity against neutrophil migration. Int J Biol Macromol 2016;89:111-7.
32. Sung NY, Yang MS, Song DS, Byun EB, Kim JK, Park JH, *et al.* The procyanidin trimer C1 induces macrophage activation via NF-κB and MAPK pathways, leading to th1 polarization in murine splenocytes. Eur J Pharmacol 2013;714:218-28.
33. Ying ZL, Li XJ, Dang H, Wang F, Xu XY. Saikosaponin-d affects the differentiation, maturation and function of monocyte-derived dendritic cells. Exp Ther Med 2014;7:1354-8.
34. Hatchwell L, Collison A, Girkin J, Parsons K, Li J, Zhang J, *et al.* Toll-like receptor 7 governs interferon and inflammatory responses to rhinovirus and is suppressed by IL-5-induced lung eosinophilia. Thorax 2015;70:854-61.
35. Singh BN, Rawat AK, Bhagat RM, Singh BR. Black tea: Phytochemicals, cancer chemoprevention, and clinical studies. Crit Rev Food Sci Nutr 2017;57:1394-410.
36. Ragupathi G, Gardner JR, Livingston PO, Gin DY. Natural and synthetic saponin adjuvant QS-21 for vaccines against cancer. Expert Rev Vaccines 2011;10:463-70.
37. Marty-Roix R, Vladimer GI, Pouliot K, Weng D, Buglione-Corbett R, West K, *et al.* Identification of QS-21 as an inflammasome-activating molecular component of saponin adjuvants. J Biol Chem 2016;291:1123-36.
38. Ikeda Y, Murakami A, Fujimura Y, Tachibana H, Yamada K, Masuda D, *et al.* Aggregated ursolic acid, a natural triterpenoid, induces IL-1beta release from murine peritoneal macrophages: Role of CD36. J Immunol 2007;178:4854-64.
39. Alva-Murillo N, López-Meza JE, Ochoa-Zarzosa A. Nonprofessional phagocytic cell receptors involved in *Staphylococcus aureus* internalization. Biomed Res Int 2014;2014:538546.
40. Silverstein RL, Febbraio M. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. Sci Signal 2009;2:re3.