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Prevotella induces IL-10 production in monocytic cells*

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Abstract

Objective: Clinical remission after immunotherapy for allergic rhinitis is based on an increase in Interleukin-10 (IL-10) production, a regulatory cytokine. We focused on the effect of the oral microbiome on sublingual immunotherapy and investigated the IL-10 production from human monocytic cell line (THP-1 cell) stimulated by one of the commensal gram-negative *Prevotella* species.

Methods: We measured IL-10 production in THP-1 cells stimulated by *P. melaninogenica*, one of the major bacteria in the *Prevotella* genus. Lipopolysaccharides (LPS) from *Escherichia coli* (*E. coli*) were used to confirm the validity of the experiments. Polymyxin B sulfate salt (PMB), an antibiotic that interacts with LPS, was added to counteract the effect of LPS and *P. melaninogenica*.

Results: P. *melaninogenica* induced IL-10 production in THP-1 cells ($P < 0.01$). The IL-10 production in THP-1 cells stimulated with *P. melaninogenica* was substantially increased when combined with PMB ($P < 0.01$), whereas IL-10 induced by *E. coli* LPS was decreased when combined with PMB.

Conclusion: The IL-10 production in THP-1 cells was induced by *P. melaninogenica* and was increased when combined with PMB. Further investigations into the mechanisms of IL-10 production induced by *Prevotella* are needed.

Key words: sublingual immunotherapy, allergic rhinitis, polymyxin B, lipopolysaccharides, immune regulation

Introduction

The mechanism of sublingual immunotherapy (SLIT) is thought to be associated with a post-therapy increase in the production of interleukin 10 (IL-10), a regulatory cytokine [(1)]. Our research has focused on the salivary microbiome because the antigens supplied during SLIT first interact with immune cells on the oral mucosa. In fact, we found in a previous study that expectorated saliva induced greater IL-10 production in THP-1 cells from a human monocytic cell line than saliva collected directly from the parotid duct, which has a less diverse microbiome compared to expectorated saliva [(2)]. Our research also showed that the composition ratio of *Prevotella* in the salivary microbiome is positively correlated with the amount of IL-10 production by saliva-stimulated THP-1 cells and is higher in the patients with clinical remission after SLIT [(3)]. Based on these results, we hypothesize that *Prevotella* induces IL-10 production and contributes to a good response to SLIT.

Methods

THP-1 cells were provided by Dr. Hideo Takahashi from Okayama University. PBMCs were isolated from peripheral blood of two healthy volunteers using Lymphoprep™ (STEMCELL Technologies, Vancouver, BC, Canada) in accordance with the manufacturer's instructions. The RPMI-1640 culture medium was supplemented with 10% fetal calf serum and L-glutamine-penicillin-streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA). LPS from *E. coli* O111:B4 (Sigma-Aldrich) and *Prevotella melaninogenica* ATCC® 25845™ (Microbiologics, St. Cloud, MN, USA) were used for the cell stimulation experiments. A pellet of *P. melaninogenica* was placed in 0.5 mL of sterile water and gently crushed with a sterile swab. The hydrated material was transferred to agar medium (sheep blood agar medium for CDC anaerobes, modified BD BBL™ 252203, Becton Dickinson Company, Franklin Lakes, NJ, USA) with the same swab, and immediately placed into a pouch bag with an O_2 absorber-cum-CO_2 genera-

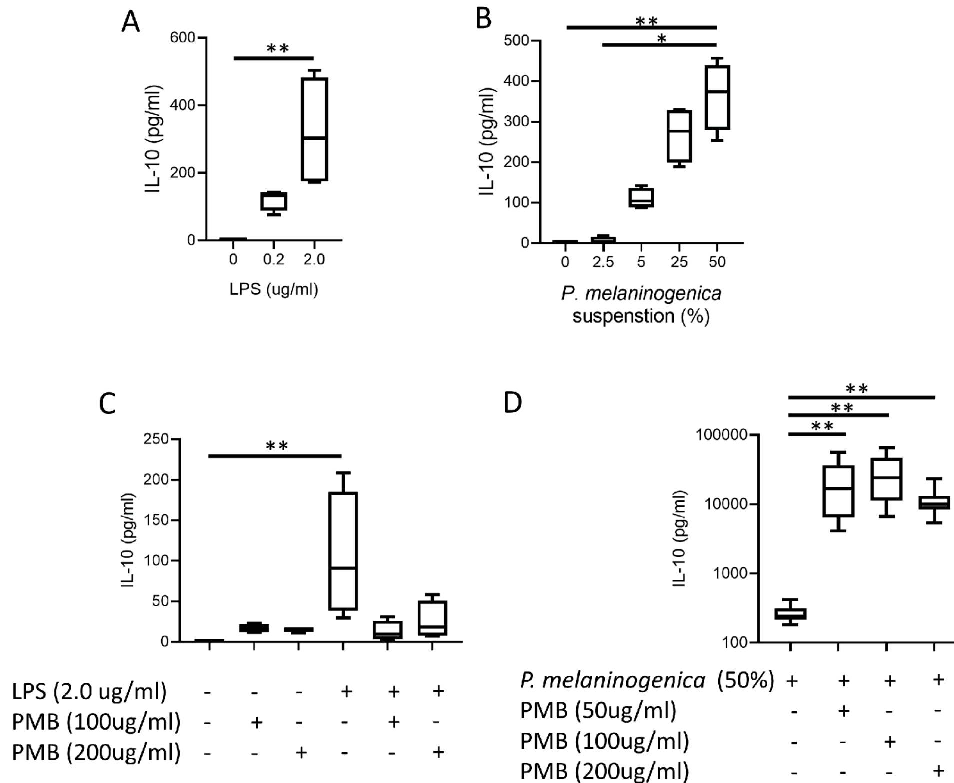

Figure 1: IL-10 production from THP-1 cells. THP-1 cells (0.8 x 10^6/ml) were incubated with 0, 0.2 and 2.0 μg/ml LPS (A), 0, 2.5, 5, 25 and 50% *P. melaninogenica* suspensions (B), 0 and 2.0 μg/mL LPS in combination with 0, 100, and 200 μg/mL PMB (C) and 50% *P. melaninogenica* suspensions in combination with 0, 50, 100, and 200 μg/mL PMB (D). *<0.05 and **<0.01 in Kruskal-Wallis test followed by the Dunn test.

tor (Anaeropack® Kenki A-17, AXEL, Japan). A *P. melaninogenica* suspension from fresh plates was prepared in PBS to an optical density of 0.06 at 540 nm. THP-1 cells (0.8 x 10^6/mL) and PBMCs (0.8 x 10^6/mL) in culture medium were incubated with 0.2 μg/mL and 2.0 μg/mL LPS, and 2.5%, 5%, 25%, and 50% *P. melaninogenica* suspensions at 37°C in 5% CO_2/air mixture for 24 h. Just before the 24-h incubation, the effects of LPS and *P. melaninogenica* were counteracted with addition of PMB (Sigma-Aldrich, St. Louis, MO, USA). The supernatants were subsequently collected and stored at -80°C until they were assayed for determination of IL-10 levels. IL-10 levels were detected using Opt EIA sets (BD Biosciences, Franklin Lakes, NJ, USA) in accordance with the manufacturer's instructions. For multiple comparisons, we used the Kruskal-Wallis test followed by the Dunn test. Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). P values less than 0.05 (two-tailed) were considered statistically significant.

The ethics committee of International University of Health and Welfare Narita Hospital approved this study (22-Nr-062).

Results

In the present study, we measured IL-10 production in THP-1 cells stimulated by *P. melaninogenica*, one of the major bacteria in the *Prevotella* genus. The composition ratio of *P. melaninogenica* (5.68% of all salivary microbiome) was greater than those of other *Prevotella* species (up to 0.43% of all salivary microbiome) in our past study, and this was why we selected *P. melaninogenica* as a stimulant in the present study. Lipopolysaccharides (LPS) from *Escherichia coli* (*E. coli*) were used to confirm the validity of the experiments because LPS are surmised to be the main endotoxin of *Prevotella*, a gram-negative microbe. We also added polymyxin B sulfate salt (PMB), an antibiotic that interacts with LPS, to counteract the effect of *P. melaninogenica*. In addition, peripheral blood mononuclear cells (PBMCs) were stimulated with *P. melaninogenica* to ascertain its effect on IL-10 production. THP-1 cells stimulated with LPS and *P. melaninogenica* suspensions produced IL-10 in a concentration-dependent manner (Figure 1A, B). Significant differences were seen between no stimulation and stimulation with 2.0 μg/mL LPS ($P < 0.01$), and between either no stimulation or stimulation with 2.5% *P. melaninogenica* suspension and stimulation with 50% *P. melaninogenica* suspension ($P < 0.01$ and $P = 0.02$, respectively). PBMCs stimulated with LPS and *P. melaninogenica* suspensions also exhibited increased IL-10 production (Figure 2A, B). Significant differences were seen between no stimulation and stimulation with 0.2 μg/mL LPS ($P = 0.02$), and between no stimulation and stimulation with 25%

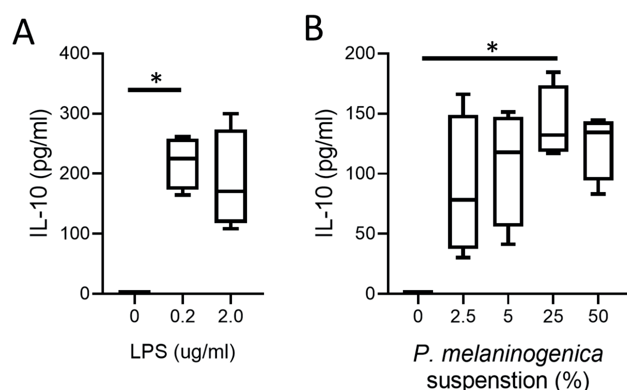

Figure 2. IL-10 production in PBMCs stimulated with LPS and *P. melaninogenica* suspensions. PBMCs (0.8 x 10^6/mL) were incubated with 0, 0.2, and 2.0 μg/mL LPS (A) and 0, 2.5, 5, 25, and 50% *P. melaninogenica* suspensions (B). *<0.05; Kruskal-Wallis test followed by the Dunn test.

P. melaninogenica suspension (P = 0.03). The production of IL-10 by THP-1 cells stimulated with LPS was reduced in the presence of PMB (Figure 1C). In contrast, the production of IL-10 by THP-1 cells stimulated with *P. melaninogenica* suspension was significantly increased in the presence of PMB (Figure 1D, P < 0.01).

Discussion

The oral and gut microbiomes have been reported to be associated with various autoimmune diseases, including rheumatoid arthritis and Sjögren's syndrome [(4)]. *Prevotella* are considered commensal bacteria due their presence in the microbiome of healthy individuals and rare involvement in infections [(5)]. Co-culture experiments found that *P. melaninogenica* reduced *Haemophilus*-induced IL-12 production [(6)]. These studies suggest that *P. melaninogenica* has anti-inflammatory functions. Surprisingly, increased production of IL-10 was observed following *P. melaninogenica* stimulation in combination with PMB. This phenomenon was contrary to what was seen after stimulation with LPS from *E. coli* and PMB. Based on this result, we hypothesize that the addition of *P. melaninogenica* in combination with PMB causes THP-1 cells to differentiate into M2b macrophages, which secrete substantial amounts of IL-10 and low levels of IL-12 [(7)]. To confirm this hypothesis, we are planning to use flow cytometry assays to detect M2b macrophages in the cultured cells. The different results between *E. coli* LPS and *P. melaninogenica* might be caused by the structural differences in LPS recognized by PMB [(8)]. Other researchers have found that the *P. melaninogenica* signal that activates the toll-like receptor (TLR) 2 on dendritic cells induces the production of IL-10 [(9)]. Their results indicate that TLR2 signaling induced by *P. melaninogenica* could be associated with immune tolerance.

Our study has some limitations. Since IL-10 production was compared between the condition of *P. melaninogenica* co-culture with that of *E. coli* derived LPS stimulation, it should be compared between *P. melaninogenica* co-culture with *E. coli* co-culture as well as the stimulation between LPS from *E. coli* and from *P. melaninogenica*. Future studies comparing IL-10 production in these conditions are expected. In addition, THP-1 cell is a monocytic cell line and it is uncertain that it shows the same effects as macrophages. It is reported that THP-1 cells differentiated in phorbol 12-myristate 13-acetate (PMA) show antimicrobial effect similar to primary human macrophages [(10)]. We confirmed that THP-1 cells differentiated in PMA induced IL-10 production after stimulation of saliva. We are planning to use this THP-1 macrophage model for further study of *Prevotella*-induced IL-10 production.

Conclusions

P. melaninogenica induced IL-10 production in THP-1 cells. Together with our previous findings, the present result may suggest the future application of *P. melanigogenica* as a microbial adjuvant for SLIT. The IL-10 production in THP-1 cells stimulated with *P. melaninogenica* was increased when combined with PMB, whereas that induced by *E. coli* LPS was decreased when combined with PMB. Further investigations into the mechanisms of IL-10 production induced by *Prevotella* are needed.

List of abbreviations

SLIT: sublingual immunotherapy; IL-10: interleukin 10; LPS: lipopolysaccharides; E. coli: *Escherichia coli*; PMB: polymyxin B sulfate salt; TLR: toll-like receptor; PMA: phorbol 12-myristate 13-acetate

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Authorship contribution

AO designed the study, performed the experiments and wrote the manuscript. KK and MO contributed to analyze the data. T.H, SM, YN, SK and MA provided valuable comments on the discussion section. All authors read, approved, and signed the final manuscript.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interest

The authors have no conflict of interest to declare.

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