Difference in microbiome among house dust mite species causes difference in induction of allergic responses in vitro

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Difference in microbiome among house dust mite species causes difference in induction of allergic responses in vitro

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House dust mite is one of the major allergens causing allergic diseases. Although allergens of house dust mites have been studied extensively, research on the differences of microbiome among house dust mite species has been studied insufficiently. The purpose of this study is to compare the microbiota composition according to the species of mites and to determine whether the microbiome causes the difference in inducing allergic responses. *Dermatophagoides farinae*, *D. pteronyssinus*, and *Tyrophagus putrescentiae*, which are known to cause allergic diseases, were grown for 18 weeks in autoclaved medium. Next-generation sequencing (NGS) that complemented Sanger sequencing was used to analyze the differences of microbiome according to the mite species. Human bronchial epithelial cell line were equally treated with protein extracts from three species of mites to compare cytokine expression levels. Analysis of the microbiome of three species of mites revealed that the different types of microbiota composition according to the species. Gram negative bacteria accounted for the most of the microbiota composition in the *D. fariane* and *T. putrescentiae*. The microbiome of *D. pteronyssinus* cultivated on the autoclaved medium was different from the other two species and barely existed. The amount of endotoxin of the three species corresponded to the pattern of the microbiome. However, the expression pattern of pro-inflammatory cytokines was similar when the same amount of protein extract of each mite species was treated equally to human bronchial epithelial cell line. In summary, the distribution of microbiota of house dust mites cultured on the same conditions in autoclaved media was different according to species and the concentration of endotoxin was corresponded to the microbiota composition. However, the expression of pro-inflammatory cytokines in human bronchial epithelial cell was similar to each other.

Key words : house dust mite, allergy, NGS, microbiome, pro-inflammatory cytokine

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I. INTRODUCTION

Allergies are caused by hypersensitivity of host immune system to typically harmless substances in the environment.[1] House dust mite (HDM) is well-known allergen source to cause allergic diseases, such as asthma, atopic dermatitis, and rhinitis.[2] The quantity of allergens differ according to the mite species and breeding environment.[3] The response to skin prick tests varies according to species, and the degree of sensitization to allergies also varies.[4] The main sources of allergens are fecal pellets of mites.[2] Furthermore, adjuvants of allergic responses, such as lipopolysaccharide (LPS) or bacterial DNA, are also enriched in the feces of mites.[3]

LPS is a critical promoter of various immunological outcomes.[3] Endotoxin affects the allergic response in different ways, depending on the concentration.[5] Indeed, low concentrations of endotoxin promote Th2 responses or allergic disease, whereas high concentrations of endotoxin relieve airway inflammation.[5] The low level of LPS activates mast cells and NK-T cells to produce inflammatory cytokines like TNF-α. Dendritic cells (DCs) uptake allergens and TNF-α facilitates DC maturation. Activated DCs migrate into draining lymph node and activate naïve T cells to differentiate into Th2 cells.[6]

There are two groups of major allergens in house dust mites. Group 1 allergen, Der f 1 and Der p 1, is cysteine protease to destroy the tight junction of epithelial cells and located in the gut of mites.[2] Group 2 allergen, Der f 2 and Der p 2, is MD-2 like lipid binding protein which is similar to the component

of TLR4 which binds to LPS and located also in the gut of mites.[2] Group 2 allergen is lipid-carrying protein to induce the innate immune response and type 2 immune response by binding to TLR2 of bronchial epithelial cell and TLR4 of DCs.[2] Allergens in the same group have same IgE-binding epitopes for cross-reactivity.[2]

Group 1 allergen and endotoxin from house dust mites activate the expression of pro-inflammatory cytokines, such as IL-6 and IL-8, via stimulating protease-activated receptor 2 (PAR-2) and toll-like receptor 4 (TLR4).[7] There are studies that therapy targeting IL-6 is valid in patients with asthma with mixed neutrophilic/eosinophilic bronchitis. IL-8 is a major mediator of neutrophilia in acute severe asthma.[8-9] House dust mite induces a type 2 immune response and the elevated expression level of IL-6 and IL-8 in airway- derived epithelial cell.[10-11] Calcium level in the airway epithelial cell was elevated by HDM extract, and then activates NFAT/calcineurin signal pathway to induce the transcriptional production of pro-inflammatory cytokine, IL-6 and IL-8.[12]

There are few studies about differences of the microbiome and the ability to induce allergic responses according to mite species. Accordingly, the microbiome of three species of HDMs, i.e., *Dermatophagoides farinae*, *D. pteronyssinus*, and *Tyrophagus putrescentiae*, was analyzed and the expressed pro-inflammatory cytokine in human bronchial epithelial cell treated by each species was measured to see differences in inducing allergic responses.

II. MATERIALS AND METHODS

1. Mite cultivation

Inactive dry yeast (Ottogi, Seoul, Korea) was mixed with fish food at a ratio of 1:1, and autoclaved twice. *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, storage mites most commonly found in Korean houses, were inoculated into autoclaved medium in each of five 75T cell culture flasks. They were cultivated at 25℃ for 18 weeks, resulting in three generations and each generation were grown for 6 weeks.

2. Isolation of DNA and preparation of protein extracts

Autoclaved saturated salt water was used to harvest mites separated from culture medium and debris. DNA was isolated from mites using a NucleoSpin DNA Insect Kit (MACHEREY-NAGEL, Düren, Germany). The extract was obtained by removing foreign substances including bacteria with a sterile Millex-GP syringe filter with a 0.22-μm pore-sized hydrophilic polyethersulfone membrane (EMD Millipore, Darmstadt, Germany).

3. Amplification of 16S rRNA by polymerase chain reaction (PCR)

DNA, forward primer, reverse primer, dNTP mixture, 10X Ex Taq buffer, TaKaRa Ex Taq polymerase (TaKaRa, Kusatsu, Japan) and distilled water were mixed. DNA was amplified by using Veriti 96 well thermal cycler (Applied Biosystems, CA, USA) as followed conditions: for initial denaturing; 95℃ 5 minutes, for denaturing and extension; 95℃ 30 seconds, 55℃ 30 seconds, 72℃ 45 seconds by 34 cycles, for final extension; 72℃ 5 minutes. The V3–V4 region of 16S rRNA was amplified by PCR using a

pair of primers: forward primer, 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse primer, 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′ (Illumina MiSeq V3 cartridge (600 cycles); Illumina, CA, USA).

4. Next-generation sequencing (NGS) and bioinformatics

A limited-cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. Libraries were normalized, pooled, and sequenced on the MiSeq platform (Illumina MiSeq V3 cartridge (600 cycles); Illumina, CA, USA) in accordance with the manufacturer′s instructions. Sequencing data obtained from the Illumina MiSeq system were processed using Mothur v1.39.5. Contigs between paired-end reads were assembled and aligned to EzBioCloud′s 16S database from ChunLab (ChunLab Inc., Seoul, Korea). Chimeras were removed using UCHIME, and remaining sequences were classified using the EzBioCloud reference.[13] Heatmap analysis and principal components analysis (PCA) were performed using R 2.15.1 with the Vegan package 2.0-10 v2.15.3.[14]

5. Protein concentration measurement by Bradford assay

The concentration of protein in the extract of mites were measured. One % bovine serum albumin (BSA) diluted in phosphate-buffered saline (PBS) was as standard dyed by protein assay dye reagent concentrate (Bio-Rad, CA, USA).

6. LPS measurement

The mite extracts were diluted 1:2,000 in Limulus Amebocyte Lysate (LAL) water, and the concentration of LPS was measured using an LAL QCL-1000 Kit (LONZA, Basel, Switzerland).

7. Real-time PCR

The genes for house dust mites were amplified using the forward primer 5′-ACCCGTGAACATGCTTTGCT-3′ and reverse primer 5′-CACCATTCTCTCAAGCTCGT-3′, and the genes for bacteria were amplified using the forward primer (BACT1369) 5′-CGGTGAATACGTTCYCGG-3′ and reverse primer (PROK1492R) 5′-GGWTACCTTGTTACGACTT-3′ from XenoTech with AMPIGENE qPCR Mixes (ENZO, NY, USA) for the all bacterial 16S rRNA.

8. Enzyme-linked immunosorbent assay (ELISA)

Der f 1, 2 and Der p 1, 2, the major allergens of *D. farinae* and *D. pteronyssinus* respectively, was quantified using the Der f 1, 2 and Der p 1, 2 ELISA kit (Indoor Biotechnologies, VA, UK) with a 1:1,000 diluted extract of *D. farinae* and *D. pteronyssinus* cultivated in autoclaved media.

9. Cytokine array

Protein extracts (10 μg/mL) of each species of mites, cultured in normal medium, were treated into the human bronchial epithelial cell line BEAS-2B (American Type Culture Collection, VA, USA) cultured in a six-well cell culture plate (SPL, Pocheon, Korea). To identify the types of cytokines expressed,

cytokine assay was performed with supernatants of cell culture medium by using Human Cytokine Array Kit (R&D Systems, MN, USA).

10. Cytokine measurement

The human bronchial epithelial cell line BEAS-2B (American Type Culture Collection, VA, USA) was cultured in a six-well cell culture plate (SPL, Pocheon, Korea) in Dulbecco's modified Eagle medium/F12 (1:1) with 10% fetal bovine serum and 1% penicillin (Thermo Fisher Scientific, MA, USA) for 24 h at 37°C in a 5% CO2 incubator. Each extracts (100 μg/mL) were applied to each well, and the supernatant was collected after 24 h of culture. The concentrations of cytokines secreted from BEAS-2B cells were measured from the supernatants with the DuoSet ELISA human IL-6 and IL-8 kit (R&D Systems, MN, USA).

11. Statistical analysis

Results are presented as the mean ± standard deviation, and statistical significance was determined using Student′s t-test or analysis of variance, with Bonferroni correction as a post-hoc analysis. Differences with p values of 0.05 or less were considered statistically significant.

III. RESULTS

1. The microbiome of mites

To compare the microbiota compositions among three strains of mites, *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, EzBioCloud′s 16S database with MOTHUR was used for analyzing the microbiota composition at species level (Fig. 1). As a result, *D. farinae* showed 500-times higher read count than *D. pteronyssinus*, and the microbiota composition of *D. farinae* was also very different from *D. pteronyssinus*. HQ806746 belonging to the genus *Bartonella*, gram-negative bacteria, was accounted for 97.9% of *D. farinae* and *Enterococcus faecalis* were accounted for 1.6%. *Klebsiella pneumonia*, accounted for 64.6% of *D. pteronyssinus*, was presumably from culture medium origin or contamination in the experimental process (Table 1). *T. putrescentiae* had moderate amount of bacteria and the gram-negative bacteria, *Bartonella* and *Sphingobacteriaceae*, were mainly detected in 6 to 4 ratio. JX001274 belonging to the genus *Bartonella* was accounted for 63.2% and JN236497 belonging to the *Sphingobacteriia* class was accounted for 35.5% of *T. putrescentiae*. Especially, the concentration of extracted DNA and the read count of raw data from MiSeq were very low and almost free of bacteria in *D. pteronyssinus*. (The average of DNA concentration(ng/㎕): *D. farinae*; 4.625, *D. pteronyssinus*; 0.073, *T. putrescentiae*; 1.206.[15])

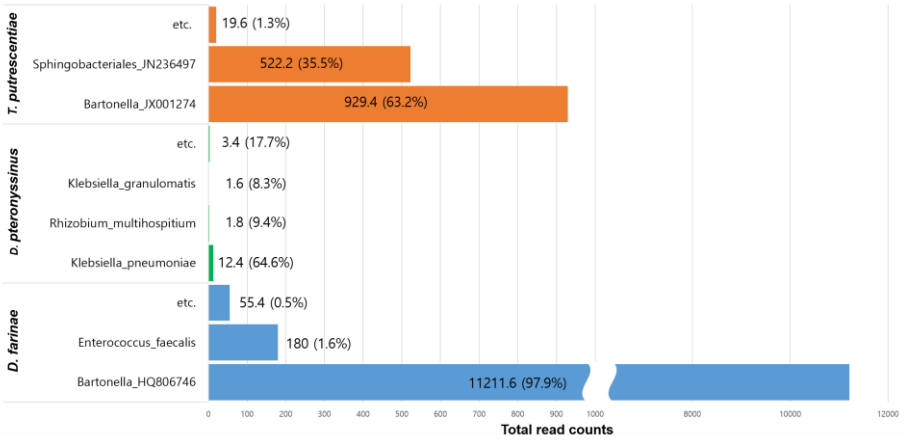

Figure 1. Bar chart based on species-level classifications. Read counts (percentages of bacteria) are indicated next to each bars. Organisms are only indicated when they are accounted for more than 1% of the total in *D. farinae* and *T. putrescentiae*. The microbiome of each species was calculated as the average of five different batches.

Table 1. Read counts at the species and genus levels

(A) Species level

Taxon	D.f[1]	D.p	T.p
Actinomyces_odontolyticus	0.6	0.2	0
Corynebacterium_glutamicum	1.4	0	0.2
Microbacterium_kyungheense	0.2	0	0
Asaccharobacter_celatus	0.6	0	0
Bacteroides_vulgatus	5	0	0
Pedobacter_panaciterrae	0.4	0	0
Anaerobacillus_macyae	1	0	0.4
Bacillus_bingmayongensis	0.8	0	0
Bacillus_pseudofirmus	1.2	0	0
Enterococcus_faecalis	180	0	0
Lactobacillus_apodemi	7.2	0	1.4
Streptococcus_mutans	0.8	0	0
*Lachnospiraceae*_EU509291[2]	0.4	0	0
*Acetatifactor*_EU006478	0.4	0	0
*Clostridium*_HM124173	0.6	0	0
*Oscillibacter*_FJ678358	0.2	0	0
*Bartonella*_HQ806746	11211.6	0	0
*Bradyrhizobium*_APJD	0.2	0	0
Devosia_chinhatensis	0.2	0	0
Phyllobacterium_brassicacearum	0.2	0	0
Rhizobium_laguerreae	1.4	0.2	0.4
Rhizobium_multihospitium	0.6	1.8	0.4
Rhizobium_nepotum	0.4	0	0
Rhizobium_tropici	0.8	0	0
Sphingomonas_leidyi	0.6	0	0
Delftia_acidovorans	2.6	0.4	0.6
Variovorax_gossypii	0.4	0	0

Enterobacter_cloacae	6.6	0.6	1.6
Escherichia_coli	0.4	0	0
Klebsiella_granulomatis	2.6	1.6	0.4
Klebsiella_pneumoniae	16.4	12.4	12.2
Leclercia_adecarboxylata	1.2	0.8	0.2
Propionibacterium_acnes	0	0.2	0
*Anaerobacillus*_AY642552	0	0.2	0.2
Mesorhizobium _AVBN	0	0.4	0
Burkholderia_contaminans	0	0.4	0
*Sphingobacteriales*_JN236497	0	0	522.2
Bacillus_nanhaiisediminis	0	0	0.2
Lysinibacillus_sphaericus	0	0	0.2
Lactobacillus_salivarius	0	0	0.4
*Ruminococcaceae*_AB606382	0	0	0.2
*Bartonella*_JX001274	0	0	929.4
Delftia_lacustris	0	0	0.6

(B) Genus level

Taxon	D.f	D.p	T.p
Actinomyces	0.6	0.2	0.2
Corynebacterium	1.4	0	0
Microbacterium	0.2	0	0
Adlercreutzia	0.6	0	0
Bacteroides	5	0	0
Pedobacter	0.4	0	0
Anaerobacillus	1	0.2	0.8
Bacillus	0.8	0	0
Bacillus	1.2	0	0
Enterococcus	180	0	0
Lactobacillus	7.2	0	1.8
Streptococcus	0.8	0	0
AB626912	0.4	0	0

Acetatifactor	0.4	0	0
Clostridium	0.6	0	0
Oscillibacter	0.2	0	0
Bartonella	11211.6	0	929.4
Bradyrhizobium	0.2	0	0
Devosia	0.2	0	0
Phyllobacterium	0.2	0	0
Rhizobium	3.2	2	0.8
Sphingomonas	0.6	0	0
Delftia	2.6	0.4	1.2
Variovorax	0.4	0	0
Enterobacter	6.6	0.6	1.6
Escherichia	0.4	0	0
Klebsiella	19	14	12.6
Leclercia	1.2	0.8	0.2
Burkholderia	0	0.4	0
Mesorhizobium	0	0.4	0
Propionibacterium	0	0.2	0
JN236497	0	0	522.2
Lysinibacillus	0	0	0.2
JN713389	0	0	0.2

Results represent the average total reads for the three species of mites as determined by next-generation sequencing of 16S rRNA amplicons.

[1]D.f, D.p, and T.p means *D. farinae*, *D. pteronyssinus* and *T. putrescentiae.*

[2]The names are species or genus from EzBioCloud database.

Indeed, the read count of *Klebsiella pneumoniae*, which accounts for a significant proportion of the microbiota composition of *D. pteronyssinus*, was similarly distributed in each species of mites. To confirm whether the amount of bacteria in *D. pteronyssinus* was actually lower than other species, real-time PCR was performed with *D. farinae* and *D. pteronyssinus* relative to the body of *D. fariane* (Fig. 2). Despite using the same amount of mites was used in the experiment, the amount of bacteria in *D. pteronyssinus* was considerably smaller than that in *D. farinae*.[15]

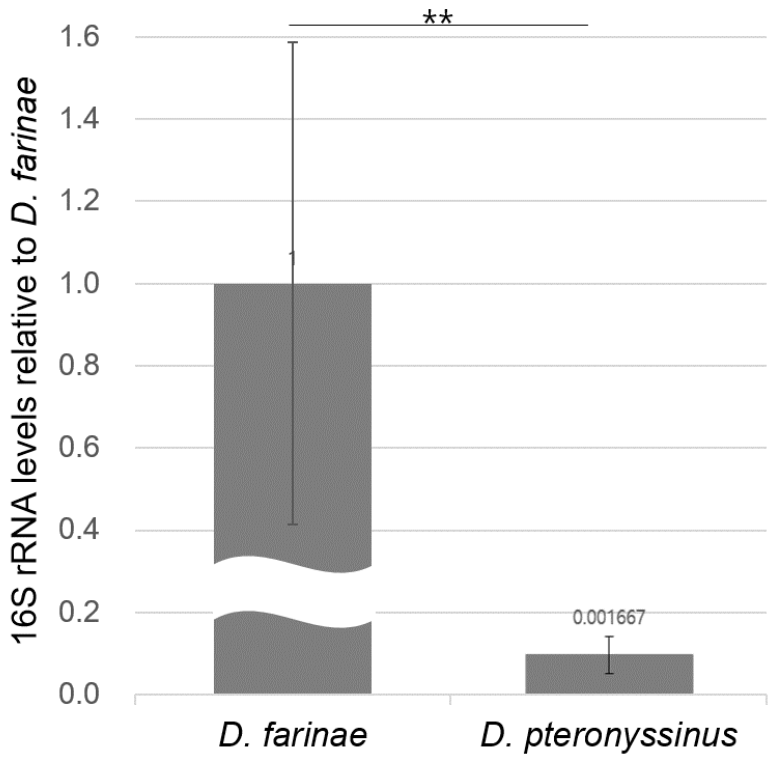

Figure 2. Relative 16S rRNA levels in *D. farinae* and *D. pteronyssinus*. Each bar shows the average rRNA level from bacteria in five different batches (n = 5 per group). **p < 0.01.

To compare microbiota composition, heatmap and PCA to cluster existing bacterial profile considering phylogenetic distance was analyzed (Fig. 3-4). As a result, the microbiomes in the same species were similar to each other and the microbiome between mite species showed significantly different patterns correlated to those microbiota compositions.[15]

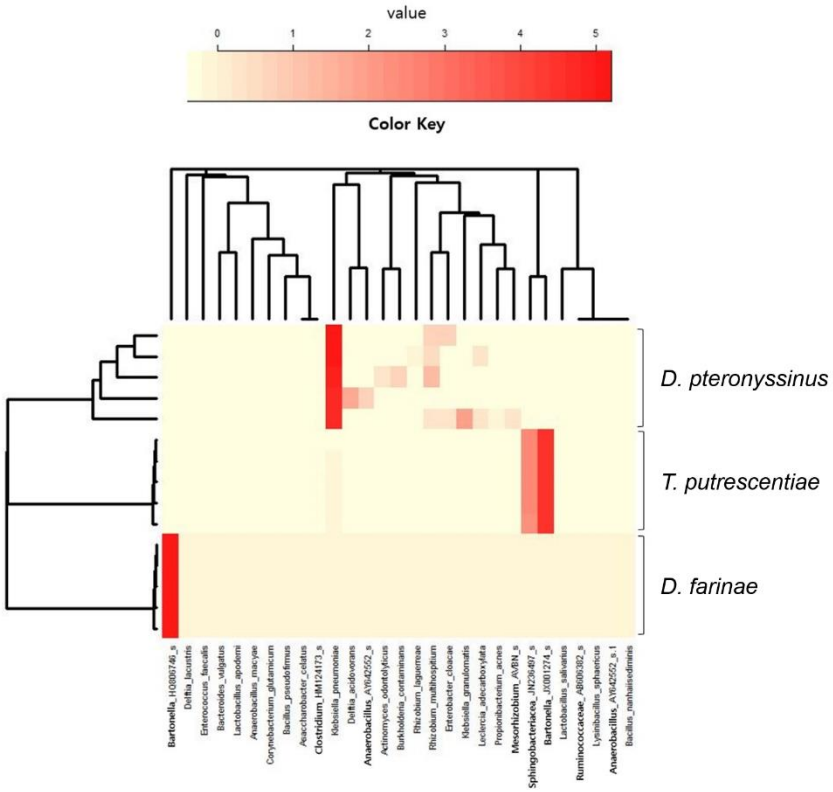

Figure 3. Heatmap and dendrogram for mite microbiomes. The rows in the dendrogram indicate hierarchical clustering of the three species of mites, *D. farinae* (Df), *D. pteronyssinus* (Dp), and *T. putrescentiae* (Tp), and the columns indicate clustering of species that occurred more often together.

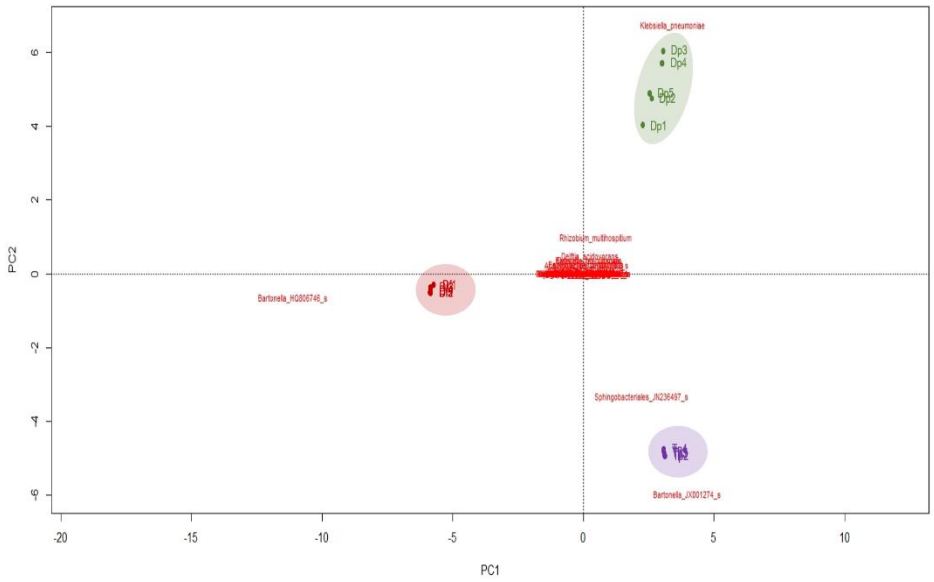

Figure 4. Analysis of the variance of microbial communities in three species of mites using principal components analysis (PCA). PCA score plots for *D. farinae* (red), *D. pteronyssinus* (green), and *T. putrescentiae* (purple) are shown. Each point represents one batch (n = 5).

2. The concentration of endotoxin and allergens

The concentration of endotoxin in the same amount of mite-derived extracts (1 mg/ml) was measured to examine whether the distinct profile of microbiota composition affects the level of endotoxin, which may act as an adjuvant for inducing allergy (Fig. 5). Consistent to previous results, there was a high concentration of endotoxin in *D. farinae* and *T. putrescentiae*, whose microbiota composition were highly composed of gram-negative strains such as HQ806746, JX001274 and JN236497. In contrast, *D. pteronyssinus* with few bacteria showed corresponding result with microbiota composition analysis with a low concentration of endotoxin.[15]

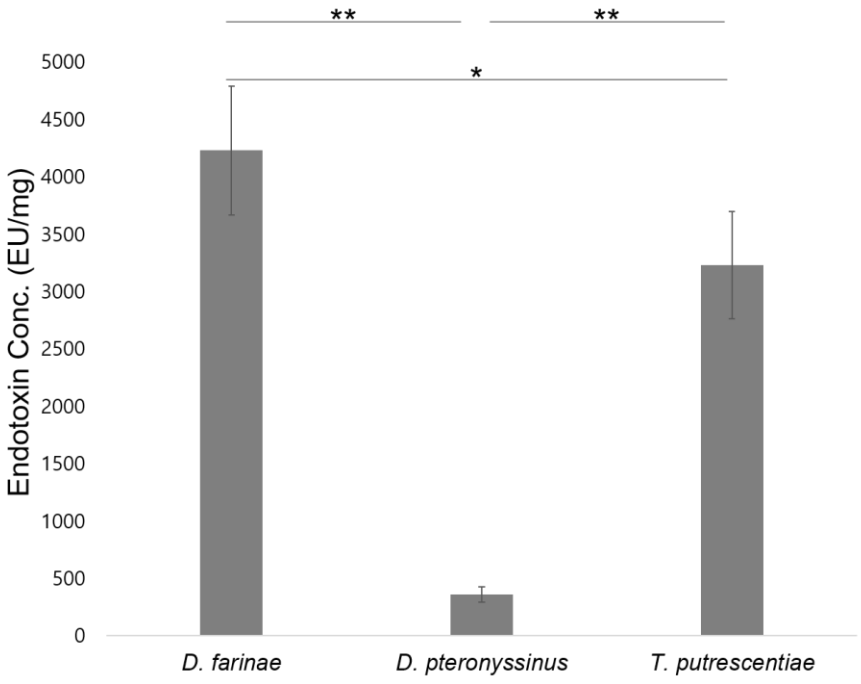

Figure 5. Concentration of endotoxin in 1 mg/mL of mite body extract (EU/mL). Each bar represents the average of five different batches of each species of mites. *$p < 0.05$, **$p < 0.01$.

The concentration of major allergens of *D. farinae* and *D. pteronyssinus* in each extracts (1 mg/ml) were measured (Fig. 6). The concentration of major allergens in *D. farinae* were lower than that in *D. pteronyssinus*. In each species, group 1 allergens were less than group 2 allergens.

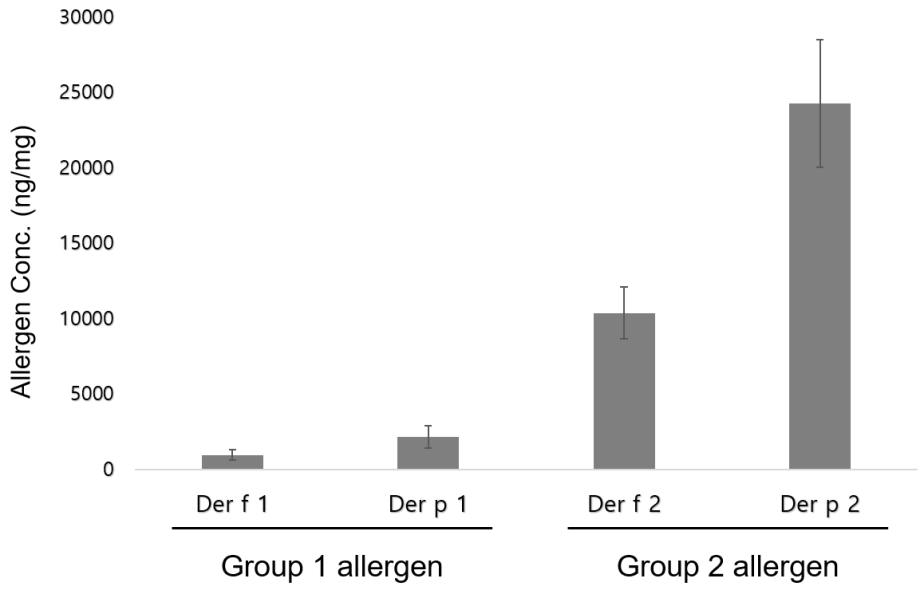

Figure 6. The concentrations of major allergens in *D. farinae* and *D. pteronyssinus*. Concentrations of Der f 1, Der f 2, Der p 1 and Der p 2 in 1 mg/mL of *D. farinae* and *D. pteronyssinus* extracts (ng/mL).

3. The level of expressed pro-inflammatory cytokines

To examine the differences in the concentrations of endotoxin and allergens among the mite species affect the extent of allergic responses, extracts derived from each species of mites were treated into the human bronchial epithelial cell line (BEAS-2B cell line). I measured the expression level of pro-inflammatory cytokines, IL-6 and IL-8, which were different in the amount of expression level between HDM extract treatment and PBS treatment in BEAS-2B cell (Fig. 7-8). When I treated the same concentration of extracts into BEAS-2B cell, the expressed cytokine levels were similar in each species of mites.

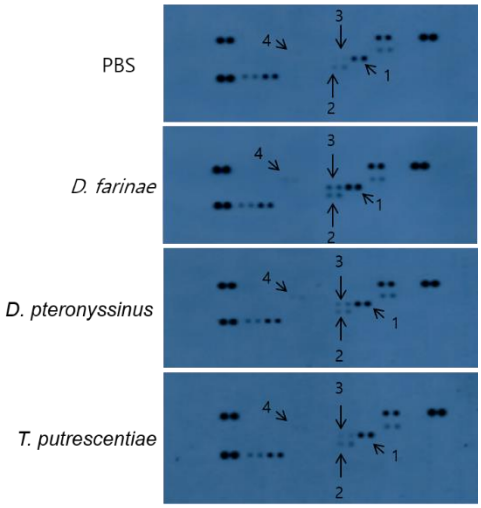

Figure 7. The screening of expressed cytokines after treatment of extracts of mites into the human bronchial epithelial cell line (BEAS-2B cell). Mites were cultured in normal medium, not autoclaved. The amount of extract treated in BEAS-2B cell was 100 μg. 1,2,3 and 4 means IL-8, IL-18, IL-6 and G-CSF, respectively.

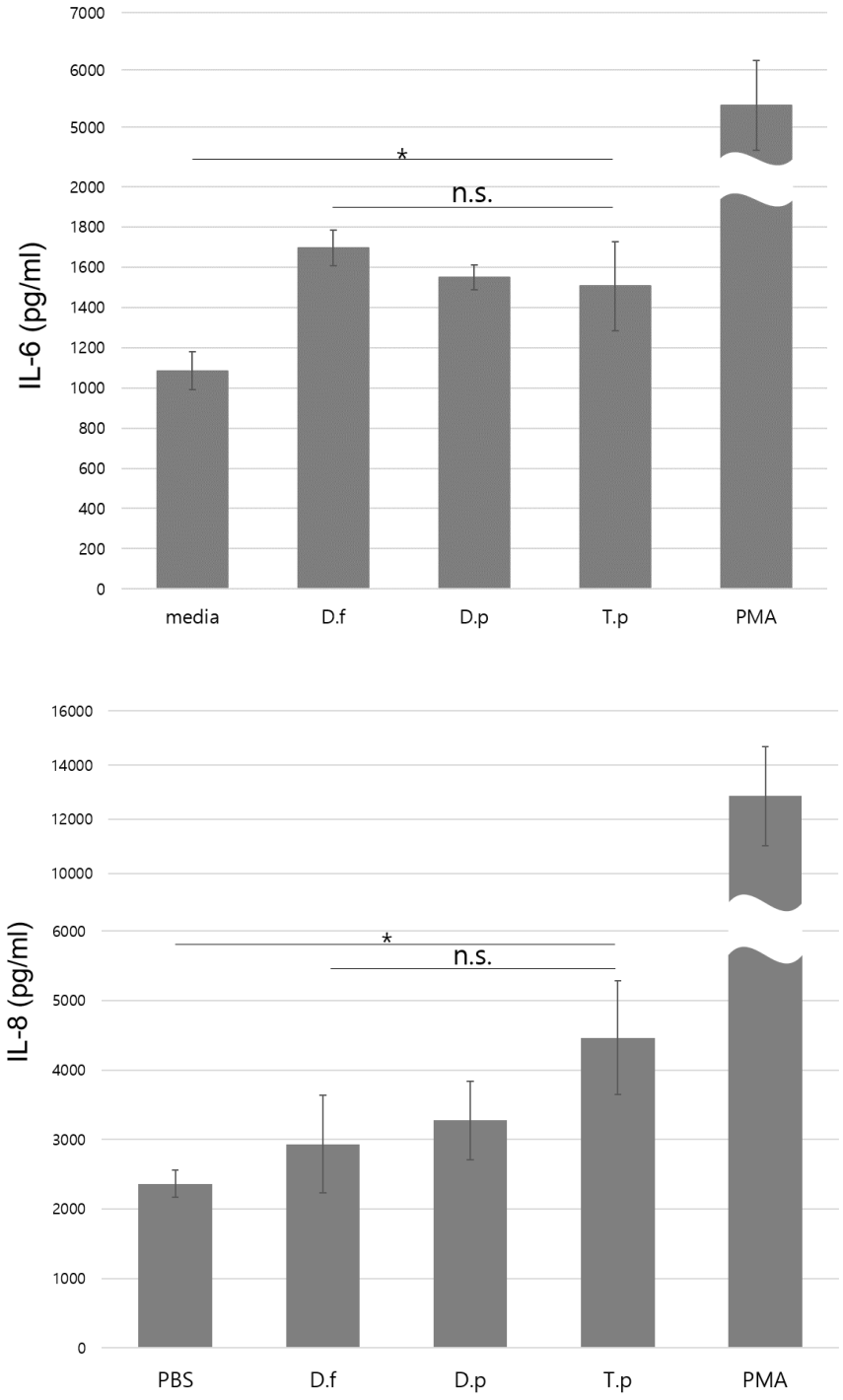

Figure 8. The level of expressed pro-inflammatory cytokines from human bronchial epithelial cell line (BEAS-2B cell). BEAS-2B cell were treated by extracts (50 μg/ml) of three species of mites. PBS and PMA are negative and positive control, respectively. Each bar shows the average of different batches (n = 5 per group). *p < 0.05, **p < 0.01.

IV. DISCUSSION

This study examined whether different species of mites have distinct microbiomes and whether the differences affect the extent to induce allergic reactions. Results showed that three species of mites, i.e., *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, exhibited different patterns of microbiota composition, even though they were grown under the same conditions for a long period over 15 years. *D. pteronyssinus* grown on an autoclaved medium harbored few bacteria compared with the other two species. The concentration of endotoxin derived from the extract of mites was correlated with the quantity of the microbiota composition.

In this study, *K. pneumoniae* was detected in all three species of mites; it is unclear whether this bacterium was present in the culture medium or derived from experimental contamination. Additionally, differences in bacterial amounts in *D. farinae* and *D. pteronyssinus* were not caused by differences in the amounts of mites. Obviously *D. farinae* and *D. pteronyssinus* are commonly known morphologically and phylogenetically similar, however, their own microbiota composition was quite different to each other shown by this study.

Previous studies have shown that allergens and the microbiome influence the allergic reaction.[16] Additionally, *Lactobacillus* isolated from house dust and *Staphylococcus sciuri* isolated from farm dust mitigate the symptoms of asthma.[17-18] Some studies have shown that the distributions of microbiota composition vary according to the mite species. The 16S rRNA genes of *Acarus siro*, *D. farinae*, *Lepidoglyphus* destructor, and *T. putrescentiae* were sequenced and microbiota compositions were analyzed; each species showed a different bacterial community pattern.[19]

There were some studies about the microbiome of mites. In several studies, *Bartonella* (or *Bartonella*-like bacteria) was composed of the majority in the microbiome of *D. farinae* and *T. putrescentiae*. [14,20-22] *Bartonella* was also detected in *D. pteronyssinus* in the other study based on the 16s rRNA cloning.[20] *T. putrescentiae* originated from different countries was composed of *Solitalea*-like bacteria belonging to *Sphingobacteriaceae*.[22]

However, in these previous works, the analysis was not comprehensive. For this study, 16S rRNA gene of mites were sequenced by using MiSeq, a machine for next-generation sequencing, and assessed the microbiome using EZBioCloud database and Mothur pipeline, more effective tools for the investigation of the microbiome.

Importantly, cultivated *D. pteronyssinus* did not harbor many bacteria, suggesting potential applications as a therapeutic agent. This organism could be used to study the relationship between allergy induced by mite-derived allergens and the microbiota composition. Bacterial strains related to inducing allergy could be identified by monocolonization of *D. pteronyssinus* with a candidate strain.

While concentrations of endotoxin and allergens measured in same amount of protein extract (1mg/ml) were different to each species, the level of expressed pro-inflammatory cytokines from BEAS-2B cell were similar. Endotoxin and allergen might stimulate different signaling pathway to induce immune response by binding to TLR4 and PAR-2, respectively. LPS or other factors in PBS could induce immune reaction, and the expressed cytokine levels, treated by extracts of mites, were not significantly higher than negative control.

Because it was not able to measure the allergen of *T. putrescentiae*, the concentration of major allergen, which could affect to the difference in inducing allergic reaction, among three species of mites was not comparable. While only one kind of cell line was used, it would be better to use different cell lines or co-culture with DCs or $CD4^+$ T cell to identify the changes of various cytokines. For further study, to make TLR4 knock-out (KO) / PAR-2 KO cell line would be a good method to study the own effects of endotoxin and allergen.

V. CONCLUSION

D. farinae, *D. pteronyssinus* and *T. putrescentiae* accommodate distinct microbiota composition. The level of endotoxin in each species is correlated to the microbiome. Endotoxin and major allergen in mites affect complementarily to the extents of inducing allergic responses on human bronchial epithelial cells in vitro.

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APPENDICES

The concentrations of protein extracts derived from three species of mites.

D. farinae	Conc.(mg/ml)
D.f1	4.106701
D.f2	4.089966
D.f3	4.307517
D.f4	4.42466
D.f5	4.106701

D. pteronyssinus	Conc.(mg/ml)
D.p1	3.002209
D.p2	2.449963
D.p3	4.441395
D.p4	2.734453
D.p5	3.654863

T. putrescentiae	Conc.(mg/ml)
T.p1	7.922217
T.p2	2.232412
T.p3	6.248745
T.p4	1.178124
T.p5	6.683848

ABSTRACT(IN KOREAN)

진드기 종 간 세균총 차이에 의한 시험관 내에서의 알레르기성 질환 유발 차이

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이진영

집먼지진드기는 알레르기 질환을 일으키는 주요한 알레르겐 중 하나이다. 집먼지진드기의 알레르겐은 많이 연구되어 왔지만, 집먼지진드기의 세균총 집먼지진드기 종에 따른 세균총의 차이에 대한 연구는 아직 미흡한 상태이다. 같은 조건에서 오랜 기간 동안 사육 하였을 때 진드기의 종에 따른 세균총의 분포를 비교 분석하고 세균총의 차이가 알레르기를 유발하는 정도에 차이를 일으키는지 확인하는 것이 본 연구의 목적이다. 이 연구를 위하여 알레르기 질환을 일으키는 원인이 된다고 잘 알려진 큰다리먼지진드기, 세로무늬먼지진드기, 긴털가루진드기를 같은 조건으로 무균처리한 배지에서 18주간 사육하였고 기존의 Sanger시퀀싱을 보완한 차세대 염기서열 분석법을 이용하여 진드기 종에 따른 세균총 차이를 분석하였다. 세 종의 진드기에서 얻은 단백질 추출물을 사람기도상피 세포주에 동량 처리하여 사이토카인 발현 정도를 비교하였다. 세 종 진드기의 세균총을 분석한 결과 종에 따라 서로 다른 패턴의 세균총이 분포하는 것을 확인하였다. 큰다리먼지진드기와 긴털가루진드기는 세균총의 거의 대부분을 그람음성균이 차지한 것을 확인하였으나, 무균배지에서 사육한 세로무늬먼지진드기의 세균총은 다른 두 종과 다른 패턴이며 세균이 거의 존재하지 않았다. 세 종의 내독소 양은 세균총의 패턴과 상응하였다. 각 종의 단백질 추출물을 사람기도상피세포주에 동량 처리하였을 때 전염증성 사이토카인 발현이 비슷하게 일어났다. 이 연구를 통하여 무균배지에서 같은 조건으로 사육한 세 종 집먼지진드기의 세균총의 분포는 종에 따라 다르며 내독소의 양도 세균총 분포 결과와 상응하게 다르지만, 사람기도상피세포에서 전염증성 사이토카인의 발현은 비슷하게 일으키는 것을 확인하였다.

핵심되는 말 : 집먼지진드기, 알레르기, 차세대 염기서열 분석법, 세균총, 전염증성 사이토카인

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