A novel human nasal *Staphylococcus epidermidis* strain shapes respiratory immunity by driving protective Type 17 responses

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Like anyone else, I spent this time constantly reflecting on what this period would mean in the grand scheme of my life, and I still cannot give a clear answer. Sometimes, I wonder if, weighed down by a heavy sense of responsibility and burden, I lost sight of the true meaning or value of what I was doing. Whatever the case, I hope that through this thesis, I will be able to regain the simple happiness I once lost and forgot, and that my heart, which has long been unhealed, will finally find some measure of healing.

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ABSTRACT

A novel human nasal *Staphylococcus epidermidis* strain shapes respiratory immunity by driving protective Type 17 responses

Upper respiratory tract infections (URIs), predominantly occurring in the nasal cavity, represent a significant global health burden, accounting for over 17 billion cases annually. The nasal mucosa provides a critical immunological barrier through epithelial defenses, antimicrobial peptides, and mucociliary clearance, supported by diverse T helper subsets (Th1, Th2, Th17, Treg). Among these, IL-17a driven Type 17 immunity, mediated by Th17, Tc17, and γδ17 T cells, is particularly crucial for mucosal defense. Recent evidence highlights the essential role of the commensal nasal microbiome, notably *Staphylococcus epidermidis* (*S. epidermidis*), in modulating this immune balance through enhanced IL-17a production and antimicrobial activity. However, the precise immunomodulatory mechanisms and strain-specific immune effects of nasal *S. epidermidis* remain poorly understood. This thesis aims to identify and characterize immunologically potent *S. epidermidis* strains isolated from human nasal samples, elucidating the molecular and cellular mechanisms underlying their ability to enhance Type 17 immune responses. Ultimately, this study provides critical insights into commensal-host interactions in the nasal cavity, paving the way for novel pharmabiotic approaches to treat respiratory diseases.

Key words: nasal immunity, nasal commensals, *Staphylococcus epidermidis*, T cell, Type 17 immune response

1. Introduction

1.1. Research background

The upper respiratory tract (URT) serves as a primary site of infection for viruses and bacteria[1]. Between 1990 and 2019, the incidence of upper respiratory tract infections (URIs) increased by 37.07%, reaching 17.2 billion cases in 2019[2]. URIs accounted for 42.82% of all disease and injury cases. These infections reduce quality of life, impose a significant social and economic burden for prevention and treatment, and can lead to high mortality rates.

The nasal cavity plays a crucial role in filtering, heating, and humidifying inhaled air, and also supports olfaction[3]. As the first point of contact in the URT with environmental pathogens, it acts as a key immunological barrier[4-5]. Nasal epithelial cells, forming a physical barrier through tight junctions, defend against external pathogens both directly, by secreting antimicrobial compounds, and indirectly, by triggering immune responses through secreted substances. Secretory cells, such as club and goblet cells, produce mucus, which traps particles and pathogens. Secreted IgA dimers bind to these trapped pathogens, preventing them from entering the respiratory system. Ciliated cells, another component of the epithelial layer, remove trapped particles from the body via ciliary motion[3].

In addition to its physical and chemical defenses, in the nasal mucosa, the immune system engages a diverse array of immune responses to maintain a balance between defense against pathogens and tissue homeostasis[6]. The mucosa is exposed to various environmental threats, such as viruses, bacteria, and allergens, making a multi-faceted immune strategy essential. Among these, T helper cells, including Th1, Th2, Th17, and Treg cells, mediate different immune pathways to protect the host. Th1 cells activate macrophages and promote IFN-γ production to eliminate intracellular pathogens, while Th2 cells are involved in responses to extracellular parasites and allergic inflammation by promoting cytokines such as IL-4 and IL-5. The IL-17a-driven Type 17 immune response, mediated by Th17 cells, Tc17 cells, and γδ17 T cells, is particularly crucial in the nasal mucosa[7-8]. Th17 cells release IL-17a, which plays a vital role in recruiting neutrophils to infection sites, thus facilitating the rapid clearance of bacterial and fungal pathogens. Additionally, IL-17a stimulates nasal epithelial cells to secrete antimicrobial peptides and mucus, which enhance the physical barrier by trapping pathogens. The presence of Tc17 cells further contributes by providing cytotoxic responses, while γδ17 T cells act swiftly at mucosal sites, providing an early defense

against invaders. This complex interplay of immune cells ensures not only the elimination of harmful pathogens but also supports the repair and regeneration of nasal tissues. By modulating inflammation and promoting tissue integrity, the Type 17 response helps maintain a healthy equilibrium in the nasal cavity, allowing it to perform its vital roles in both immunity and olfaction effectively.

Together with immune responses mediated by the epithelial and immune cells in the nasal mucosa, the nasal microbiome plays a crucial role in maintaining immune homeostasis and enhancing host defense[9]. *Staphylococcus epidermidis*, a predominant member of the nasal and skin microbiome, has been increasingly recognized for its role in immune regulation and pathogen resistance. It has been shown to interact with immune cells to shape both innate and adaptive immune responses, promoting barrier integrity and preventing pathogen colonization[10-11]. Recent research indicates that *S. epidermidis* not only provides colonization resistance by inhibiting opportunistic pathogens such as *Staphylococcus aureus*, but also modulates local immune responses[12]. It achieves this by promoting the activation of dendritic cells and T cells, particularly through the induction of IL-17A-producing cells, which are critical for enhancing mucosal immunity[13]. This IL-17A-driven response facilitates the recruitment of neutrophils and the secretion of antimicrobial peptides, bolstering the nasal barrier against invading pathogens.

In addition to its role in direct immune modulation, *S. epidermidis* has been found to stimulate epithelial cells to release cytokines that activate macrophages and other immune cells, further enhancing the immune landscape of the nasal mucosa[3]. Moreover, recent studies have highlighted its ability to generate a non-inflammatory, commensal-specific T cell response that contributes to tissue homeostasis without triggering excessive inflammation[11, 14].

Despite the growing evidence of *S. epidermidis*'s immunomodulatory functions, the precise mechanisms through which it strengthens nasal immune responses remain underexplored. Understanding these pathways is crucial for developing new therapeutic strategies, such as pharmabiotics—commensal-based therapies designed to enhance mucosal immunity. The potential of *S. epidermidis*-derived bioactive molecules, such as lipopeptides that regulate inflammation and promote tissue repair, presents an exciting avenue for the development of novel treatments aimed at preventing or mitigating respiratory infections[10]. By leveraging these insights, pharmabiotics could offer a natural and targeted approach for maintaining nasal immune balance, reducing infection risk, and improving overall respiratory health.

1.2. Research objectives

Based on the *S. epidermidis* library that has been established by isolating *S. epidermidis* from healthy human nasal, this research aims to figure out functional *S. epidermidis* that could regulate immune response by assessing the ability in vivo. Developing first-in-class pharmabiotics is expected to be achieved by conducting mechanistic study of the selected *S. epidermidis.* For this research, there are three hypotheses.

Hypothesis I: There are immunomodulatory bacteria among human nasal commensal, and they interact with host immune system to maintain tissue homeostasis in respiratory tract.

Hypothesis II: *S. epidermidis,* the most abundant species of human nasal commensal, has a role in inducing specific type of immune response to maintain tissue homeostasis in respiratory tract.

Hypothesis III: Each subspecies of *S. epidermidis* has own immunomodulatory function and mechanism to be developed as pharmabiotics for treating various respiratory tract diseases.

1.3. Research scope

This study is divided into three parts: selection of *S. epidermidis* strains for further investigation, the type of immune responses elicited by *S. epidermidis* colonization, and the cellular mechanism to induce the response.

1.3.1. Screening of immunomodulatory *S. epidermidis* strains

Given *S. epidermidis* has a high genetic diversity in a strain level, and in vivo screening processes should be conducted among forty-nine human-isolated strains to figure out functional strain.

1.3.2. In vivo immune modulation induced by *S. epidermidis*

To investigate the effect of intranasally colonized *S. epidermidis* in a commensal bacteria-independent manner, flow cytometry and transcript analysis are conducted. They give a clue of prtein level change in a cell and tissue level, respectively. Moreover, to connect these results with the pathway of proein expression, bulk RNA-seq is conducted.

1.3.3. Elucidating cellular mechanism of *S. epidermidis* for the immune modulation

Based on the specific interaction of the bacterial components and receptors on APCs, receptor knock out system is a useful tool to figure out the bacterial PAMP and the mechanism of *S. epidermidis* to induce the immune responses in nasal tissue. To verify characteristics of the key component, heat-killed *S. epidermidis* and conditioned medium of cultured bacteria are used.

2. Research method

2.1. Mice

C57BL/6 specific pathogen-free (SPF) mice are purchased from Orient Bio or Central Laboratory Animal Incorporation. C57BL/6J-[KO]MYD88 (*Myd88*$^{-/-}$) and C57BL/6J-[KO]TLR4 (*Tlr4*$^{-/-}$) mice are provided by You-Me Kim (KAIST). C57BL/6J-[KO]IL6 (*Il6*$^{-/-}$) mice are purchased from The Jackson Laboratory. All mice are bred and maintained under SPF conditions at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at Yonsei Biomedical Research Institute. Germ-free (GF) C57BL/6 mice are maintained in the gnotobiotic animal facility and provided by Sin-Hyeog Im (POSTECH). C57BL/6J-[KO]TLR2-[KO]TLR6 (*Tlr2*$^{-/-}$ *Tlr6*$^{-/-}$) mice are provided by Sin-Hyeog Im (POSTECH). Their C57BL/6J *wild-type* controls are purchased from Central Laboratory Animal Incorporation.

2.2. *S. epidermidis* library

S. epidermidis library is provided by Hyun Jik Kim (Seoul National University). For constructing the library, *S. epidermidis* was isolated from healthy human nasal by swabbing mucus on individuals' middle turbinate. Isolated *S. epidermidis* was cultured on LB plates followed by isolation of bacterial colony. Following 16s rRNA sequencing, forty-nine strains of *S. epidermidis* were identified and the library of human nasal-isolated *S. epidermidis* was established.

2.3. Bacteria preparation

Frozen *S. epidermidis* stock is thaw and dispensed on a tryptic soy agar (TSA) plate after diluting with tryptic soy broth (TSB), with a proper dilution factor. The plate is incubated at 37℃ for 16 hours. Colony is randomly picked and dispensed into a bottom tube filled with TSB after diluting with a proper dilution factor. The tube is incubated at 37℃ for 16 hours with 200 rpm shaking. For a preparation of bacterial conditioned medium, cultured broth is centrifuged at 13,000 rpm for 3 minutes, and the supernatant is collected and centrifuged at 8,000 g, 4℃ for 75 minutes using Vivaspin® for concentrating. For a preparation of heat-killed bacteria, cultured broth is centrifuged at 13,000 rpm for 3 minutes and the supernatant is removed. The pellet is resuspended with PBS and heated at 95℃ for 30 minutes. After removing PBS, the heat-killed pellet is stored at -80℃ for

future use. To derive growth curve based on the relationship between OD_{600} and colony forming units (CFUs) to calculate bacterial CFU from OD_{600} of cultured broth before treatment in mice, the cultured broth is serially diluted with PBS. OD_{600} of diluted cultured broth is measured with OD spectrophotometer and bacterial CFU is assessed and calculated after cultured on a TSA plate.

2.4. Intranasal inoculation of *S. epidermidis*

Cultured *S. epidermidis* is 0.05X diluted with TSB, and OD_{600} is measured with OD spectrophotometer using TSB as a blank. After centrifuging at 13,000 rpm for 3 minutes, supernatant is removed and 2×10^8 CFU of *S. epidermidis* resuspended in 30 μL of PBS is intranasally treated to SPF or GF mice. All SPF mice are euthanized the next day after 5 consecutive days of inoculation, whereas GF mice are sacrificed 14 days after colonization once. 30 μL of filtered conditioned medium or 2×10^8 CFU of heat-killed *S. epidermidis* is intranasally treated to SPF mice.

2.5. Murine tissue processing

Respiratory epithelium and olfactory epithelium are harvested and combined for integrated analysis. Nasal tissue is digested with digestion media (RPMI 1640 supplemented with 2% DNase, 2% fetal bovine serum (FBS), and 1 mg ml^{-1} collagenase II), and incubated 15 minutes at 37℃ with 200 rpm shaking. Digested tissue is meshed thorough 40-micron cell strainer to generate a single-cell suspension.

2.6. Phenotypic analysis

Murine single cell suspensions are incubated with fluorochrome-conjugated antibodies against surface markers CD45, NK1.1, CD19, TCRβ, TCRγδ, CD4, CD8α, CD8β, Gr1, Ly-6G CD11b, CD11c, F4/80, and MHCII in PBS for 20 minutes at room temperature and then washed. LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit is used to exclude dead cells. Cells are fixed overnight at 4℃ using Fixation/Permeabilization Buffer and washed with permeabilization buffer supplied with the eBioscience™ Foxp3/Transcription Factor Staining Buffer Set (Invitrogen™). For simultaneous transcription factors and intracellular cytokine staining, cells are stained with fluorochrome-conjugated antibodies against FOXP3, RORγt, IL-17a, IFNγ, and TNFα in

permeabilization buffer for 40 minutes at room temperature and then washed. Every washing process is preceded twice. (PBS) Cell acquisition is performed on a SONY ID7000 flow cytometer and data are analyzed using FlowJo software.

2.7. Cytokine measurement

Respiratory epithelium and olfactory epithelium are harvested and combined for integrated analysis after intranasally treated with *S. epidermidis*. Proteins are extracted from nasal tissue by homogenizing with RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate; tissue:RIPA buffer ratio, 1:20 in milligrams to microliter). The extracted proteins are sent for quantitation using Olink technique, which gives information of the forty-three cytokines (CCL2, CCL4, CCL5, CCL11, CCL12, CCL17, CCL22, CD274, CSF1, CSF2, CSF3, CTLA4, CXCL1, CXCL2, CXCL9, CXCL11, CXCL12**,** FGF21, HGF, IFNα2, IFNγ, IFNL2, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12A/IL-12B, IL-16, IL-17A, IL-17F, IL-21, IL-22, IL-27, IL-31, IL-33, PDCD1LG2, and TNF) amount in the tissue simultaneously compared to controls.

2.8. Bulk RNA-sequencing

RNA is extracted from nasal tissue of *S. epidermidis*-treated mice with RNeasy® Micro kit (QIAGEN) following the manufacturer's protocols and sent for bulk RNA-sequencing.

2.9. Statistical analysis

Groups are compared with Prism software (GraphPad) using the multiple unpaired t-test or one-way ANOVA. Data are presented as mean ± standard deviation of the mean. P values of less than 0.0332 are considered significant. The significance of the difference between groups was analyzed as described in the figure legends.

3. Results

3.1. SE13 has an ability to elicit Type 17 immune response following intranasal colonization.

To identify functional strains that proliferate immune cells in nasal tissue after colonization, we cultured and intranasally inoculated nine strains of *S. epidermidis*—SE09, SE10, SE11, SE13, SE17, SE18, SE32, SE36, and SE47—isolated from healthy human nasal tissue (**Figure 1A).** FACs analysis showed that SE09, SE11, SE13, SE18, SE32, and SE47 elevated the absolute number of live $CD45^+$ cells relative to control in the tissue, unlike SE10, SE17, and SE36 (**Figure 1B**). We also verified by multiplex analysis that these strains increased IL-17a level in the tissue compared to vehicle treatment (**Figure 1C**).

For further assessment of immunomodulatory effects, we selected SE13 as a functional strian and SE17 and SE36 as non-functional strains. Using Olink technique, we performed proteomic analysis and verified SE13 specifically increased the level of IL-1β, which is critical for IL-17a production from immune cells, CXCL1 and CXCL2, which are necessary for neutrophil recruitment into the tissue. We also observed an increase in IL-17a levels in nasal tissue after intranasal treatment (**Figure 2A-C**).

From RNA bulk-sequencing analysis, several gene sets and pathways related to T cell co-stimulation and regulation of T-helper 17 type immune response were upregulated in nasal tissue after SE13 intranasal treatment (**Figure 3A-C**).

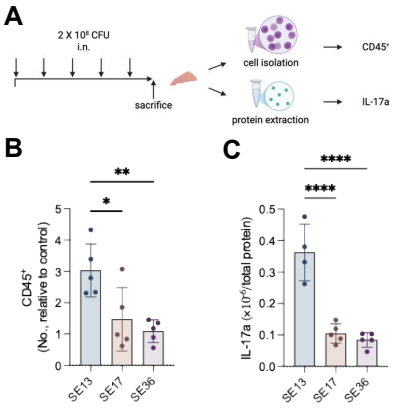

Figure 1. SE13 specifically elevates immune cell numbers and IL-17a levels. (A) Schematic of the experiment. **(B)** Absolute number of $CD45^+$ cells relative to control in nasal tissue after intranasal treatment with SE09, SE10, SE11, SE13, SE17, SE18, SE32, SE36 or SE47. **(C)** IL-17a level in nasal tissue after intranasal treatment with SE09, SE10, SE11, SE13, SE17, SE18, SE32, SE36 or SE47. Means were compared with the mean of control group using One-way ANOVA. *P<0.0332, **P<0.0021, ****P<0.0001.

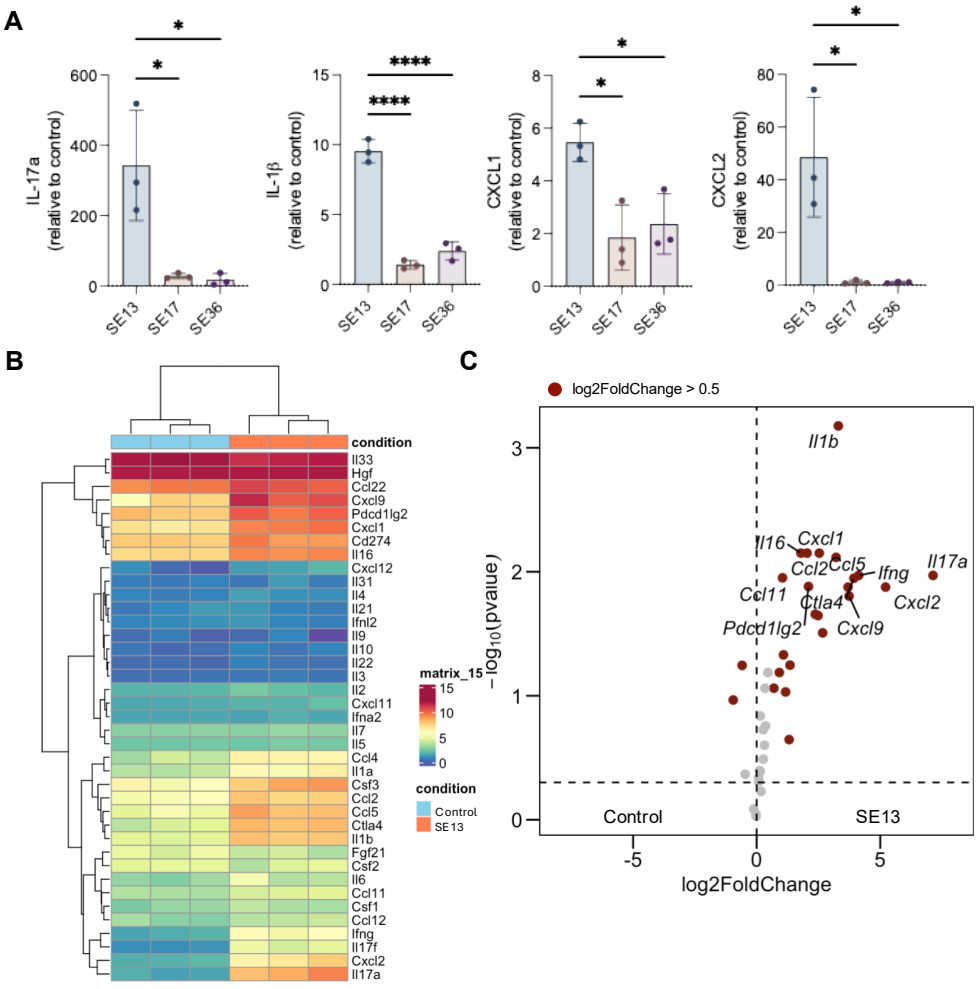

Figure 2. SE13 specifically increases the levels of Type 17 immune response-related factors. (A) The level of cytokines relative to control in the tissue after intranasal treatment with SE13, SE17, or SE36. Means were compared between the three groups using One-way ANOVA. *P<0.0332, ****P<0.0001. **(B)** Hierarchical clustering of differentially expressed genes (DEGs) from SE13 and control group and **(C)** fold change plot of DEGs from SE13 and control group after intranasal treatment with SE13, SE17, or SE36.

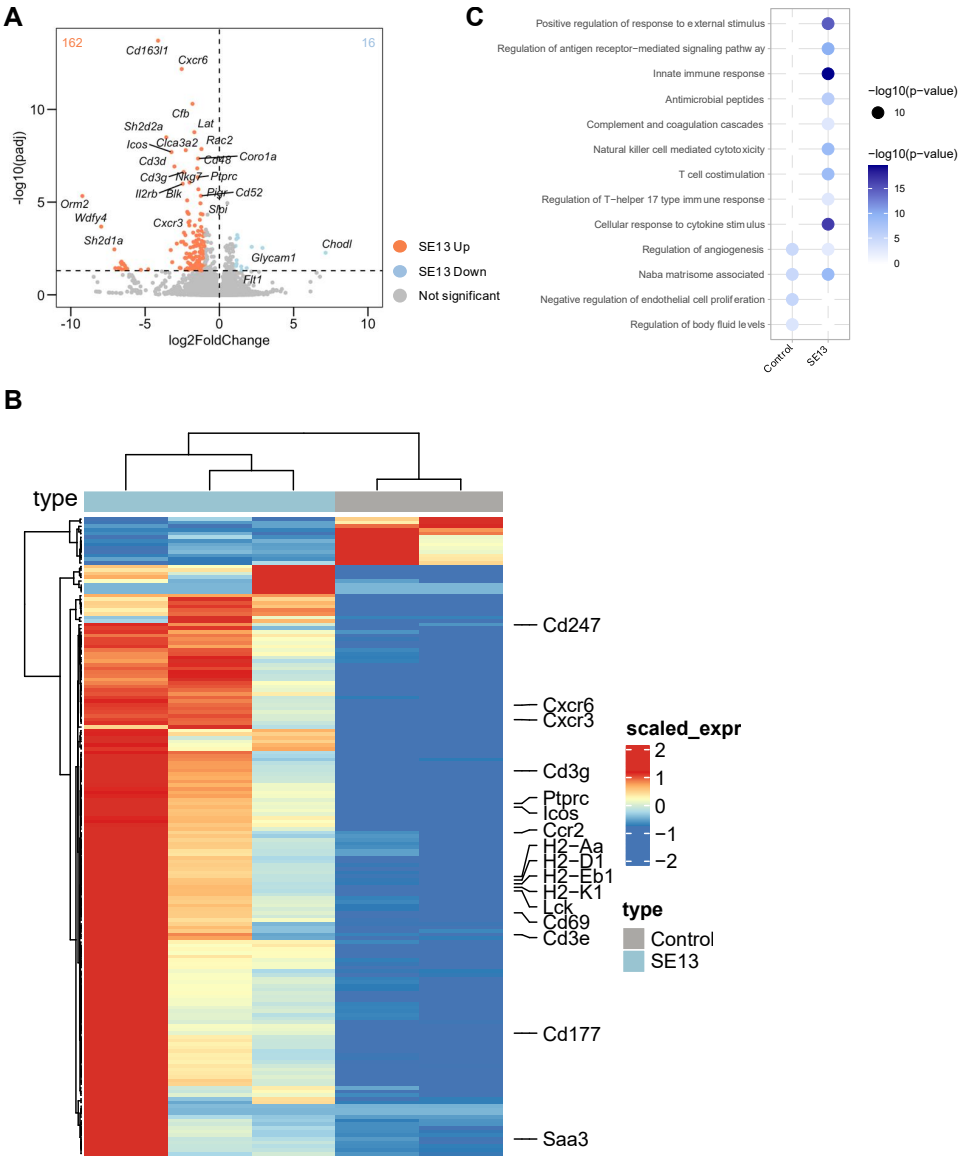

Figure 3. SE13 has an ability to elicit Type 17 immune response. (A) Fold change plot of DEGs from SE13 and control group, **(B)** hierarchical clustering depicting upregulated (orange dots) and downregulated (blue dots) DEGs from SE13 and control group, and **(C)** gene ontology (GO) enrichment analysis performed on upregulated genes in each group after intranasal treatment with SE13.

3.2. SE13 is an intrinsic inducer of Th17 and γδ17 T cells, but not that of Tc17 cells.

To verify change in immune cell population after SE13 treatment, FACs analysis was conducted (**Figure S1A-B, Table 1**). It identified primary IL-17a-secreting immune cells before and after SE13 treatment: intranasal colonization with SE13 led T cells, including Th17 cells, Tc17 cells, and γδ17 T cells to predominantly secrete IL-17a accounting 83.7% of all IL-17a-secreting immune cells, thereby diminishing the proportion of IL-17a-secreting macrophages (**Figure 4A**). In addition to IL-17a-secreting T cells, it increased the proportion of T cells out of all immune cells from 3.61% to 8.53% (**Figure 4B**).

To identify the specific T cell subsets that induce the Type 17 immune response, we analyzed flow cytometry after SE13 colonization and found there were increased frequencies and absolute numbers of IL-17a-secreting, RORγt-expressing $CD4^+$ T cells, $CD8^+$ T cells and γδT cells in nasal tissue (**Figure 5A-D**).

To exclude extrinsic effects arising from interaction with other bacteria, we intranasally treated SE13 to GF mice instead of SPF mice. As a result, Th17 cells and γδ17 T cells, but not Tc17 cells, expanded upon colonization, indicating that their induction occurs independently of pre-existing microbiota (**Figure 6A-D).** This guided us to focus on Th17 cells and γδ17 T cells for further mechanistic study.

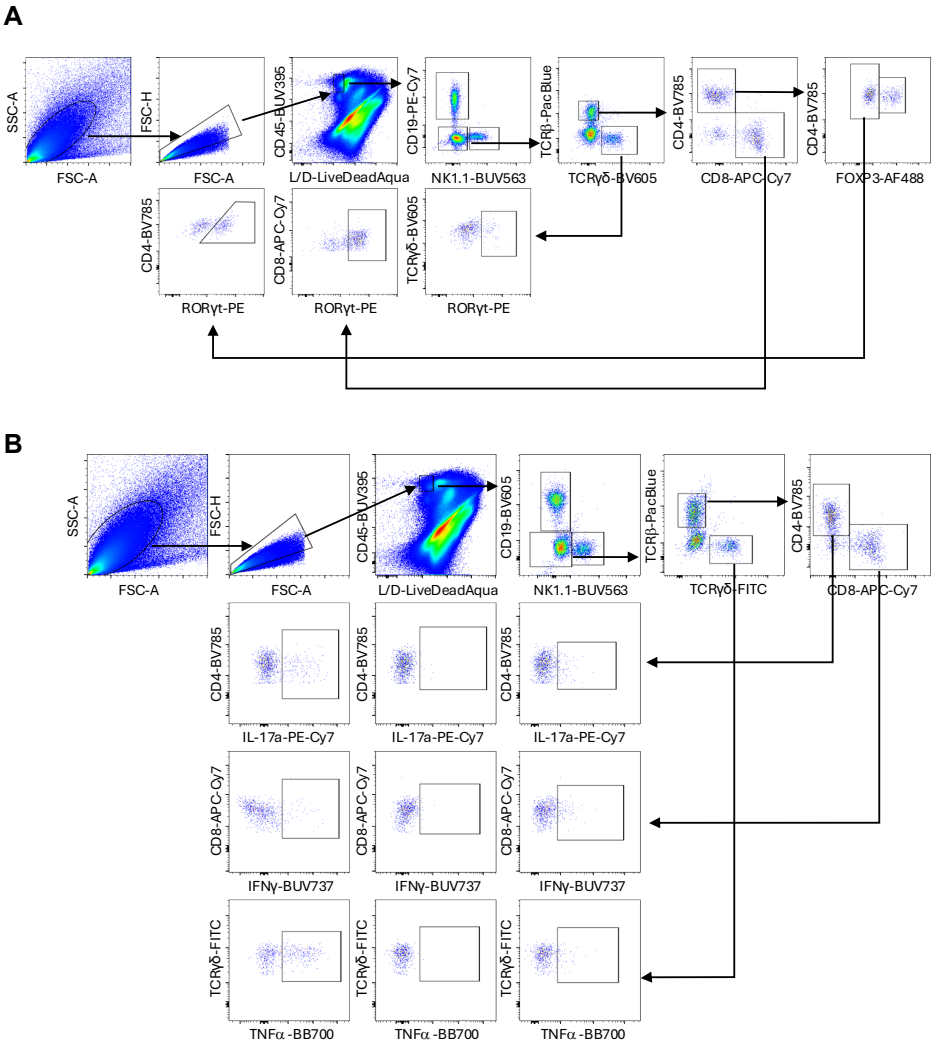

Figure S1. Gating strategy for FACs analysis. Gating strategy for **(A)** the transcription factor panel and **(B)** the cytokine panel.

Table 1. List of antibodies for FACs analysis.

No.	Antigen	Fluorophore
1	Cell Viability	Fixable Aqua
2	CD45	BUV395
3	NK1.1	BUV563
4	CD19	PE-Cy7
5	CD19	BV605
6	TCRβ	Pacific Blue
7	TCRγδ	BV605
8	TCRγδ	FITC
9	CD4	BV785
10	CD8α	APC-Cy7
11	CD8β	APC
16	Gr1	APC
17	Ly-6G	BV570
18	CD11b	Spark Blue550
19	CD11b	PerCp-Cy5.5
20	CD11c	BV480
21	F4/80	BUV661
22	MHCII	BUV496
23	FOXP3	AF488
24	RORγt	PE
28	IL-17a	PE-Cy7
29	IFNγ	BUV737
30	TNFα	BB700

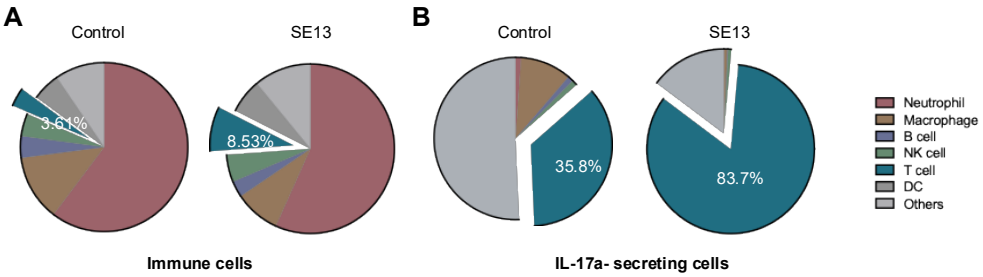

Figure 4. SE13 promotes the proliferation of T cells, with a significant effect on IL-17a-secreting T cells. (A) Composition in immune cell population and **(B)** IL17-secreting immune cells after intranasal treatment with vehicle (left) or SE13 (right).

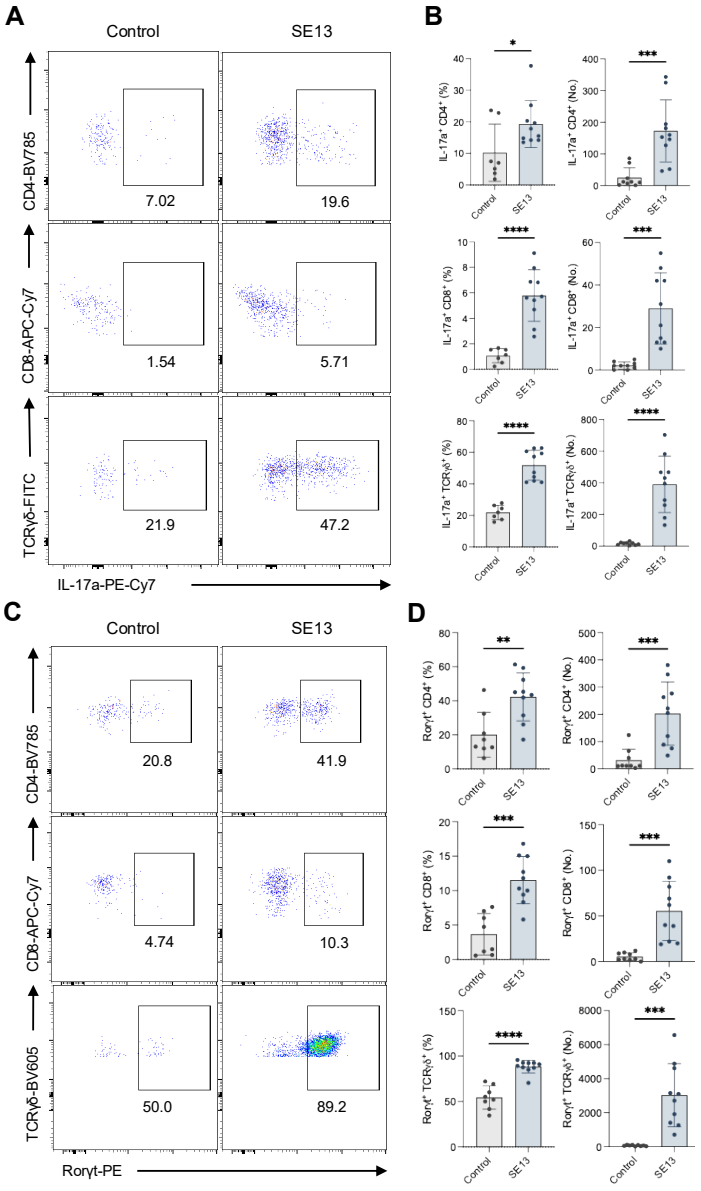

Figure 5. SE13 is an inducer of Th17, Tc17, and γδ17 T cells. (A-B) Frequencies and absolute numbers of IL-17a-secreting T cells and **(C-D)** RORγt-expressing T cells after intranasal treatment with vehicle or SE13 to SPF mice. Means were compared between the two groups using Student's t test. *P<0.0332, **P<0.0021, ***P<0.0002, ****P<0.0001.

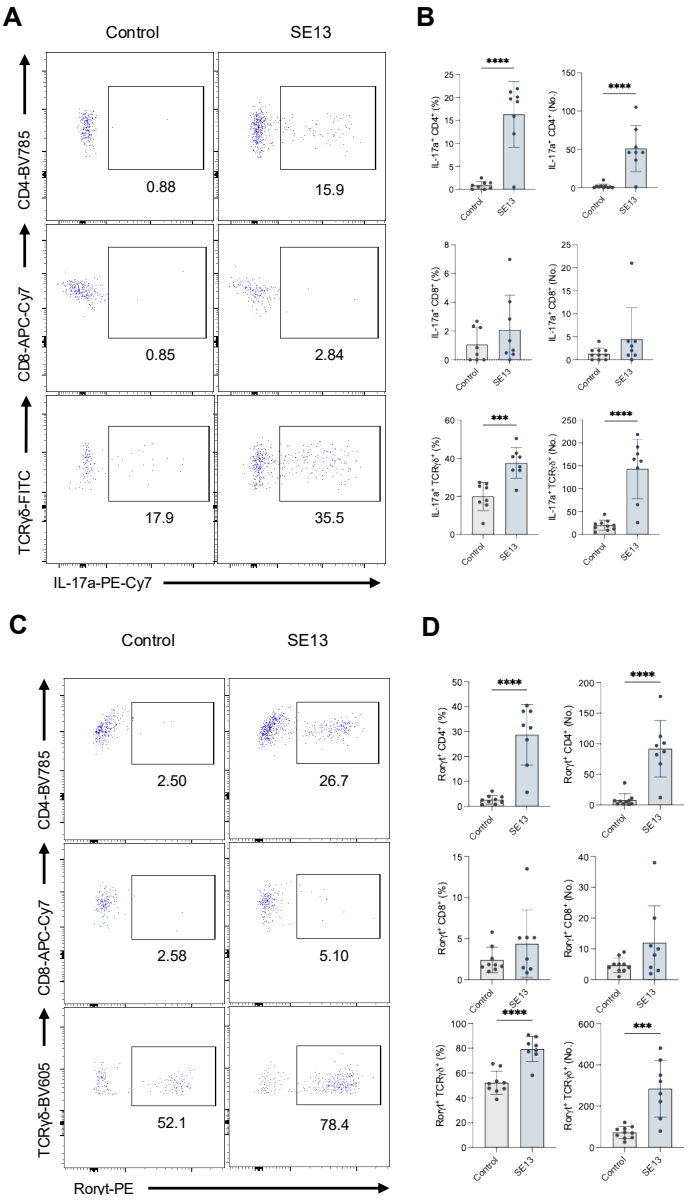

Figure 6. SE13 is an intrinsic inducer of Th17 and γδ17 T cells, but not that of Tc17 cells. (A-B) Frequencies and absolute numbers of IL-17a-secreting T cells and **(C-D)** RORγt-expressing T cells after intranasal treatment with vehicle or SE13 to GF mice. Means were compared between the two groups using Student's t test. ***P<0.0002, ****P<0.0001.

3.3. Th17 cells are induced in an IL-6-dependent manner by SE13 treatment, whereas γδ17 T cells are not.

To verify the mechanism by which SE13 boosts Type 17 immune responses, we used TLR2/6 or TLR4 knockout mice for APC receptor deficiency, MYD88 knockout mice for APC adapter deficiency, and *Il6* knockout mice for deficiency in a stimulus for T cell differentiation into Type 17 immune cells.

Fluctuations in the frequencies of IL-17a-secreting, RORγt-expressing $CD4^+$ T cells and γδT cells were not observed in the transgenic mice in the tissue, regardless of intranasal treatment with vehicle or SE13, except for increased RORγt-expressing $CD4^+$ T cells in TLR2/6 knockout mice and decreased IL-17a-secreting cells in MYD88 knockout mice compared to those of *wild-type* mice (**Figure 7A-B**). However, unlike γδT cells, $CD4^+$ T cells of IL6 knockout mice showed decreased frequencies of IL-17a secretion and RORγt expression compared to those of *wild-type* mice after intranasal treatment with SE13 in the tissue, meaning Th17 cells are induced in an IL-6-dependent manner. IL-17a secretion from $CD4^+$ T cells of IL6 knockout mice also decreased after treatment with vehicle compared to that of *wild-type* mice, though (**Figure 7A-B**).

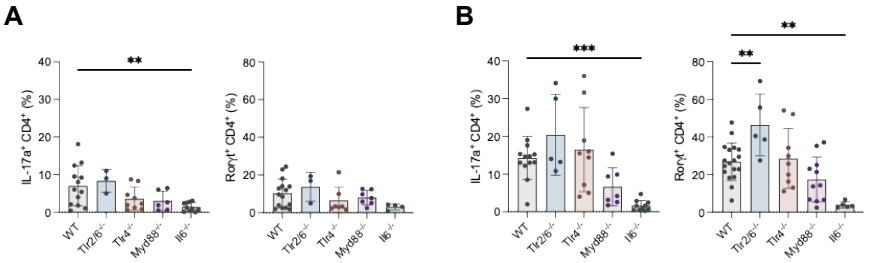

Figure 7. Th17 cells are induced in an IL-6-dependent manner by SE13 treatment. (A) Frequencies of IL-17a-secreting T cells and **(B)** RORγt-expressing T cells after intranasal treatment with vehicle (left) or SE13 (right) to *wild-type, Tlr2/6*$^{-/-}$*, Tlr4*$^{-/-}$*, Myd88*$^{-/-}$*,* and *Il6*$^{-/-}$ mice. Means were compared with the mean of WT group using One-way ANOVA. **P<0.0021, ***P<0.0002. WT: *wild-type*; *Tlr2/6*$^{-/-}$: TLR2/6 knockout; *Tlr4*$^{-/-}$: TLR4 knockout; *Myd88*$^{-/-}$: MYD88 knockout; *Il6*$^{-/-}$: IL6 knockout

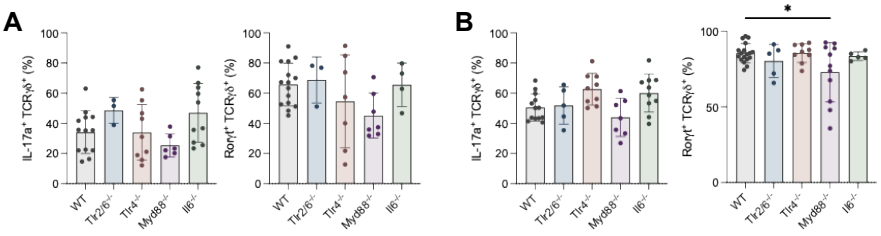

Figure 8. The induction of γδ17 T cells by SE13 treatment occurs independently of IL-6. (A) Frequencies of IL-17a-secreting T cells and **(B)** RORγt-expressing T cells after intranasal treatment with vehicle (left) or SE13 (right) to *wild-type, Tlr2/6*$^{-/-}$*, Tlr4*$^{-/-}$*, Myd88*$^{-/-}$*,* and *Il6*$^{-/-}$ mice. Means were compared with the mean of WT group using One-way ANOVA. *P<0.0332. WT: *wild-type*; *Tlr2/6*$^{-/-}$: TLR2/6 knockout; *Tlr4*$^{-/-}$: TLR4 knockout; *Myd88*$^{-/-}$: MYD88 knockout; *Il6*$^{-/-}$: IL6 knockout

3.4. Th17 cells are induced by SE13-secreting molecules, whereas induction of γδ17 T cells requires live component of the bacteria.

To identify the bacterial component detected by host cells, we used heat-killed bacteria and conditioned medium of SE13. Live bacteria increased the frequencies and absolute numbers of IL-17a-secreting, RORγt-expressing $CD4^+$ T cells and γδT cells, confirming the previous results (**Figure 9A-D, 10A-D**). Intranasal treatment with heat-killed bacteria showed unchanged frequencies and absolute numbers of Type 17 cells, regardless of $CD4^+$ T cells and γδT cells (**Figure 9A-D**). However, treatment with conditioned medium resulted in increased IL-17a secretion and RORγt expression in $CD4^+$ T cells, but not in γδT cells (**Figure 10A-D**).

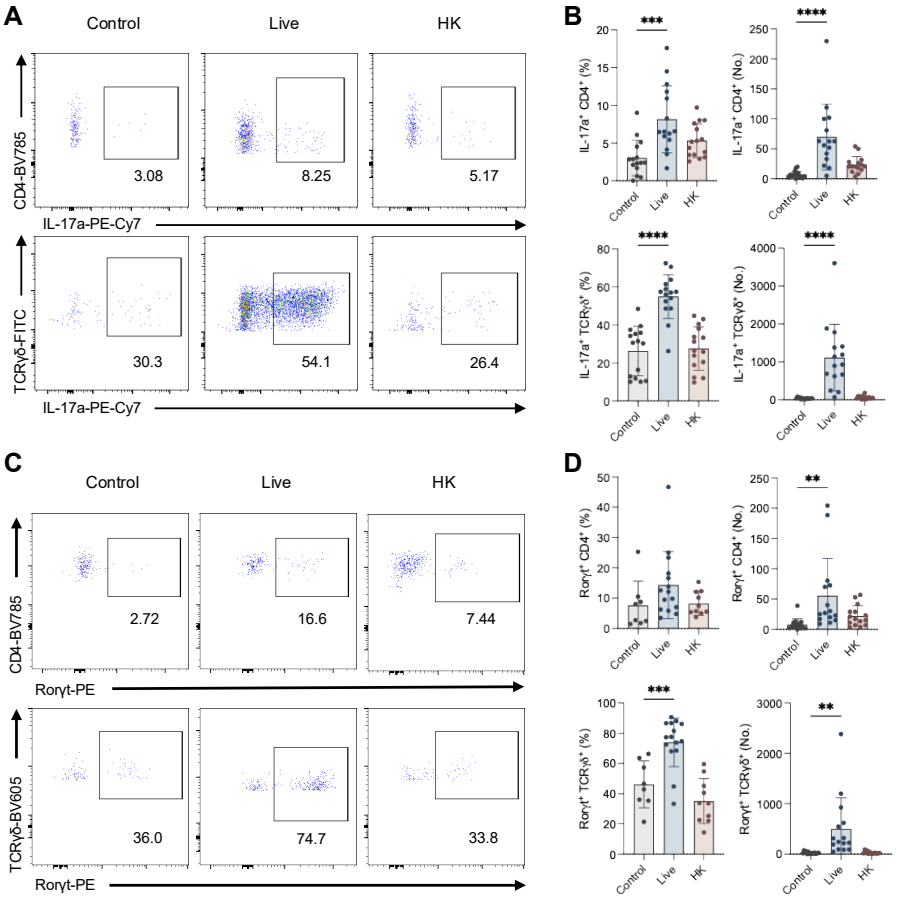

Figure 9. Th17 cells are induced by SE13-secreting molecules. (A-B) Frequencies and absolute numbers of IL-17a-secreting T cells and **(C-D)** RORγt-expressing T cells after intranasal treatment with vehicle, Live, or HK. Live: live SE13; HK: heat-killed SE13. Means were compared with the mean of control group using One-way ANOVA. **P<0.0021, ***P<0.002, ****P<0.0001.

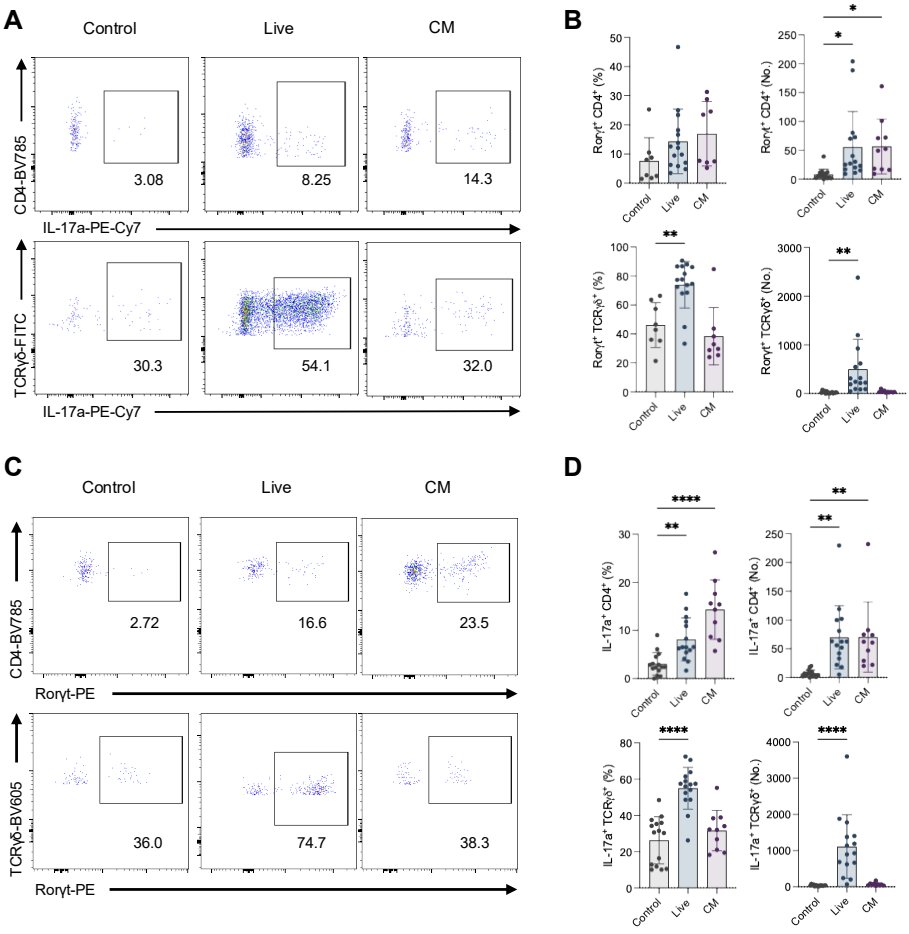

Figure 10. Induction of γδ17 T cells requires live component of the bacteria. (A-B) Frequencies and absolute numbers of IL-17a-secreting T cells and **(C-D)** RORγt-expressing T cells after intranasal treatment with vehicle, Live, or CM. Live: live SE13; CM: conditioned medium of SE13. Means were compared with the mean of control group using One-way ANOVA. *P<0.0332, **P<0.0021, ****P<0.0001.

4. Discussion

S. epidermidis, a commensal bacterial species residing in human skin and nasal tissue, exhibited high genetic diversity in its population, with approximately 20% of variable genes out of whole genome[15]. Each *S. epidermidis* strain showed unique colonization and adaptation abilities, forming its own microenvironment in the tissue[16]. In our study, among the *S.* epidermidis strains tested, SE13 identified as one of the most potent strains because it elevated the absolute number of immune cells (live $CD45^+$ cells) in nasal tissue. Based on these findings, we selected SE13 as a functional strain from our *S. epidermidis* library, which was constructed by isolating strains from human nasal tissues. FACs and multiplex analysis, proteomic analysis, and transcriptomic analysis indicated that SE13 specifically and intrinsically induces Type 17 immune response after colonization in nasal tissue, particularly by Th17 cells and by γδ17 T cells, confirming human nasal commensal bacteria, particularly *S. epidermidis*, interact with the immune system to maintain respiratory tissue homeostasis and induce Type 17 immune responses to support this balance.

In skin, *S. epidermidis* is detected by TLR2 of dendritic cells, thereby stimulating $CD8^+$ T cells to maintain tissue homeostasis[17]. However, since there was no consistent change in IL-17a secretion and RORγt expression in $CD4^+$ T cells and γδT cells in the tissue after intranasal treatment of SE13 to TLR4 and MYD88 as well as TLR2/6 knockout mice**,** we concluded that they are not involved in the increased Type 17 immune responses we observed. However, the results of SE13 treatment in *Il6* knockout mice suggested that Th17 cells were IL-6-dependently proliferated, unlike γδ17 T cells. Although the frequency of IL-17a-secreting $CD4^+$ T cells decreased after intranasal treatment of vehicle compared to *wild-type* mice, the implication of the frequency after SE13 treatment was not observed in *Il6* knockout mice, indicating the proliferated Th17 cells were IL-6-dependently induced.

The results of intranasal treatment with heat-killed bacteria and conditioned medium suggested that Th17 cell activation was mainly induced by secreted factors from SE13, whereas γδ17 T cell activation required heat-labile components of live bacteria. Thus, Th17 cells and γδ17 T cells have distinct mechanisms for the Type 17 immune responses.

Given *S. epidermidis* conferred protection against respiratory pathogens such as H1N1 influenza virus and *Streptococcus pneumoniae*, these findings reveal a key role for nasal commensals in promoting protective immunity and highlight their potential in enhancing resistance to respiratory

infections. For further therapeutic implication for respiratory infection, study on whether Type 17 immune response has a critical role for the restriction against respiratory pathogens is necessary, and if so, the primary T cell subsets or effecting molecules for the response induction should be investigated.

5. Conclusion

In this research, we identified functional commensal bacteria in nasal tissue, especially SE13, which was selected from a library of human nasal isolates. SE13 possesses an intrinsic ability to enhance Type 17 immune responses through Th17 cells and γδ17 T cells in the tissue following intranasal colonization. Mechanistic studies revealed that these two responses are induced by different components of the bacteria and involve distinct mechanisms: Th17 cells are induced by SE13-secreting molecules and differentiated in an IL-6-dependent manner, whereas the induction of γδ17 T cells requires heat-labile components of live bacteria, regardless of IL-6 presence in the tissue. These findings may contribute to the development of novel pharmabiotics that potentiate Type 17 immune responses for the treatment of respiratory diseases.

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Abstract in Korean

호흡기 점막에서 Type 17 면역반응을 유도하는 인간 비강 유래 신규 *Staphylococcus epidermidis* 균주의 발굴 및 면역조절 기전 규명

본 논문은 인간 비강에서 분리한 *Staphylococcus epidermidis* 아종 중 면역조절 기능을 가지는 아종을 선별하고, 이들의 면역조절 매커니즘을 설명하는 데에 목적을 가지고 있다. 비강 공간은 여과, 가습 뿐만 아니라, 흡입한 공기와 함께 침투한 병원균에 대하여 방어하면서 면역학적 장벽으로 작용한다. 비강 상피세포는 융합막(tight junction), 항미생물 화합물, 분비세포 및 섬모세포ㄹ가 형성하는 물리적 장벽에 더해 Th17 세포, Tc17 세포, γδ17 T 세포가 분비한 IL-17a에 의해 유도된 Type 17 면역 반응을 통해 비강 공간 내에서 병원균을 제거하고 조직을 보수(repair)한다. 비강 공생세균 중 우세종인 *S. epidermidis*는 선천 면역과 후천 면역을 조절함으로써 면역 균형을 유지하는 데 중요한 역할을 하며, IL-17a 생산과 항미생물 펩타이드 분비를 통해 병원균의 집락화(colonization)를 방지하고 점막 면역을 향상시키는 것으로 보여져 왔다. *S. epidermidis*가 면역조절에 있어서 이러한 역할을 하는 것이 알려져 있음에도 불구하고 *S. epidermidis*가 비강 면역 방어를 강화하는 정확한 매커니즘은 연구된 바가 없다. 본 연구에서는 인간 비강 조직 유래 *S. epidermidis* 중 비강 집락화 후 Th17 세포와 γδ17 T 세포를 통해 Type 17 면역 반응을 강화하는 SE13을 규명하였다. 또한, 매커니즘 연구를 통해 Th17 세포는 SE13으로부터 분비된 물질에 의해 IL-6 자극을 받아 활성화되고, γδ17 T 세포는 생균이 가지는 열에 불안정한 물질에 의해 조직 내 IL-6 유무에 무관하게 유도됨을 보여줌으로써 이들이 Type 17 면역반응을 일으키기 위해 다른 매커니즘을 가지고 있음을 확인하였다. 이 결과는 Type 17 면역반응 강화를 통한 호흡기 감염을 치료하기 위한 새로운 파마바이오틱스(pharmabiotics, 의약용 프로바이오틱스) 개발에 기여할 것으로 기대된다.

핵심되는 말 : 비강 면역, 비강 공생미생물, *Staphylococcus epidermidis*, T 세포, Type 17 면역반응