4. Hawro T, Ohanyan T, Schoepke N, et al. The Urticaria Activity Score—Validity, Reliability, and Responsiveness. *J Allergy Clin Immunol In Pract*. 2018;6(4):1185-1190.
5. Bernstein JA, Singh U, Rao MB, Berendts K, Zhang X, Mutasim D. Benralizumab for Chronic Spontaneous Urticaria. *N Engl J Med*. 2020;383(14):1389-1391.
6. Kaplan AP, Joseph K, Maykut RJ, Geba GP, Zeldin RK. Treatment of chronic autoimmune urticaria with omalizumab. *J Allergy Clin Immunol*. 2008;122(3):569-573.
7. Kolkhir P, Church MK, Altrichter S, et al. Eosinopenia, in Chronic Spontaneous Urticaria, Is Associated with High Disease Activity, Autoimmunity, and Poor Response to Treatment. *J Allergy Clin Immunol Pract*. 2020;8(1):318-325.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Interplay between skin microbiota and immunity in atopic individuals

To the Editor,

The prevalence of allergic diseases continues to increase worldwide along with industrialization and urbanization. Emerging evidence provides insights into the connection between the development of chronic inflammatory disorders, such as asthma and allergies, and the human microbiota through the immune-modulatory function of the commensal microbes.[1,2] In addition to the well-established evidence on the role of the gut microbiota in human health and disease, other microbial communities, such as cutaneous microbiota, play an important role in establishing and tuning the host immune system toward a balanced response against commensal and pathogenic microorganisms.[3]

Additionally, the skin microbiota may promote immune tolerance toward environmental allergens both locally and systemically.[4] Interindividual differences and alterations in the skin microbiota composition and its associations with human health and disease are evident, but a greater clarification of the factors that modify the composition of microbiota is needed.[1,2] Factors that have been investigated for their potential interaction with the microbiota include lifestyle, use of medication, and the exposure to a changing environmental biodiversity.[1,5]

Here, we explore the composition of skin microbiota in 62 healthy and atopic asymptomatic young adults, and associate that with the characteristics of their childhood and present living environments, allergen-specific serum IgE levels, and the immune function of their peripheral blood mononuclear cells (PBMCs) (see Figure 1A and supplementals for detailed methodological description). We observed distinct associations between the skin microbiota and allergen-specific serum IgE levels and the expression of cytokines, and identified several taxa that may play an important role in augmenting or suppressing the systemic inflammatory immune responses.

Allergen-specific serum IgE measurements revealed that approximately half of the subjects were sensitized to airborne and food allergens (aIgE > 0.35 kU_A/L, n = 30), and of these, 43% were sensitized to birch pollen (bIgE > 0.35 kU_A/L, n = 13), and 50% to timothy pollen (tIgE > 0.35 kU_A/L, n = 15) (Figure S1A–C). Principal component analysis (PCA) of the allergen-specific IgE levels revealed clustering of all subjects, except those with very high (>12.5 kU_A/L) aIgE, bIgE, or tIgE (Figure 1B), which are referred to as highly sensitized (HS; n = 11) individuals in subsequent analyses. Subjects with aIgE below 0.35 kU_A/L are referred to as non-sensitized (NS; n = 32) individuals. A total of 19 subjects exhibited intermediate allergen-specific IgE levels (0.35–12.5 kU_A/L).

The relative levels of *IL10*, *IL4*, *IL5*, *IL13*, and *IFNG* mRNA were measured in PBMCs after 6 h of stimulation with either birch (Bet v 1) or timothy (Phl p 1) pollen allergen, and compared between the groups. HS individuals showed significantly higher expression of Th2-type cytokine *IL4* in Bet v1 and Phl p 1 stimulated cells (p < 0.05) and Th1-type cytokine *IFNG* in Phl p 1 stimulated cells (p < 0.05) compared with NS. There was no difference in *IFNG* expression between HS and NS after Bet v 1 stimulation. Furthermore, the expression of anti-inflammatory cytokine *IL10* was significantly lower at the mRNA level in HS individuals compared with NS (in Phl p 1 stimulated PBMCs, p < 0.05; but not in Bet v 1 stimulated PBMCs, p < 0.1) (Figure 1C, results from Bet v 1 stimulated PBMCs in Figure S2).The mRNA levels correlated closely with protein levels measured in cell supernatants (Figure S3). In conclusion, we show that high allergen-specific serum IgE levels in our study subjects

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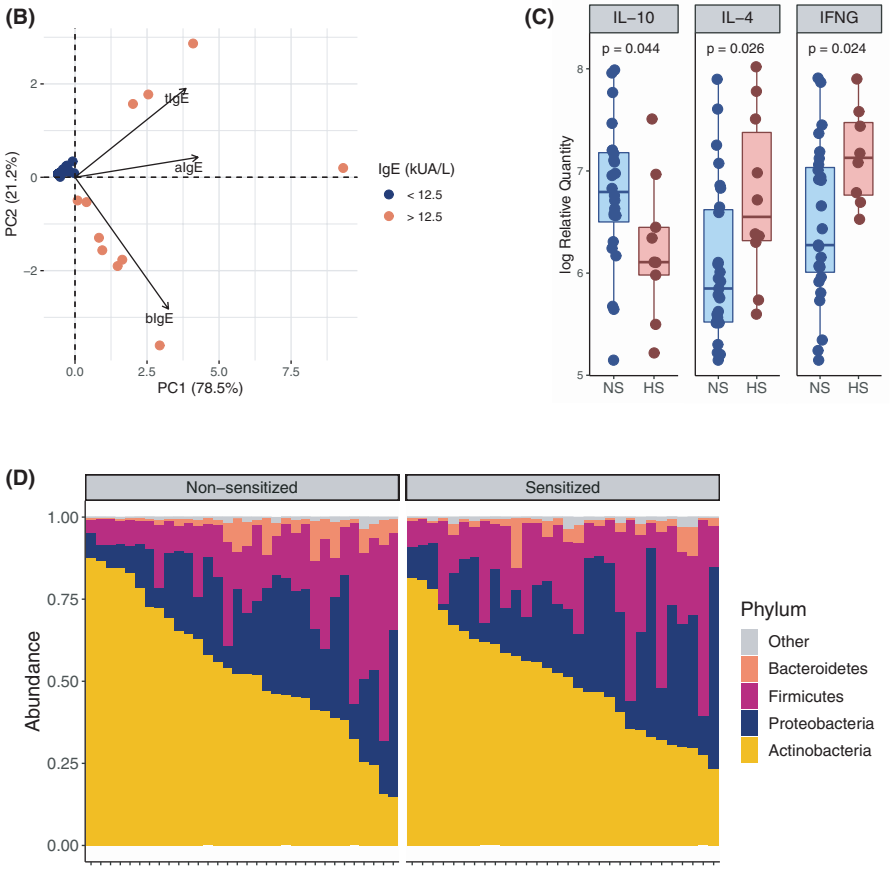
FIGURE 1 Serum IgE levels, immune function, and skin microbiota composition. (A) Study population characteristics: group size (*n*), female ratio (F%), mean IgE levels specific for airborne and food (aIgE), birch (bIgE), and timothy (tIgE) allergens. (B) Principal component analysis of IgE levels (IgE < 12.5 kU_A/L [blue]; >12.5 kU_A/L [pink]). (C) *IL10*, *IL4*, and *IFNG* mRNA levels in Phlp1 stimulated peripheral blood mononuclear cells. *P*-values calculated with Wilcoxon rank-sum test. Log transformation using natural logarithm. (NS, non-sensitized; HS, highly sensitized individuals). (D) Taxa relative abundance at phylum level in non-sensitized (aIgE < 0.35 kU_A/L) and sensitized (aIgE > 0.35 kU_A/L) individuals

(A)

	Sensitization†		Childhood living environment		
	Non-sensitized	Sensitized	Urban	Agriculture	Forest
n	32	30	30	15	17
F%	65.5	46.7	44.8	80	58.8
aIgE mean	0.11	11.59	6.73	6.90	2.71
bIgE mean	n/a	14.70	12.54	4.17	0.13
tIgE mean	n/a	6.92	2.65	5.93	2.32
Sensitized %			56.7	40.0	41.2

†Sensitization is defined according to the levels of serum IgE specific for airborne allergens. Non-sensitized: aIgE <0.35 kUA/L; sensitized: aIgE ≥0.35 kUA/L.



are associated with potent pro-inflammatory, and attenuated anti-inflammatory responses to allergens.

The skin microbiotas, analyzed by 16S rRNA gene sequencing, mainly consisted of four phyla, including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Figure 1D), representing typical skin microbial communities consistent with previous observations.[2] We further explored the microbial compositions using distance-based redundancy analysis (dbRDA), testing the possible influence of living environments and allergen-specific IgE levels. Our results suggest that childhood living environments (urban, agricultural, or forest) may have impacted the skin microbiota (Figure 2A) in a manner which is still discernible when constraining the analysis by the effect of current living environment (Figure S4). Notably, the study subjects had moved from their first childhood homes since several years before the current study (Table S1). Finally, the constrained ordination analysis (dbRDA) suggested that high allergen-specific serum IgE levels may influence skin microbial compositions (Figure 2B). However, the results did not reach statistical significance, likely due to the low number of HS individuals.

To further explore associations between the skin microbiota and host immunity, we analyzed links between serum aIgE levels, cytokine expression in PBMCs, and the relative abundance of microorganisms, using Spearman's rank correlation. Our exploratory analysis revealed several taxa, including *Burkholderia–Caballeronia–Paraburkholderia*, *Ralstonia*, *Pelomonas*, *Curvibacter*, *Sediminibacterium*, and *Thermoactinomyces vulgaris*, which correlated positively with aIgE levels and *IL5* mRNA expression, and negatively with the expression of*IL10* ($p < 0.05–0.1$) (Figure 2C–E, Table S2). *Burkholderia–Caballeronia–Paraburkholderia*, *Ralstonia*, *Pelomonas*, and *Curvibacter* are members of the *Burkholderiaceae* family, which includes organisms recognized as pathogens in the lungs, and an increase in their abundance has been associated with decreased pulmonary function in cystic fibrosis patients.[5] Furthermore, *T. vulgaris* is known as one of the causative organisms in farmer's lung disease, which is a form of hypersensitivity pneumonitis.[6]

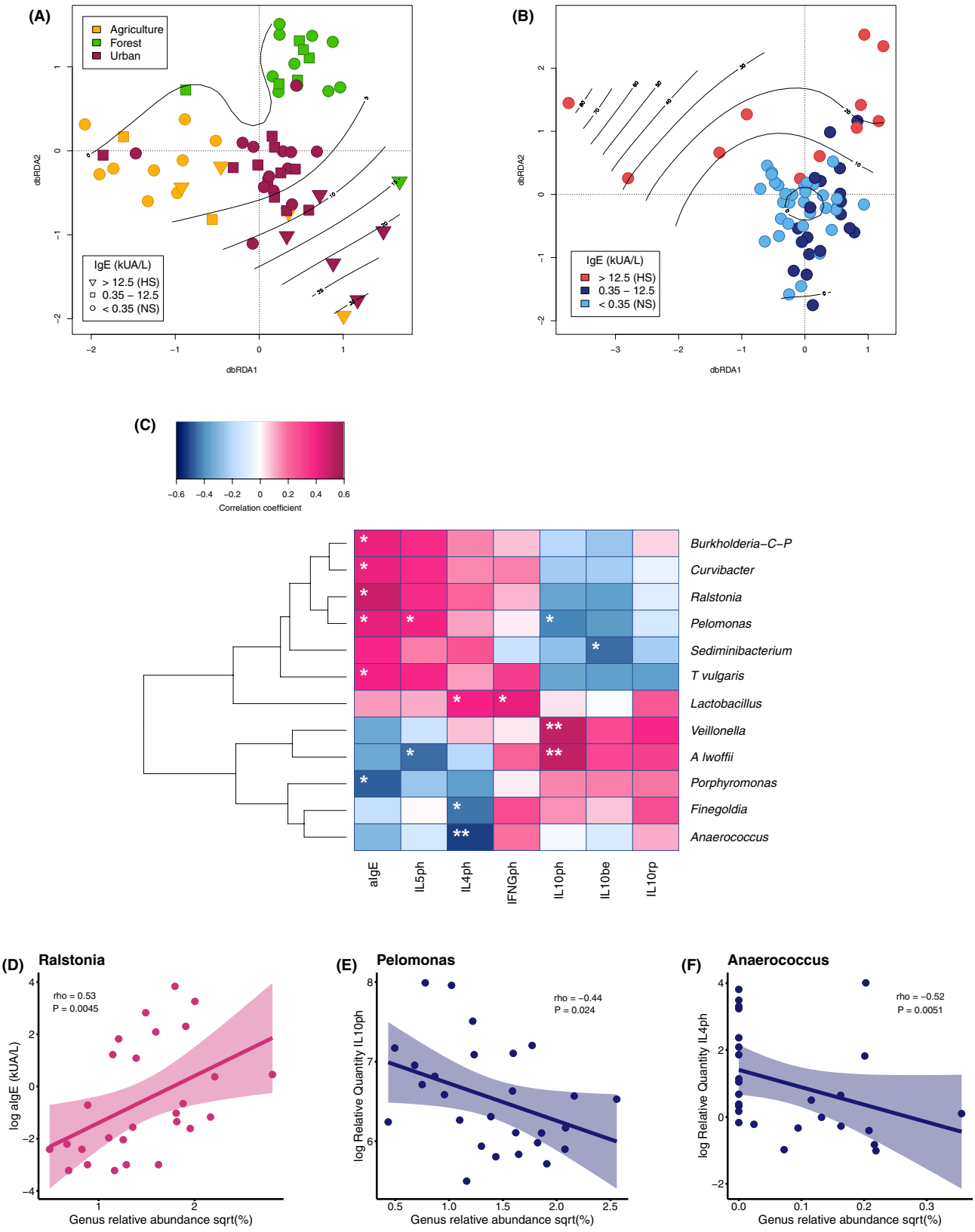


FIGURE 2 Association between skin microbiota and immune function. (A, B) Distance-based redundancy analysis (dbRDA) constrained by (A) aIgE and childhood environment (aIgE, black lines; agriculture, yellow; forest, green; urban, purple; NS, non-sensitized, circles; HS, highly sensitized, triangles; intermediate IgE levels, squares), and by (B) IgE levels (aIgE, black lines; birch; timothy; HS, pink symbols, NS, light blue; intermediate, dark blue). (C) Hierarchical clustering of taxa correlating with aIgE and cytokines (rp; non-stimulated; ph, Phlp1; be, Betv1 stimulated peripheral blood mononuclear cells (*$p < 0.05$, **$p < 0.01$). (D–F) Spearman's rank correlation between the relative abundance of (D) *Ralstonia* and aIgE, (E) *Pelomonas* and *IL10*, (F) *Anaerococcus* and *IL4* levels. Log transformation using natural logarithm

In contrast, *Acinetobacter lwoffii* correlated positively with Phl p 1 induced *IL10* ($p < 0.01$) and negatively with *IL5* ($p < 0.05$), (Figure 2C, Table S2). *Acinetobacter* species are known to stimulate anti-inflammatory immune signaling in the skin, induce immune tolerance, and protect against allergies.[4,7] Further, *Porphyromonas*, which negatively regulates airway allergic inflammation,[8] correlated negatively with aIgE levels ($p < 0.05$).Moreover, *Anaerococcus* and *Finegoldia* correlated negatively with *IL4* expression (Figure 2CF) ($p < 0.01$ and $p < 0.05$, respectively). These gram-positive anaerobic cocci have been found to be scarce in filaggrin-deficient ichthyosis vulgaris patients, suggesting that they may have protective effects in the skin.[9] Furthermore, *Lactobacillus* and *Veillonella* correlated positively with *IFNG* and *IL10* expression, respectively ($p < 0.05$). An increase in *Veillonella* abundance is associated with decreased inflammation in the lungs of cystic fibrosis patients.[5]

In conclusion, we have identified distinct associations between the composition of the skin microbiota and immune signaling by comparing atopic individuals with healthy individuals. Our results suggest that the skin of allergen-sensitized asymptomatic individuals may be colonized by less favorable, pro-inflammatory microbial species, and is characterized by a general scarcity of beneficial anti-inflammatory microbes.

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KEYWORDS

16S rRNA gene sequencing, allergen-specific IgE, amplicon sequence variant (ASV), host–microbe interactions, skin microbiota

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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REFERENCES

1. Huang YJ, Marsland BJ, Bunyavanich S, et al. The microbiome in allergic disease: current understanding and future opportunities—2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. *J Allergy Clin Immunol*. 2017;139(4):1099-1110. https://doi.org/10.1016/j.jaci.2017.02.007
2. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol*. 2011;9(4):244-253. https://doi.org/10.1038/nrmicro2537
3. Naik S, Bouladoux N, Wilhelm C, et al. Compartmentalized control of skin immunity by resident commensals. *Science*. 2012;337(6098):1115-1119. https://doi.org/10.1126/science.1225152
4. Fyhrquist N, Ruokolainen L, Suomalainen A, et al. Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation. *J Allergy Clin Immunol*. 2014;134(6):1301. https://doi.org/10.1016/j.jaci.2014.07.059
5. Hanski I, von Hertzen L, Fyhrquist N, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. *PNAS*. 2012;109(21):8334-8339. https://doi.org/10.1073/pnas.1205624109
6. Rossi GA, Morelli P, Galietta LJ, Colin AA. Airway microenvironment alterations and pathogen growth in cystic fibrosis. *Pediatr Pulmonol*. 2019;54(4):497-506. https://doi.org/10.1002/ppul.24246
7. Erkinjuntti-Pekkanen R, Kokkarinen JI, Tukiainen HO, Reiman M, Terho EO. IgG antibodies, chronic

bronchitis, and pulmonary function values in farmer's lung patients and matched controls. *Allergy*. 1999;54(11):1181-1187. https://doi.org/10.1034/j.1398-9995.1999.00275.x

8. Card JW, Carey MA, Voltz JW, et al. Modulation of allergic airway inflammation by the oral pathogen *Porphyromonas gingivalis*. *Infect Immun*. 2010;78(6):2488-2496. https://doi.org/10.1128/IAI.01270-09
9. Zeeuwen PLJM, Ederveen THA, van der Krieken DA, et al. Gram-positive anaerobe cocci are underrepresented in the microbiome of filaggrin-deficient human skin. *J Allergy Clin Immunol*. 2017;139(4):1368-1371. https://doi.org/10.1016/j.jaci.2016.09.017

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