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ORIGINAL ARTICLE

Allergen-Specific Immunotherapy and Biologics

Dupilumab but not cyclosporine treatment shifts the microbiome toward a healthy skin flora in patients with moderate-to-severe atopic dermatitis

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Abbreviations: AD, atopic dermatitis; ASV, amplicon sequence variant; CCW, completely controlled weeks; DLQI, Dermatology Life Quality Index; EASI, Eczema Area and Severity Index; IGA, Investigator Global Assessment; oSCORAD, objective SCORing Atopic Dermatitis; POEM, patient-oriented eczema measure; rRNA, ribosomal RNA; TEWL, transepidermal water loss; WCW, well-controlled weeks.

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Abstract

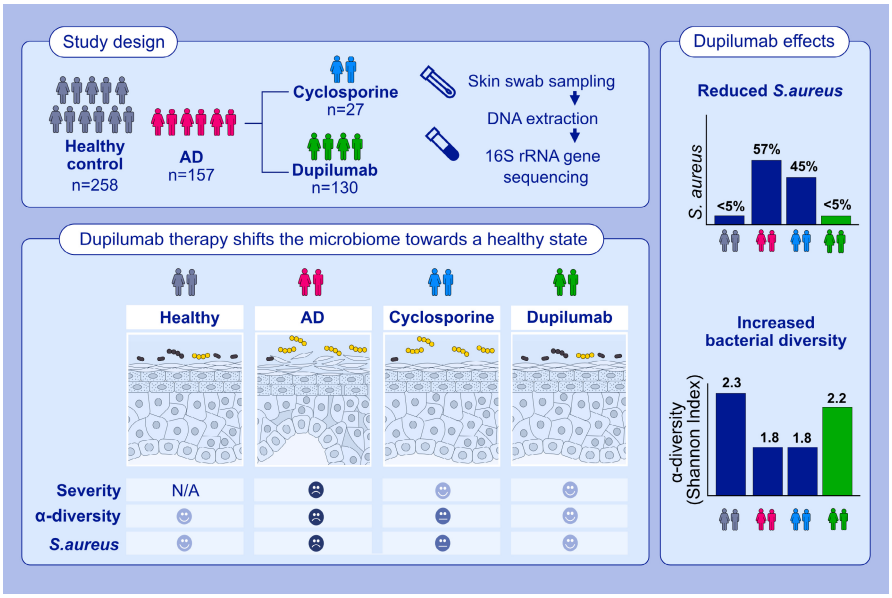
Background: Atopic dermatitis (AD) patients display an altered skin microbiome which may not only be an indicator but also a driver of inflammation. We aimed to investigate associations among AD patients' skin microbiome, clinical data, and response to systemic therapy in patients of the TREATgermany registry.

Methods: Skin swabs of 157 patients were profiled with 16S rRNA gene amplicon sequencing before and after 3 months of treatment with dupilumab or cyclosporine. For comparison, 16s microbiome data from 258 population-based healthy controls were used. Disease severity was assessed using established instruments such as the Eczema Area and Severity Index (EASI).

Results: We confirmed the previously shown correlation of *Staphylococcus aureus* abundance and bacterial alpha diversity with AD severity as measured by EASI. Therapy with Dupilumab shifted the bacterial community toward the pattern seen in healthy controls. The relative abundance of Staphylococci and in particular *S. aureus* significantly decreased on both lesional and non-lesional skin, whereas the abundance of *Staphylococcus hominis* increased. These changes were largely independent from the degree of clinical improvement and were not observed for cyclosporine.

Conclusions: Systemic treatment with dupilumab but not cyclosporine tends to restore a healthy skin microbiome largely independent of the clinical response indicating potential effects of IL-4RA blockade on the microbiome.

KEYWORDS
atopic dermatitis, dupilumab, inflammation, microbiome



GRAPHICAL ABSTRACT

One hundred fifty-seven atopic dermatitis patients were microbiome profiled before and after 3 months of systemic therapy with either dupilumab or cyclosporine. Dupilumab but not cyclosporine therapy largely restored a healthy skin microbiome by reducing *Staphylococcus aureus* abundance and increasing diversity. The microbiome after 3 months of dupilumab therapy resembled healthy controls.
Abbreviations: AD, atopic dermatitis; *S. aureus*, *Staphylococcus aureus*

1 | INTRODUCTION

The bidirectional crosstalk between bacterial commensals and host immunity is crucial for skin homeostasis, and many chronic inflammatory skin disorders are associated with shifts in the resident microbiota composition.[1] In particular, atopic dermatitis (AD) is characterized by a profound dysbiosis with a reduction of microbial diversity, an overgrowth of *Staphylococcus aureus*, and a relative reduction of commensal species.[2] Although it is yet unclear whether this imbalance is a cause or rather consequence of the disease, there is growing evidence that the microbiome harbors at least a proportion of causality in skin inflammation, as shown by microbiome transfer experiments in mouse models.[3–5] Furthermore, the microbiome is reciprocally associated with both epidermal barrier dysfunction[6,7] and T-cell-driven inflammation,[8–10] which are key pathophysiological features. In established disease, the altered skin microbiome appears to be an important mediator and trigger factor.[11] Thus, modulation of the skin microbiome is an attractive approach for improving AD or enhancing response to immunomodulating therapies.[12–15]

The currently most widely used targeted immunomodulating drug for moderate-to-severe AD is the anti-IL-4RA-antibody dupilumab.[16] Multiple studies have shown that dupilumab treatment leads to clinical improvements[17] along with reductions of inflammatory cell infiltrates and proinflammatory cytokine and chemokine expression in lesions[18] and an enhanced skin barrier function with decreased transepidermal water loss (TEWL) and normalized epidermal lipid composition.[19–24] Likewise, an increased microbial diversity and decrease of *S. aureus* abundance during dupilumab treatment was reported.[25] Moreover, clinical signs of skin infections (mostly caused by *S. aureus*) were less frequent in dupilumab treated patients as compared to controls in prospective placebo-controlled studies.[26,27] However, it remains unclear if and to which extent there might be a direct impact of IL-4RA-blockade on the skin microbiome beyond indirect effects through clinical improvements and resolution of lesions.

Here, we set out to characterize the microbiome of patients with AD on non-lesional and lesional skin and its dynamics over time under systemic therapy with dupilumab and cyclosporine.

2 | PATIENTS AND METHODS

2.1 | Study design and participants

Adult patients with moderate-to-severe AD from the TREATgermany registry were recruited between August 2017 and March 2019 and agreed to participate in an optional bioanalytics part. The bioanalytics module was approved by the Medical Faculty of the Christian-Albrechts-University, Kiel, Germany (B 261/16), as well as the responsible local ethics committees at participating sites. Details on the study design, clinical assessments, and biosampling were provided elsewhere.[28] For the current analysis, skin swabs were taken from $n = 157$ patients before and 3 months after the initiation of systemic therapy. A total of 130 and 27 patients received therapy with dupilumab and cyclosporine, respectively. About 95% of the dupilumab-treated patients reported having received any systemic treatment for AD (including systemic steroids) in the past, and 25% reported previous treatment with cyclosporine. Disease severity (as measured by the Eczema Area and Severity Index, EASI and Objective SCORing of Atopic Dermatitis, oSCORAD) was evaluated using established assessment instruments.[29] Response to therapy was defined as achieving at least a 75% improvement in EASI (EASI75-responder) 3 months after therapy initiation, while nonresponse was defined as not achieving at least a 50% improvement in EASI (EASI50-nonresponder) 3 months after therapy initiation. Details on patients are given in Table 1. For comparison, we used 16S rRNA gene sequencing data from antecubital skin swabs of 258 adults without skin or allergic diseases from the population-based PopGen cohort.[30]

2.2 | Sampling collection and microbial profiling

Participants were asked to avoid bathing/showering and application of any topical agents 24 h prior to the sampling visit. A 4 cm^2 area from the antecubital fossa, volar forearm, and lower back was firmly swabbed for at least 30 s. Immediately prior to collection, swabs (BD BBL Culture Swab EZ [Becton, Dickinson and Company]) were soaked in specimen collection fluid. Sampling negative controls were swabs exposed to ambient air for 5 s. DNA was isolated from samples with QIAamp UCP Pathogen Mini Kit on an automated QIAcube system (QIAGEN). The V1 and V2 variable regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) with the universal primer pair 27F and 338R. Sequencing was performed with MiSeq Reagent Kit v3 on the Illumina MiSeq (Illumina Inc.). For sample processing, FastQC (version 0.11.9[31]) was used for quality evaluation, DADA2 (version 1.10[32]) to dereplicate, remove bimeras and infer amplicon sequence variants (ASVs). For ASV classification the RDP classifier algorithm (version 16) was used.[33] For comparison, similarly sampled, extracted, and processed 16S rRNA sequence data from antecubital fossa skin swabs of 258 healthy participants of the PopGen cohort, with no history of atopic or chronic inflammatory disease, were included in this study (sampling, extraction, and processing are described in detail in Moitinho-Silva et al.[34]). Given the specific association between *S. aureus* and AD, we classified the Staphylococcus sequences to the species level using manual blast analysis to resolve unclassified Staphylococcus species with high confidence (see Table S1).

2.3 | Statistical analysis and microbiome diversity parameters

Given that the lesional status of skin areas has a considerably stronger impact on the skin microbiome in AD than the body site

TABLE 1 Baseline characteristics of included patients.

Characteristic	Dupilumab-treated patients ($n=130$)	Cyclosporine-treated patients ($n=27$)	Controls ($n=258$)
Female sex, no. (%)	50 (38%)	10 (37%)	108 (42%)
Age, mean ± SD	43 ± 16	41 ± 14	65 ± 11
BMI, mean ± SD	26 ± 5	24 ± 4	27 ± 4
oSCORAD, mean ± SD at baseline	43 ± 15	41 ± 9	-
EASI, mean ± SD at baseline	20 ± 13	15 ± 7.6	-
Responder/nonresponder	70/28	12/7	-
Physician-diagnosed asthma (%)	61%	49%	-
Physician-diagnosed rhinitis (%)	60%	62%	-
Total IgE, mean ± SD[a]	5247 ± 7203	2689 ± 1622	-
Allergen-specific IgE (% positive)[a]	86%	100%	-
Dustmite-specific (% positive)	70%	80%	-
Pollen-specific IgE (% positive)	80%	100%	-
Food-specific IgE (% positive)	34%	20%	-

Abbreviations: BMI, body mass index; EASI, Eczema Area and Severity Index; IgE, immunoglobin E; oSCORAD, objective SCORing of Atopic Dermatitis; SD, standard deviation.

[a]Physician-reported results from IgE testing in the past 12 months prior to inclusion into the registry, available from a subgroup of patients only.

and that we were interested to examine skin microbiome changes overall, for analysis within the TREATgermany study we combined all three sites on the patient level, and only differentiated between lesional and non-lesional skin.[35] For comparisons of patients to healthy population-based PopGen controls we restricted analyses to the antecubital fossa which overlapped between the studies.

Analyses used boxplots for visualization and the Wilcoxon rank-sum test, as well as PERMANOVA for significance testing. All statistical analyses were performed using R (R version 4.1.3 [2022-03-10][36]). The following general packages were used for data organization and visualization: tidyverse, phyloseq, MicroViz, microbiome, and vegan.[37–42] Shannon index and Bray–Curtis dissimilarities were calculated based on non-rarefied data.[43]

Differential ASV abundances were inferred from centered log ratio transformed data with linear models using the LinDA function implemented in the MicrobiomeStat R package.[44] Benjamini–Hochberg correction for multiple testing was employed where appropriate. If not indicated otherwise, data was presented as mean ± SD and an alpha cutoff of .05 was used to determine statistical significance for all tests.

3 | RESULTS

After quality control, skin microbiome data were available from 157 AD patients at baseline and Month 3. A total of 130 and 27 patients had received treatment with dupilumab and cyclosporine, respectively. The clinical characteristics of the patients are shown in Table 1. Response rates in the patient cohort examined here for EASI50, EASI75, and EASI90 at Month 3 were 78.5%, 53.9%, and 10% for dupilumab, and 77.8%, 44.4%, and 11.1% for cyclosporine, respectively. Samples from patients that used topical or systemic antibiotics 3 weeks prior to sampling were excluded, resulting in 833 samples with an average sequencing depth of ~25,000 reads.

3.1 | AD skin microbiome is characterized by higher Staphylococcus genus abundance, *S. aureus* presence and lower diversity than healthy control skin microbiome

The baseline bacterial genus composition of the antecubital fossa microbiome of AD patients in our study showed significantly elevated levels of Staphylococcus (64.7 ± 31.3% vs. 36.2 ± 25.5%, $p<.001$), Enhydrobacter (5.6 ± 12.7% vs. 4.8 ± 9.0%, $p<.001$), and Acinetobacter (5.3 ± 16.1% vs. 3.4 ± 8.4%, $p<.001$) compared to healthy controls (Figure S1A). Significantly lower abundances were found for Corynebacterium (6.1 ± 9.2% vs. 15.6 ± 12.8%, $p<.001$), Propionibacterium (3.5 ± 8.2% vs. 20.6 ± 18.8%, $p<.001$), and other low abundant genera (total of 14.9 ± 24.4% vs. 19.5 ± 17.3%, $p<.001$) in AD skin microbiomes compared to healthy control skin microbiomes. Furthermore, we observed a higher proportion of *S. aureus* positive samples in AD patients (98% in lesional, 87% in non-lesional skin) compared to healthy controls (28%), respectively (Figure S1B). In addition, we observed a significantly lower ($p<.001$) alpha diversity, which describes the diversity within a given microbiome sample, in AD skin microbiomes compared to healthy control microbiomes (Figure S1C). Comparing beta diversity, which describes the diversity between different microbiome samples, we found significantly different ($p<.001$) bacterial community structure with a distinct clustering between AD patients and healthy controls using PCoA (Figure S1D).

3.2 | Alpha diversity and *S. aureus* abundance correlate significantly with severity at baseline

At baseline, spearman correlation analysis between the most common clinical severity measurements included in this study and alpha diversity as well as *S. aureus* abundance resulted in significant, yet weak correlations with oSCORAD (−.18 and .22, respectively) and EASI (−.18 and .17, respectively, see Table S2).

3.3 | *S. aureus* abundance and alpha diversity do not show relevant differences between sampling sites

For our study three distinct skin sites of AD patients were sampled: antecubital fossa, volar forearm, and lower back. Comparing *S. aureus* abundance (see Figure S2A) and alpha diversity between the skin sites at baseline (see Figure S2B) we found no significant differences. Hence for our analysis focusing on only AD patients we pooled the skin samples regardless of skin site and corrected for skin site as a confounder in linear models.

3.4 | Skin lesional status has a major impact on the skin microbiome of AD patients

At baseline, we found a significantly lower abundance of *S. aureus* on non-lesional skin (11% median abundance, $p < .001$) compared to lesional skin (40% median abundance, Figure S3A). Interestingly, the median alpha diversity of non-lesional skin was higher, yet not significantly elevated compared to lesional skin ($p = .11$, Figure S3B). Bray–Curtis dissimilarity values differed significantly between non-lesional and lesional skin ($p < .001$), and visualization via PCoA revealed differences in clustering despite a substantial overlap (Figure S3C). Using linear models, we found significantly lower abundances of *S. aureus* (ASV1, $p < .001$, Log2FoldChange [LFC] −2.2, Figure S3D) and significantly higher abundances of *Staphylococcus hominis* (ASV17, $p = .03$, LFC 1.5) in non-lesional skin as compared to lesions. Comparing mean bacterial genus composition, we found lower abundances of Staphylococcus (62.3 ± 32.0% vs. 49.7 ± 33.8%) and Paracoccus (1.5 ± 4.6% vs. 1.3 ± 4.2%), and higher abundances of Acinetobacter (8.4 ± 20.5% vs. 10.4 ± 23.8%), Corynebacterium (4.6 ± 7.0% vs. 7.1 ± 10.8%), Propionibacterium (3.8 ± 8.2% vs. 5.1 ± 10.9%), Bacillus (5.7 ± 16.2% vs. 13.9 ± 28.2%), Enhydrobacter (6.0 ± 12.7% vs. 7.1 ± 16.0%) genera in non-lesional skin as compared to lesional skin, yet only the reduced Staphylococcus genus abundance reached statistical significance ($p < .001$, Figure S3E). Non-lesional skin displayed higher proportions of *Staphylococcus epidermidis* (+9.3%, $p < .001$), *S. hominis* (+4.8%, $p = .003$), and *Staphylococcus capitis/Staphylococcus caprae* (+2.7%, $p = .72$) compared to lesional skin, whereas *S. aureus* (−16.4%, $p < .001$) and *S. saprophyticus* (−2%, $p = .24$) showed lower proportions in non-lesional compared to lesional skin (Figure S3F).

3.5 | Dupilumab but not cyclosporine therapy shifts bacterial diversity toward a healthy state independent of clinical response

Patients who received dupilumab and cyclosporine did not show significant differences at baseline regarding alpha diversity (see Figure S4A), beta diversity (see Figure S4B), Staphylococcus genus abundance (see Figure S4C) or *S. aureus* abundance (see Figure S4D).

Alpha diversity (Shannon index) of lesional skin of responders, but not nonresponders to dupilumab was significantly increased compared to baseline ($p < .001$). Non-lesional skin overall did not show significant changes in alpha diversity. Cyclosporine treatment did not result in a significant change to alpha diversity regardless of skin lesional status or clinical response (Figure 1A).

Beta diversity indicated by Bray–Curtis dissimilarities showed significant changes on lesional skin in patients treated with dupilumab regardless of clinical response ($p < .001$), whereas only in responders a significant change was seen also for non-lesional skin ($p < .001$). In cyclosporine-treated patients beta diversity had shifted significantly in lesional skin, but in contrast to dupilumab-treated patients, PCoA did not reveal a clear direction of change (Figure 1B).

Compared to healthy controls, we observed a significant shift in the skin microbiome of responders to dupilumab therapy ($p < .001$), which was not observed in responders to cyclosporine therapy ($p = .94$). The shift resulted in a beta diversity closer to that of healthy controls compared to baseline (Figure 2A).

To identify therapy-specific signatures at the same clinical response we used linear models to find differentially abundant ASVs after 3 months of therapy. Comparing responders to dupilumab against responders to cyclosporine at 3 months we found significantly reduced abundances of *S. aureus* (ASV1, $p < .001$, LFC −5.5), *Paracoccus yeei* (ASV25, $p = .04$, LFC −3.1), and *Corynebacterium simulans* (ASV86, $p = .04$, LFC −1.5), whereas *S. hominis* (ASV28, $p = .04$, LFC 2.6 and ASV40, $p = .04$, LFC 2.4) was significantly increased in dupilumab responders compared to cyclosporine responders (Figure 2B).

3.6 | Dupilumab but not cyclosporine therapy decreases *S. aureus* abundance largely independent of clinical response

At Month 3 of therapy, Staphylococcus genus abundance had decreased significantly in responders to dupilumab both on non-lesional (−27%, $p < .001$) and lesional skin (−46%, $p < .001$). Nonresponders showed no significant reduction in overall Staphylococcus genus abundance (lesional: −15%, $p = .14$, non-lesional: −4%, $p = .24$, Figure 3A).

Compared to baseline, lesional skin showed a significantly reduced *S. aureus* abundance in both responders (−44.9%, $p < .001$) and nonresponders (−38.2%, $p < .001$) to dupilumab (Figure 3B). The change of *S. aureus* abundance did not correlate significantly with the decrease of EASI ($\rho < 0.2$). *S. aureus* abundance was also reduced

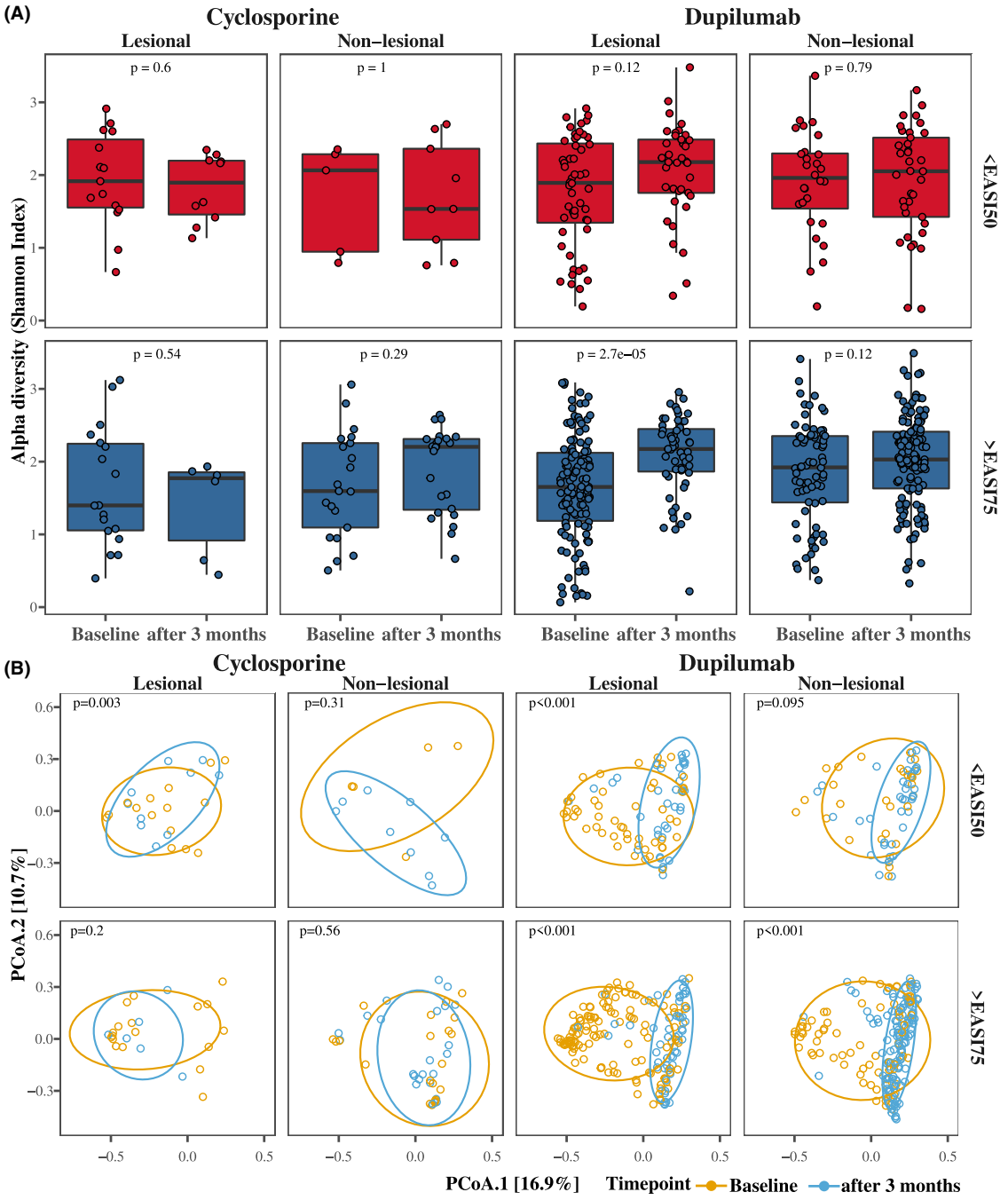


FIGURE 1 Effect of therapy on microbial diversity. Samples at baseline and Month 3 of therapy from responders (>EASI75, blue) and nonresponders (<EASI50, red) patients to either cyclosporine or dupilumab were split between lesional and non-lesional skin samples. All three sampled skin sites were pooled for this analysis. (A) Alpha diversity. Shannon index was used as a measurement of alpha diversity and Wilcoxon rank-sum test was used to infer significant differences between timepoints. (B) PCoA of beta diversity. Bray–Curtis dissimilarity was used to infer a distance matrix indicating beta diversity, using a PCoA for visual evaluation. PCoA axis indicated the proportion of variance explained by said axis. Ellipses indicate *t*-test 95% confidence interval. EASI, Eczema Area and Severity Index.

on non-lesional skin in both responders (−17.1%) and nonresponders (− 5.6%); however, this reduction was statistically significant only in responders ($p < .001$, Figure 3B). Cyclosporine treatment did not result in a significant reduction of Staphylococcus genus abundance neither *S. aureus* abundance regardless of skin lesional status or clinical response (Figure 3A,B).

3.7 | EASI90 responders to dupilumab therapy display a limited residual microbial signature

Patients that achieved an EASI90 response to dupilumab therapy showed very little differences in microbial composition to healthy controls after 3 months. Alpha diversity did not show significant

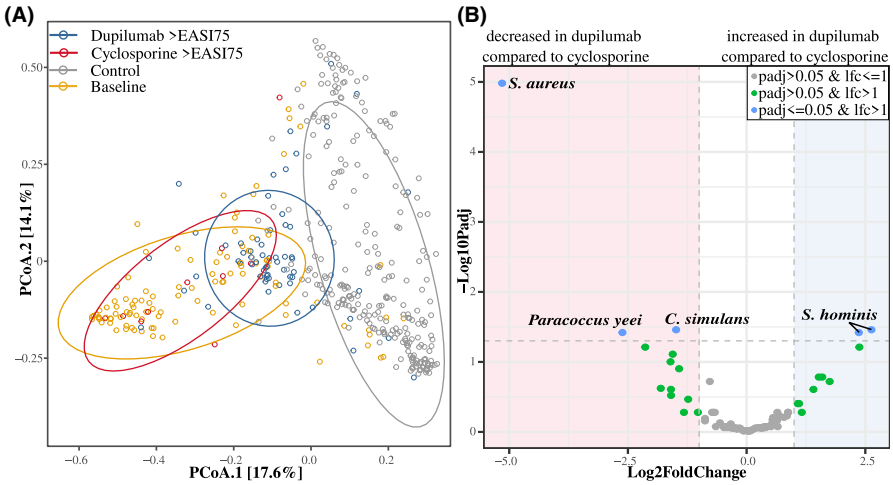


FIGURE 2 Comparative effect of therapy in context of drug specific changes and healthy skin microbiome. Samples of responders at Month 3 were compared to healthy controls and also between therapies. (A) PCoA of beta diversity. Bray–Curtis dissimilarity was used to infer a distance matrix indicating beta diversity, using a PCoA for visual evaluation. PCoA axis indicated the proportion of variance explained by said axis. Ellipses indicate *t*-test 95% confidence interval. (B) Differentially abundant ASVs. Differential ASV abundance was inferred form linear models and plotted as volcano plot with negative Log10 of Benjamini–Hochberg adjusted *p*-value over Log2FoldChange (LFC). Significantly differential ASVs with a |LFC| > 1 were indicated in blue and species level classification was indicated. Negative LFC indicated significantly decreased ASV abundance in the microbiome after 3 months of patients that received dupilumab compared to patient under cyclosporine (left side of the volcano plot), whereas positive LFC indicated significantly increased ASV abundance in the microbiome after 3 months of patients that received dupilumab compared to patients under cyclosporine (right side of the volcano plot). ASV, amplicon sequence variant; EASI, Eczema Area and Severity Index.

differences and using PCoA all samples from EASI-90-Responder patients after 3 months fell into the range of healthy patients (Figure 4A). Species significantly increased at baseline included *S. aureus* (ASV1, LFC 12.7, $p < .001$), *S. epidermidis* (ASV49, LFC 3.37, $p < .001$ and ASV80, LFC 3.38, $p < .001$) and *S. hominis* (ASV106, LFC 3.19, $p < .001$), whereas *Propionibacterium acnes* (ASV4, LFC −5.22, $p < .001$) and *Propionibacterium granulosum* (ASV199, LFC −3.03, $p < .001$) were significantly decreased (Figure 4B, left, see Table S3). At Month 3, only 36.4% of these ASVs still showed differential abundances (Figure 4B, right, see Table S4). The most pronounced change was observed for *S. aureus* abundance (ASV1, LFC decrease of 9.43). The differential abundance of *S. epidermidis* (ASV49, LFC decrease of 0.22) and *P. granulosum* (ASV199, LFC decrease of 0.68) remained largely unchanged.

4 | DISCUSSION

AD is a multifactorial disease that involves abnormalities in the immune and epidermal barrier of the skin and a microbial dysbiosis which is characterized by a reduction of microbial diversity and an overrepresentation of pathogenic *S. aureus*, the colonization with which drives skin inflammation through multiple pathways.[2,45] Blockade of IL-4RA with the monoclonal antibody dupilumab, in addition to improving AD severity, has been shown to suppress molecular markers of skin inflammation and to improve skin barrier function.[18,20] In a small phase 2 placebo-controlled trial, the microbial diversity increased, and the abundance of *S. aureus* decreased both in lesional and non-lesional skin of 26 patients treated with dupilumab.[25] More recent analyses showed lower rates of severe infections and non-herpetic skin infections in dupilumab-treated patients, suggesting that these microbiome changes may be protective against pathogenic species.[26,46] However, it remains unclear whether these observations are a secondary effect of clinical improvement or a direct effect of IL-4RA blockade.[47]

In our analysis of skin swabs collected from AD patients of the TREATgermany registry before and at 3 months after initiation of systemic treatment with either dupilumab or cyclosporine the baseline skin microbiome was consistent with findings from other studies and characterized by higher presence and abundance of *S. aureus*, reduced alpha diversity and a significantly different bacterial beta diversity compared to healthy skin.[2,6,10] Alpha diversity as well as *S. aureus* abundance showed a weak but significant correlation with measures of disease severity. Likewise, our findings confirmed an increase of the microbial diversity, a decrease of the abundance of *S. aureus*, and a higher abundance of *S. hominis* both in lesional and to a lesser degree also in non-lesional skin under treatment with dupilumab.[25] Interestingly, although these changes were more pronounced in patients showing a good clinical response (≥ EASI75 at Month 3), they were also seen in poor responders (<EASI50), that is, they were at least partially independent from the overall clinical response, and no corresponding changes were seen in patients treated with cyclosporine, including those with a good response to cyclosporine. This indirectly indicates potential direct effects of IL-4R blockade on skin microbiota. There are many potential mechanisms through which blockade of type-2-cytokine signaling apart from indirect effects could also directly impact skin microbiome composition, in particular, through direct effects on skin barrier

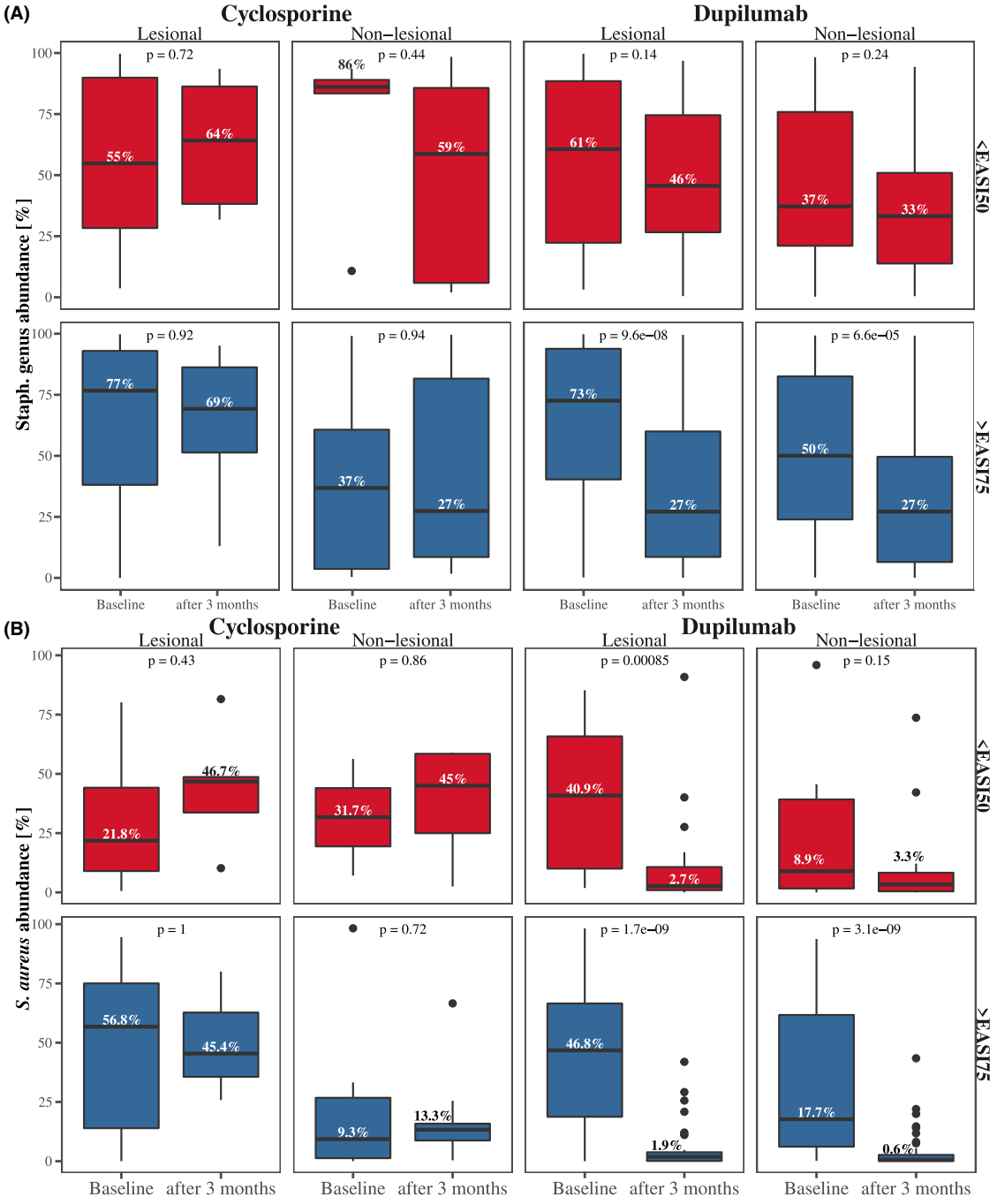


FIGURE 3 Effect of therapy on Staphylococci. Samples at baseline and Month 3 of therapy with either cyclosporine or dupilumab from responders (>EASI75, blue) and nonresponders (<EASI50, red) were split between lesional and non-lesional skin samples. All three sampled skin sites were pooled for this analysis. (A) Staphylococcus genus abundance. Relative Staphylococcus genus abundance was calculated per sample and median per responder status, therapy, lesional status, and timepoint was indicated as percentage. Statistical significance was tested using the Wilcoxon rank-sum test. (B) *Staphylococcus aureus* abundance. Relative *S. aureus* abundance was calculated per sample and median per therapy, lesional status and timepoint was indicated as percentage. Statistical significance was tested using the Wilcoxon rank-sum test. EASI, Eczema Area and Severity Index.

function such as upregulation of skin barrier protein expression, normalization of epidermal lipid composition, increased levels of natural moisturizing peptides, and increased production of antimicrobial peptides with reduced *S. aureus* uptake,[24,48,49] or skewing toward Th17 with enhanced *S. aureus* clearance.[19,50] However, the experimental setup employed here does not allow to draw robust conclusions on direct interactions, and the changes in microbiome composition observed under treatment with dupilumab may well be the consequence of both direct drug-specific effects and indirect effects of reduced cutaneous inflammation. In line with this, we observed the most pronounced restoration of bacterial diversity in patients with a strong clinical response (>EASI90). The diversity was

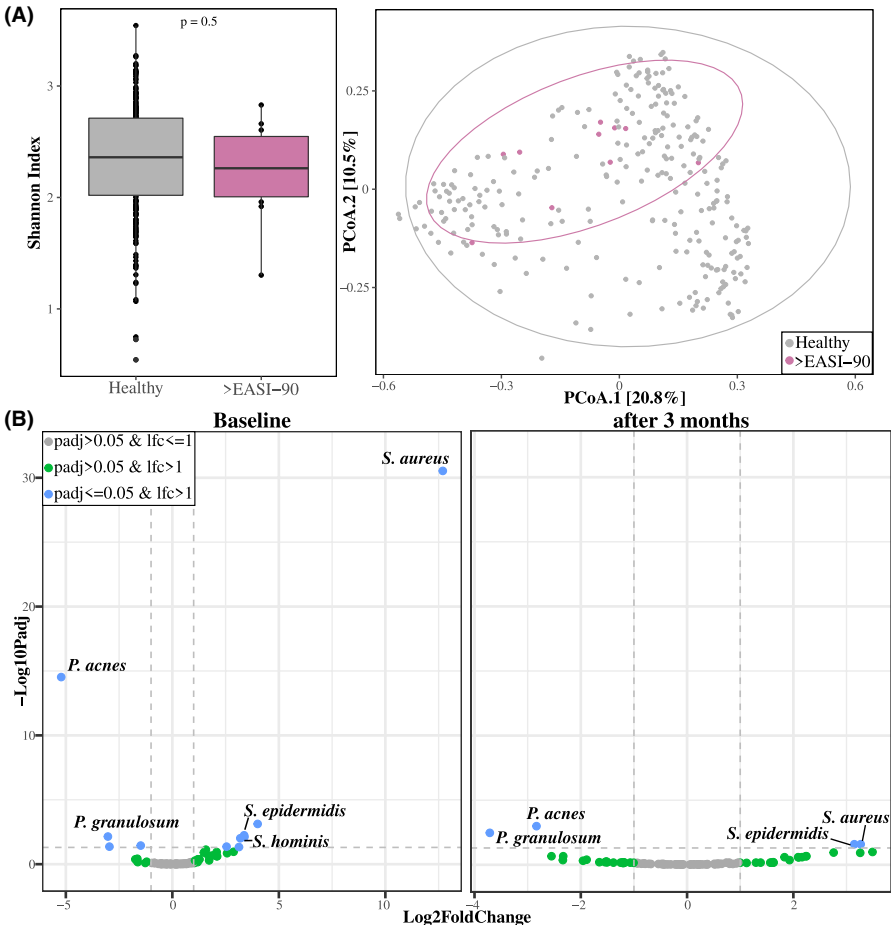


FIGURE 4 Residual signature of >EASI-90 responders to dupilumab. Samples of patients that achieved EASI-90 at Month 3 of dupilumab treatment were compared to healthy control samples. (A) Bacterial diversity. Shannon index was used as a measurement of alpha diversity and Wilcoxon rank-sum test was used to infer significant differences between timepoints (left panel), whereas Bray–Curtis dissimilarity was used to infer a distance matrix indicating beta diversity, using a PCoA for visual evaluation (right panel). PCoA axis indicated the proportion of variance explained by said axis. Ellipses indicate *t*-test 95% confidence interval. (B) Differentially abundant ASVs. Differential ASV abundance was inferred form linear models and plotted as volcano plot with negative Log10 of Benjamini–Hochberg adjusted *p*-value over Log2FoldChange (LFC). Significantly differential ASVs with a |LFC| > 1 were indicated in blue and species level classification was indicated. Negative LFC indicated significantly decreased ASV abundance in the microbiome of patients that received dupilumab compared to healthy controls, whereas positive LFC indicated significantly increased ASV abundance in the microbiome of patients that received dupilumab compared to healthy controls. Left plot shows baseline, whereas right plot shows Month 3 of dupilumab therapy; both compared to healthy controls. ASV, amplicon sequence variant; EASI, Eczema Area and Severity Index.

restored to levels found in healthy controls with a residual microbial signature which was characterized by only few AD-associated ASVs remaining differentially abundant.

Limitations of this analysis were the non-randomized non-controlled real-world setting with relatively small numbers of patients in both the dupilumab- and cyclosporine-treated groups who were allowed to use topicals as needed. However, efforts were made to minimize potential bias: skin swabs were requested from all registry patients before and 3 months after the start of systemic treatment, patients were asked to avoid application of topical agents at least 12 h before the examination visit and all samples were processed and evaluated in a blinded fashion. The use of a global EASI score for analysis rather than a target lesion score is a potential methodological limitation of this study; however, the swabbed lesional areas were representative of overall disease severity as quantified by EASI score, and we were particularly interested in associations with overall disease severity. Finally, analysis of DNA from skin surface microbial swabs does not assess bacterial survival or whether the microbes are metabolically active. Indeed, comparisons of *S. aureus* abundance measurements from lesional atopic to normal skin have shown that DNA assessments overestimate the capacity to culture *S. aureus* from healthy normal skin.[13] These observations suggest that healthy skin is more effective at killing *S. aureus* than AD skin. Indeed, type 2 cytokines have been shown to suppress antimicrobial peptide production from keratinocytes.[51] Future studies should examine the metabolic activity of bacteria and the host antimicrobial response of AD patients treated with dupilumab to assess the mechanism responsible for the improvement in the microbiome with treatment.

In conclusion, our observations from this large real-world observational study confirm and extend previous findings on skin

microbiome restoration associated with targeted type-2 cytokine blockade, which appears to be at least in part caused by direct drug effects beyond improvements of inflammation and clinical scores. Future studies should examine the overlapping and individual effects of IL-4 and IL-13 signaling as well as their blockade on cutaneous microbiota and the host antimicrobial response to assess the mechanism responsible for the improvement in the microbiome with treatment.

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CONFLICT OF INTEREST STATEMENT

The authors declare the following conflicts of interest: Thomas Werfel has received honoraria for lectures or scientific advice on atopic dermatitis from AbbVie, Almirall, Galderma, Janssen/JNJ, LEO Pharma, Leti, Lilly, Novartis, Pfizer, and Regeneron/Sanofi. Stephan Weidinger has received institutional research grants from LEO, Pfizer, and Sanofi; and has performed consulting work and lectures for AbbVie, Almirall, Boehringer, Eli Lilly, Galderma, Kymab, LEO Pharma, Pfizer, Regeneron, and Sanofi. Jochen Schmitt has received institutional grants from Novartis and Pfizer for scientifically initiated research; and has received honoraria for consulting from Sanofi, Lilly, Novartis, and ALK. Susanne Abraham has received lecture and/or consultancy fees from Novartis, LEO Pharma, Amgen, Lilly, Sanofi, Beiersdorf, Janssen, UCB, and AbbVie. Annice Heratizadeh has received lecture fees, advisory fees and/or travel grants from AbbVie, Karrer, Leo, Meda, Novartis, Sanofi-Genzyme, Ziarco, Beiersdorf, Pierre Fabre, Nutricia, Lilly, Sanofi, Almirall, Klinge Pharma, and Janssen. All of the other authors declare they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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