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ORIGINAL ARTICLE

Microbial dysbiosis in a mouse model of atopic dermatitis mimics shifts in human microbiome and correlates with the key pro-inflammatory cytokines IL-4, IL-33 and TSLP

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Abstract

Background Cutaneous bacterial dysbiosis is a characteristic hallmark of atopic dermatitis (AD), and it decisively influences the severity of the disease. Despite this, frequently used murine models of AD have not been characterized regarding the changes in skin microbiome communities.

Objective To analyse the skin microbiome of two frequently used murine models for AD for assessing their applicability in translational research.

Methods AD was induced in mice by topical application of calcipotriol or oxazolone. Following comparable elicitation of AD-like dermatitis, including IgE induction, the skin microbial communities were analysed and compared with human AD.

Results We detected critical differences in the microbiota composition of diseased skin. In contrast to calcipotriol treatment, application of oxazolone induced significant changes in the cutaneous microbiota and a drastic drop of bacterial richness. Furthermore, an expansion of *Staphylococci*, particularly *S. xylosus*, was observed in the oxazolone group, also displaying positive correlations with AD key markers including pH, TEWL, IL-4, TSLP and IL-33.

Conclusions In this article, we show that (a) the model of choice to investigate AD needs to be characterized for the cutaneous microbiota if applicable and (b) the oxazolone-mediated mixed Th1-Th2 immune response triggers microbiota-induced alterations which share similarities to dysbiosis in human AD and represents therefore a suitable model for translational research on AD if alterations of the microbiome are in the focus of the investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any conflict of interest.

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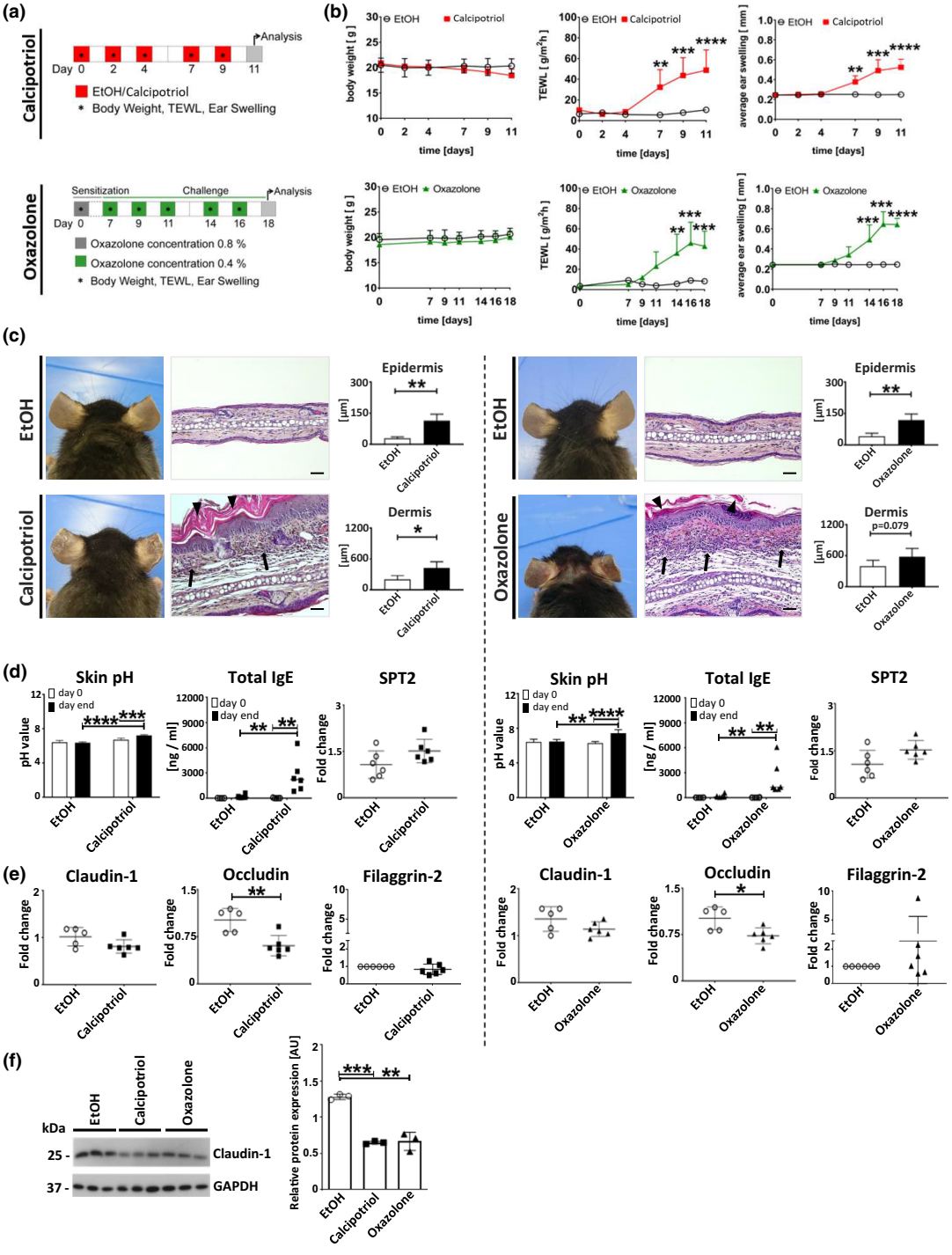
Introduction

The skin is our largest organ and fulfils various tasks. Besides the regulation of body temperature and moisture, it protects us against different environmental factors and represents the first barrier against harmful microbes, toxins or other potentially detrimental substances. The skin has its own microbiome composition, which acts together with skin cells and the immune system as a functional unit of the barrier defence.[1]



Under healthy conditions, the human skin microbiome is dominated by mainly *Firmicutes* and *Actinobacteria* such as *Staphylococci*, *Propionibacteria* and *Corynebacteria*. Furthermore, representatives of the phyla *Bacteriodetes* and *Proteobacteria* are commonly identified on the skin.[2,3] A breakdown of the balanced skin microbiome is commonly associated with skin diseases such as atopic dermatitis (AD), where *Staphylococcus aureus* and other pathogenic microorganisms colonize the skin, prevailing the healthy skin flora.[2,4,5] Alterations of skin microbiome in AD can have different origins. High levels of cytokines

Figure 1 Phenotypic characterization of two AD mouse models. (a) Experimental set-up: calcipotriol (red) and oxazolone (green). EtOH served as vehicle control. (b) Body weight (left), TEWL (centre) and ear swelling (right). (c) Visual representation (left), histology (H&E, centre) and size of epidermis and dermis (right) of mouse ears following calcipotriol (left panel) or oxazolone (right panel) treatments; arrows, inflammatory cells; arrowheads, parakeratosis. Scale bar = 50 μm. (d) left and middle: skin pH and serum total IgE at day 0 and day end (day 11 for calcipotriol and day 18 for oxazolone), right: serine palmitoyltransferase (SPT) expression values normalized to GAPDH. (e) Analysis of expression levels of barrier genes claudin-1, occludin and filaggrin-2 normalized to GAPDH/β-actin. (f) Western blot of claudin-1 from each experimental group (n = 3, left) and quantification of band intensity normalized to GAPDH (right). Data show one representative experiment from at least two and are expressed as mean ± SD. n = 6/group. (b) Two-way and (d and f) one-way ANOVA with Bonferroni post-test; (c and e) unpaired t-test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 vs. EtOH or as indicated. TEWL, transepidermal water loss.

such as IL-4/IL-13 but also IL-33 and TSLP inhibit the expression of the antimicrobial peptides (AMPs) human beta-defensin-2 (hBD-2) and hBD-3, which facilitates the colonization of the skin by pathogenic microorganisms.[6–10] Furthermore, changes in microbiome composition are driven by increased skin pH, typical for AD patients, which reduces the skin's antimicrobial abilities[11] and correlates with the significant decrease in stratum corneum hydration.[12]

Due to the heterogeneity and complexity of AD, several mouse models were developed to investigate different aspects of the disease. Transgenic or knockout mice were used to determine the biological relevance of individual genes or signalling pathways.[13–16] Moreover, mice spontaneously developing AD contributed to understand the role of specific genomic mutations in disease development.[17,18] Also, mouse AD models induced by vitamin D, its analogues, or oxazolone have been widely used to investigate mechanisms of disease progression.[19–21] Calcipotriol, through its binding to the vitamin D receptor expressed on keratinocytes, induces a TSLP-triggered inflammatory cascade, characterized by inflammatory cell recruitment and release of pro-inflammatory cytokines leading to skin barrier dysfunction.[22] Instead, repeated oxazolone application induces a systemic mixed Th1-Th2-mediated allergic response, whereby IFN-γ and TNF-α release play a critical role in IL-33 induction in keratinocytes, evoking a skin inflammatory response comparable to human AD.[21,23] However, whether these models reproduce also skin microbiome shifts as observed in human AD is still unknown.

We therefore investigated whether the induced phenotype changes in both AD models resulted in similarly altered microbiota as in human AD. Such models would be suitable for translational research on AD if microbiome alterations are in focus of the study.

Materials and methods

Human

Eight patients with moderate-to-severe AD and eleven healthy matched controls were selected according to the inclusion/exclusion criteria approved by the regional government of Oberbayern. Medical ethical committee approval (Approval n. 473/16 S) and individual written informed consent were obtained prior to sample collection. Patients were examined by an experienced dermatologist at the dermatology hospital (Technical University of Munich). Skin swabs were collected from AD lesions at the arm fossa and corresponding sites from healthy participants and processed for 16S amplicon sequencing as previously described.[24]

Mice

Eight-week-old female C57BL/6J mice (Charles River, Sulzfeld, Germany) were kept under controlled humidity, temperature and pathogen-free conditions with a 12-h light–dark cycle. To avoid cage effects, animals obtained from the same breeding compartment were distributed randomly to experimental groups. Experiments were conducted according to the European Convention for Animal Care and Use of Laboratory Animals and were approved by local ethics committee and government authorities (Approval no. 55.2-1-54-2532-198-2016).

Experimental protocols

Calcipotriol (Sigma-Aldrich, St. Louis, MO, USA), 14 μL, 1.125 nmol in 100% EtOH (Merck, Darmstadt, Germany) was topically applied five times on each mouse ear. The model was adapted from Li *et al.*[19] (Fig. 1a, top). Oxazolone (Sigma-Aldrich), 20 μL, was topically applied six times on each mouse ear at a final concentration of 0.8% (w/v) for sensitization and of 0.4% (w/v) for challenge in 100% EtOH (Merck). The model was adapted from Man *et al.*[21] (Fig. 1a, bottom). As vehicle control, the same volume of EtOH as of applied substance was used for both models. At the beginning and at the end of experiment, blood and ear skin microbiome samples were collected and skin pH was recorded. At every treatment, time point, body weight, TEWL and ear thickness were assessed. At the end of experiment, ear biopsies were taken for mRNA, protein and histological analysis. Microbial DNA was extracted from the collected skin swabs and analysed using 16S-targeted amplicon sequencing.

Data acquisition and statistical analysis

Graphical representations and statistics related to mouse AD models were done by Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Analysis of body weight, TEWL and ear swelling was performed by two-way analysis of variance (ANOVA) with post hoc Bonferroni test. Analysis of cytokines and protein expression

was performed by Student's *t*-test. Data were presented as mean ± SD. $*P < 0.05$; $**P < 0.001$; $***P < 0.005$; $****P < 0.0001$. Methodological details are described in the online supplement.

Results

Comparison of the AD-like phenotype induced in two different murine models

In this study, two different AD models were investigated, a non-antigen-specific (calcipotriol, Fig. 1a, top) and an antigen-specific (oxazolone; Fig. 1a, bottom). Application of both substances on mouse ears induced a steady increase in transepidermal water loss (TEWL) and ear swelling compared to controls (EtOH) with no alterations in body weight (Fig. 1b). Treatment of ear skin with either substance led to the characteristic dry and scaly skin (Fig. 1c, respective left panels) with typical hallmarks of human AD, including parakeratosis, hyperplasia, spongiosis and inflammatory cell infiltration (Fig. 1c, middle panels). In particular, infiltration of polymorphonuclear leucocytes, especially of eosinophils, and macrophages was strongly increased in both models compared with vehicle (EtOH), as previously reported.[21,22,25,26] Moreover, a slight increase in $CD4^+$ lymphocytes was accompanied by scant immunolocalization of natural killer cells in both models, probably due to the delayed time point of biopsy after last allergen challenge[27] (Fig. S1, Supporting Information).

In addition, a significant increase in epidermal thickness was observed in both models, while an increase in dermal thickness was only significant in calcipotriol-treated animals (Fig. 1c, right panels). Skin pH and serum total IgEs were significantly increased in both models (Fig. 1d), and a strong correlation between pH and TEWL values was noticed (Fig. S2, Supporting Information, left). Serine palmitoyltransferase (SPT-2) expression showed a mild increase in both models (Fig. 1d). Skin barrier proteins occludin and claudin-1 were clearly decreased following the treatments, while filaggrin-2 was unaffected (Fig. 1e). Contrarily, loricrin and involucrin were noticeably upregulated (Fig. S3, Supporting Information). Western blot analysis of ear lysates showed furthermore a decrease in claudin-1 protein in both models, confirming the skin barrier impairment (Fig. 1f).

Analysis of cytokines in ear tissue lysate revealed a significant increase in IFN-γ and IL-4 in both models compared with EtOH at protein as well as mRNA levels (Figs 2a,S4a, Supporting Information). While IL-33 and TSLP protein increased only in tendency and were positively correlated in both models (Figs 2a,S2, Supporting Information, right), at mRNA level, only the expression of TSLP was significantly increased, particularly by calcipotriol (Fig. S4b, Supporting Information). Although the pro-inflammatory cytokine IL-6 was similarly upregulated by both treatments, higher levels of TNF-α were induced by oxazolone at both mRNA and protein levels (Figs 2a,S4b, Supporting Information). Furthermore, IL-1β expression levels were significantly upregulated, especially in oxazolone-treated animals (Fig. S4b, Supporting Information). As the alteration of AMPs was largely reported in AD,[8] we measured their expression in ear tissue lysates. Our data showed that both treatments are potent inducers of most antimicrobial defensins, although only at the mRNA level, namely Defb1/3/4, but not Defb2 (Fig. 2b).

Treatment with calcipotriol and oxazolone differentially impacts skin microbiome diversity

The skin microbiome of AD patients is characterized by a loss of diversity and an expansion of *S. aureus*.[28] Our aim was to investigate whether the evaluated mouse models represent the human situation. We first performed a β-diversity analysis, a distance-based approach for inter-group comparison. Data revealed a significant microbiome modulation with both models (Fig. 3a). Application of merely EtOH vehicle also induced significant microbial alterations, but as displayed in the pairwise β-diversity plots (Fig. S5a–f, Supporting Information), with significant differences in comparison with the calcipotriol or oxazolone group. In addition, both treatments resulted in distinct microbial compositions, indicating that they are able to induce skin microbiome changes regardless of ethanol effects. We next analysed richness and Shannon index as indicators of the microbial α-diversity. While calcipotriol treatment or EtOH caused no differences in richness, oxazolone-treated animals showed a significant decrease (Fig. 3b). Shannon diversity tended to be reduced following oxazolone application without reaching significance (Fig. S5g, Supporting Information). These findings indicate that both treatments affect the murine cutaneous microbiota, but only oxazolone application drastically decreased the α-diversity.

Oxazolone-treatment induces an AD-like microbiome with a decreased diversity and expansion of *Staphylococcus* population

To further investigate the influence of oxazolone or calcipotriol on murine skin microbiome, we explored changes in the relative abundances of bacterial taxa. Notably, we observed an increase in *Actinobacteria* with all treatment groups compared with untreated controls. *Proteobacteria* and *Bacteroidetes* were respectively enriched in ethanol- and calcipotriol-treated animals, whereas *Firmicutes* dominated the skin landscape of the oxazolone group (Fig. 3c). At genus level (Figs 3d,S6a, Supporting Information), an increase in *Xanthomonas*, most prominent of *X. campestris* and *X. dyei*, could be detected following EtOH, at the expense of *Thiolapillus HQ191085* and *Novosphingobium sp* (Fig. S7a,b, Supporting Information). Moreover, *Fusimonas* and *Staphylococcus* relative abundances were respectively increased upon calcipotriol and oxazolone treatments (Fig. 3d).

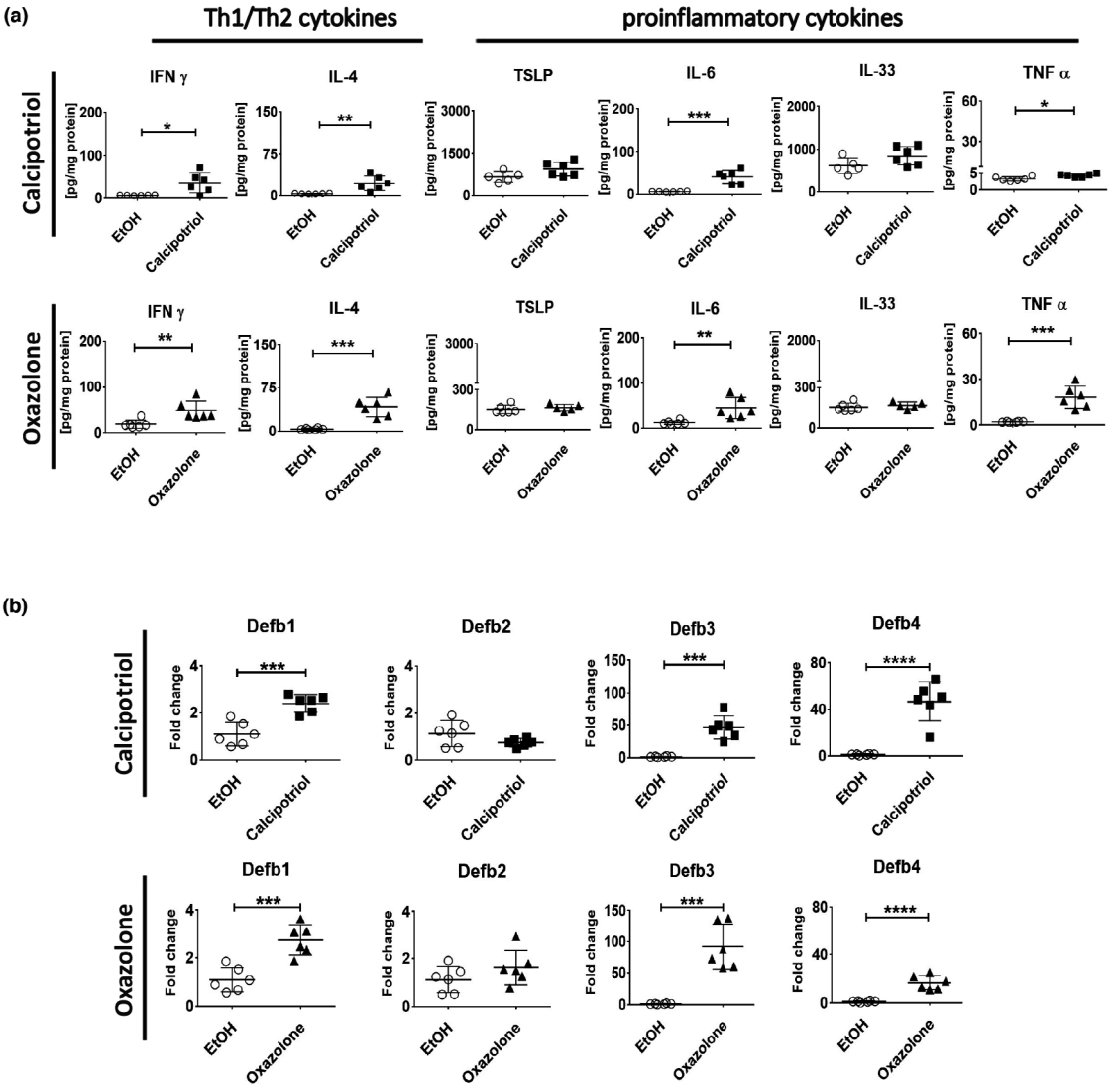


Figure 2 Analysis of cytokines and antimicrobial peptides in ear tissue lysates. (a) Measurement of Th1, Th2 and pro-inflammatory cytokines in ear tissue lysates. Diagrams represent analysis of calcipotriol-treated animals (upper row) and oxazolone-treated animals (lower row) compared with EtOH (vehicle control). Cytokine levels were normalized to total protein content. (b) mRNA level of murine defensins beta 1-4 in calcipotriol-treated animals (upper row) and oxazolone-treated animals (lower row) compared with EtOH (vehicle control). Each data point represents an individual mouse. $n = 6$/group. Data are expressed as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired t-test).

To verify whether our models reproduce skin microbiome shifts observed in human AD, we next compared skin swabs of treated animals with samples from AD patients and their matched controls. In parallel to the strong microbiome shifts observed in human AD compared with healthy volunteers' skin, AD animal models displayed clear differences to the control group (Fig. 4a). We also noticed following oxazolone application, a slight microbiome shift towards the atopic group in comparison with calcipotriol (Fig. 4b,c). Although humans and mice belong to different groups of mammals and their skin microbiome samples thus clustered separately (Fig. 4a), the oxazolone group exhibited remarkable similarities with AD hallmarks, namely the decrease in α-diversity (Fig. 4d) and expansion of *Staphylococci* (Fig. 4e). The later observation was supported by linear discriminant analysis (LDA) showing a strong association of the *Staphylococcus*

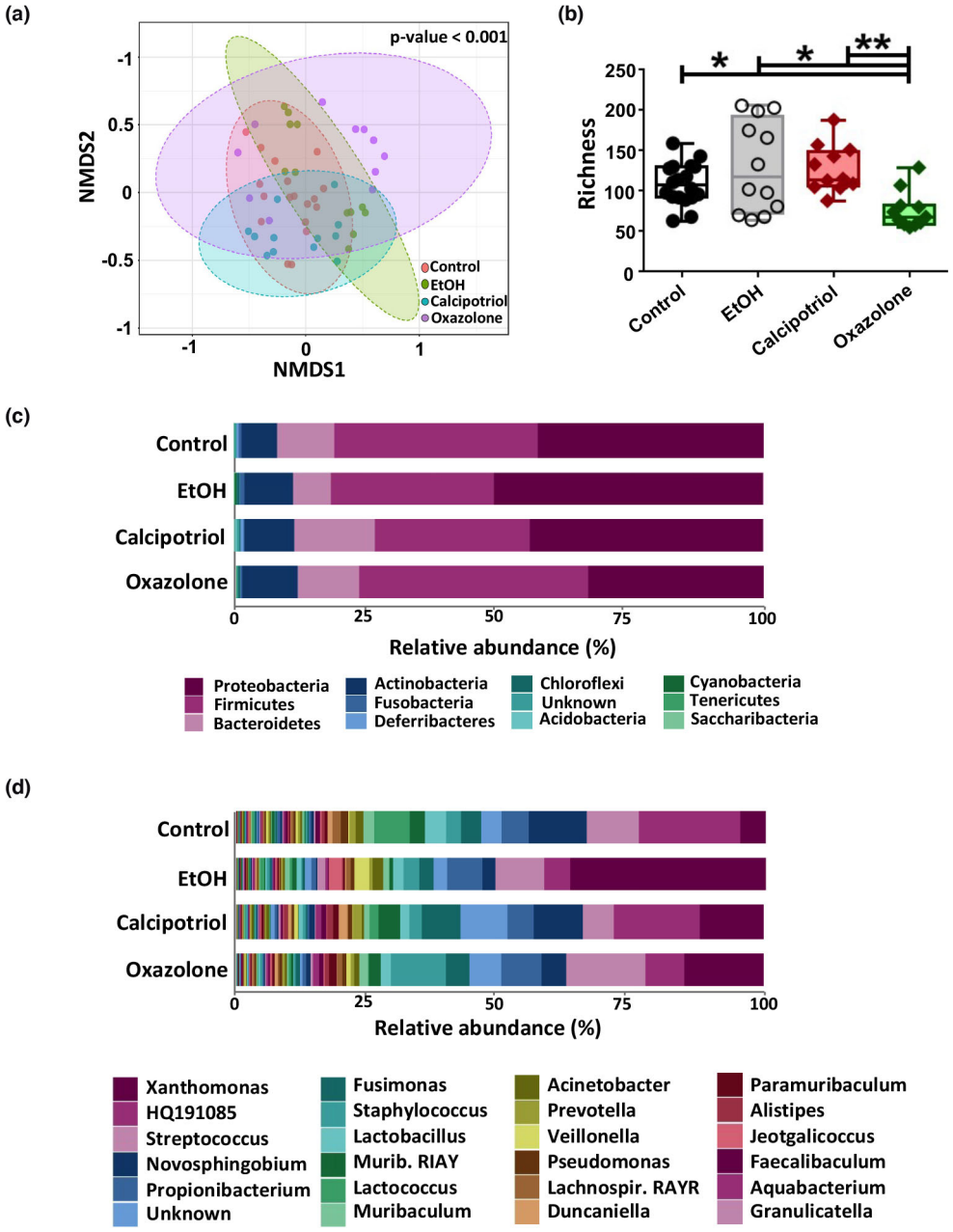


Figure 3 Diversity analysis in ear skin of calcipotriol- and oxazolone-treated animals. (a) Non-metric multidimensional scaling (NMDS) plot of β-diversity analysis shows the microbiome changes in the experimental groups compared with the untreated control. The Bray–Curtis index was used to calculate similarity between samples and the PERMANOVA (Permutational MANOVA) to test the statistical significance based on the distance matrix. (b) α-Diversity analysis expressed in terms of richness (number of different OTUs /sample). Data are presented as mean ± SD. Statistical significance of the α-diversity values was calculated using Kruskal–Wallis and Wilcoxon–Mann–Whitney tests. (c) Bar chart of relative bacterial abundance at phylum level. (d) Taxonomy analysis of taxa relative abundance displayed at genus level. Multiple test corrections were performed with the Benjamini and Hochberg procedure. The displayed data originate from combining two independent experiments. n = 11–18/group. $^{**}P < 0.001$, $^{***}P < 0.005$.

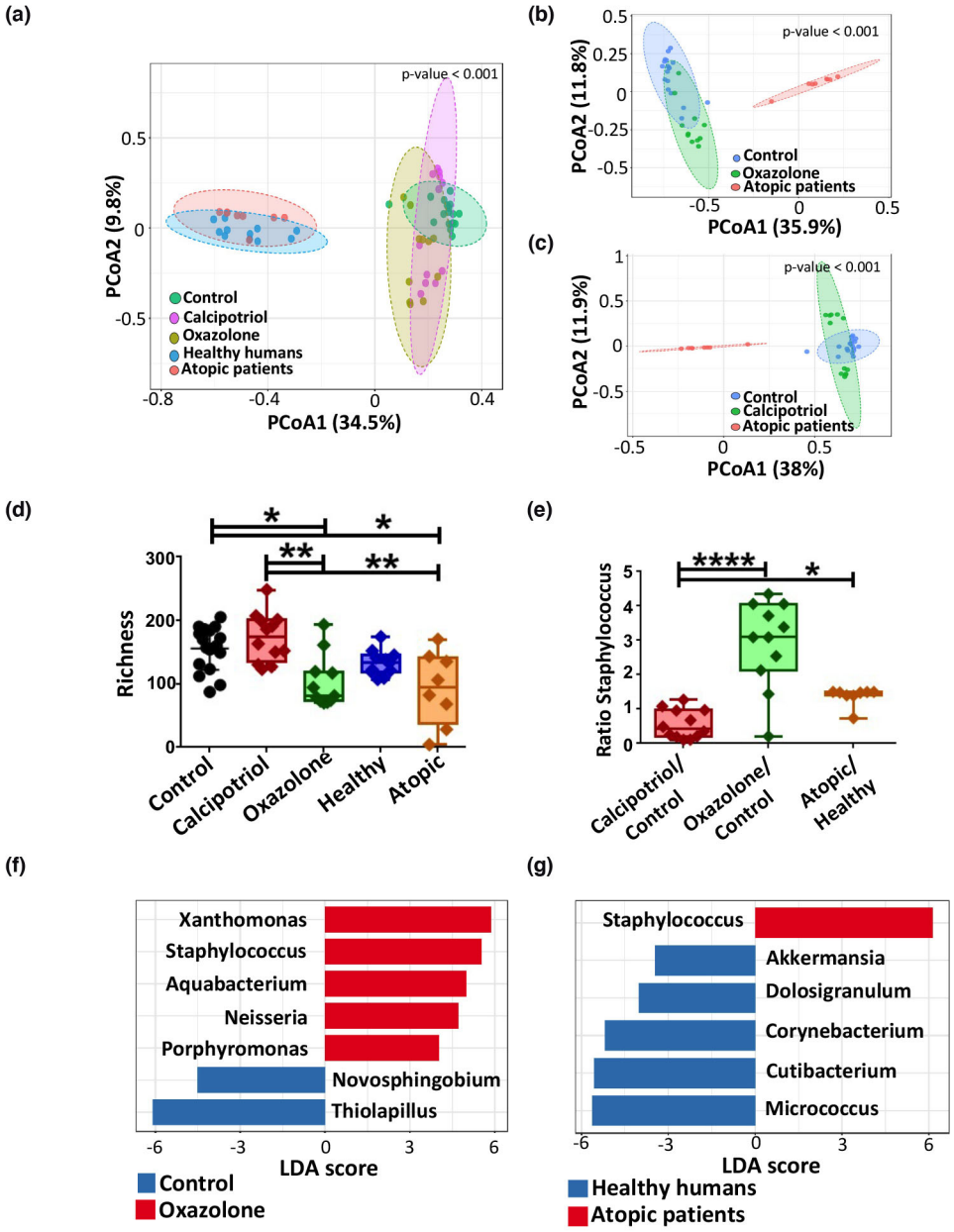


Figure 4 Comparison of AD models and human AD skin microbiota. (a) Principal Coordinates Analysis (PcoA) plots displaying microbiome comparisons of untreated control mice, calcipotriol- and oxazolone-treated animals, in addition to atopic patients and healthy matched volunteers. (b) Comparison of atopic patients with oxazolone and (c) calcipotriol-treated animals. (d) α-Diversity expressed as richness (Numbers of OTUs) for the compared groups. (e) Ratio of *Staphylococci* in treated and untreated mice as well as atopic and healthy participants. (f) Linear discriminant analysis (LDA) displaying at genus level taxa associated with control and oxazolone groups. (g) LDA plots of genera associated with atopic and healthy participants. Control (n = 18), calcipotriol (n = 12), oxazolone (n = 11), atopic patients (n = 8), healthy participants (n = 11). *P < 0.05, **P < 0.01, ****P < 0.0001.

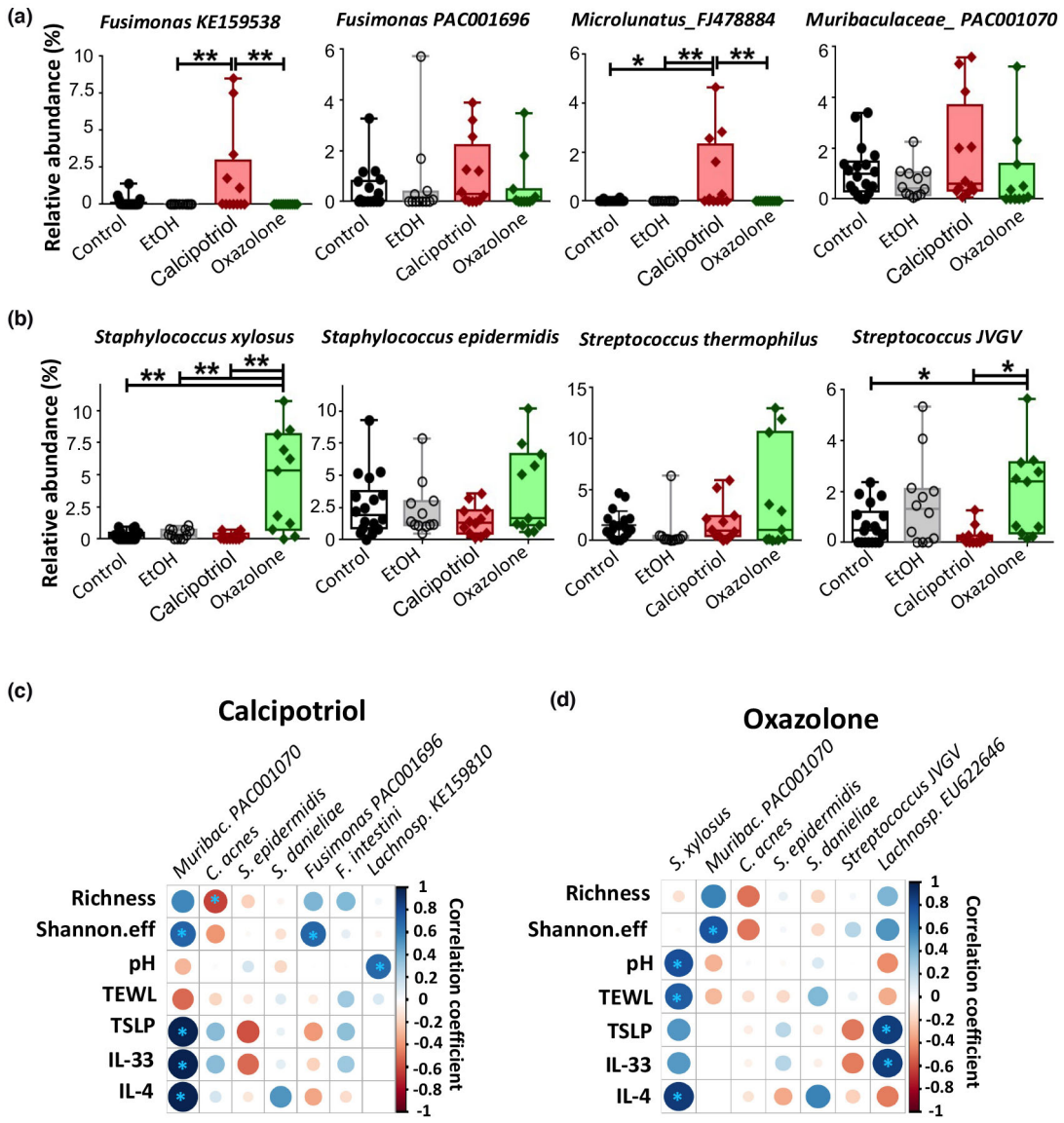


Figure 5 Relative abundance of key taxa and their correlations with AD parameters. (a–b) Box plots of relative abundance showing taxa with major shifts among the (a) calcipotriol- and (b) oxazolone-treated animals. Statistical significance was calculated using Kruskal–Wallis and Wilcoxon–Mann–Whitney tests. Multiple test corrections were performed with the Benjamini and Hochberg procedure to adjust *P*-values (n = 11–18/group, *$P < 0.05$, **$P < 0.001$, ***$P < 0.005$). (c) Correlogram showing Pearson's correlations between epidermal key taxa, α-diversity and AD markers in calcipotriol-treated mice and (d) in oxazolone-treated mice. Blue and red circles designate respectively positive and negative correlations. Empty boxes indicate that no correlation was detected (n = 5–18/group).

group with oxazolone treatment (Fig. 4f), in line with human AD (Fig. 4g).

Lachnospiraceae representatives *Fusimonas* and *Muribaculaceae RIAY* as well as *Microlunatus* were increased following calcipotriol treatment (Figs 5a, Fig. S6b, Supporting Information). Interestingly, an expansion of *Staphylococci* was only detected in oxazolone-treated animals with *S. xylosus* and *S. epidermidis* as dominant species. Additionally, an increase in *Streptococci* relative abundance, particularly *S. thermophilus* and *Streptococcus JVGV*, was observed (Figs 5b,S6c, Supporting Information). *Cutibacterium acnes*, often reduced on AD lesions, did not display a decrease in any of the experimental groups (Fig. S7c,

Supporting Information). Therefore, oxazolone treatment resulted in a skin microbiome shift similar to that observed with AD lesions.

Correlation analysis reveals *S. xylosus* as a dysbiosis marker of the oxazolone-induced AD-like phenotype

To verify whether the altered abundances of bacteria we found are indeed the most relevant for our AD models, we performed LDA analysis to identify OTU predictors that best characterize the compared groups (Fig. S8, Supporting Information). Thiolapillus *HQ191085* and *Novosphingobium sp.* were dominant in the control group while *Fusimonas KE159538* and *S. xylosus* were respectively abundant upon calcipotriol and oxazolone treatments. These taxa showing high LDA scores ranging between 5 and 5.9 appear as OTU predictors that best define the control and treatment groups, whereas *X. campestris* and *X. dyei* seem characteristic of the vehicle-treated group. Since bacterial growth is always related to ideal environmental conditions, we sought to explore the relation between key taxa and skin parameters, as comparable connections have been demonstrated in AD patients.[29,30] Correlation analysis between taxa and metavariables revealed for calcipotriol a positive correlation between *Muribaculaceae 001070* and α-diversity, reaching significance with Shannon index, while a negative association was detected with pH and TEWL. This specie also showed significant positive associations with TSLP, IL-33 as well as IL-4 and its abundance noticeably increased following calcipotriol application pointing towards a possible role in this model's pathogenesis (Fig. 5c). Interestingly, correlation data from oxazolone-treated mice corroborate results from human studies, as we detected significant positive correlations between *Staphylococci* abundance, represented by *S. xylosus* with pH, TEWL and IL-4 expression levels, in addition to positive associations with TSLP and IL-33. This strain could be linked to the oxazolone-induced skin barrier damage and dysbiotic state (Fig. 5d). Oxazolone-treated mice also showed a positive correlation between *Muribaculaceae 001070* and Shannon index (Fig. 5d).

Discussion

The need for relevant animal models for AD covering diverse aspects of disease pathogenesis is increasing.[31] Here, we used two commonly reported AD models to characterize alterations in skin microbiome along with AD phenotype. Treatment with both calcipotriol and oxazolone led to augmented epidermis and dermis thickness resulting in ear swelling and a progressive increase in TEWL, as previously reported.[22,32] Downregulation of occludin and to a lesser extent of claudin-1, both reported to be critical for cell-to-cell adhesion of keratinocytes, confirmed the skin barrier alteration in both models.[33] On the contrary, the observed upregulation of involucrin and loricrin contrasts some reports on human AD,[34,35] but is in line with other studies showing their upregulation in affected patients with extension of their expression to specific epidermal layers.[36,37] In addition, we detected a slight increase in SPT-2 following both treatments, which is known to be upregulated upon skin barrier disruption.[38] Besides the increased skin pH and serum IgE,[39] inflammatory cell infiltration, in particular of eosinophils and macrophages, was confirmed histologically in both models, as shown before.[21,22,40] An increase in keratinocyte-derived TSLP was observed, but only significantly at mRNA levels, especially in calcipotriol-treated animals. Indeed, TSLP is essential for the calcipotriol-induced AD phenotype, while IL-33 signalling is dispensable.[22,41] The levels of IL-6 and IFN-γ were comparable in both models, whereas the expression of IL-1β and IL-4 was higher in oxazolone-treated mice. IL-4 was reported to play a critical role in the T-cell response of the oxazolone model.[42–44]

The role of antimicrobial peptides at the interface between skin microbiome and the immune system has been extensively documented.[8,21,45,46] In chronic AD, high amounts of Th2 cytokines lead to a reduced expression of antimicrobial peptides hBD-2 and hBD-3[8] and weaken the skin barrier, increasing its susceptibility to allergens and infections. Our data instead showed that both treatments are potent inducers of antimicrobial defensins, namely Defb1, Defb3 and Defb4. These results corroborate previous reports on calcipotriol-[45,46] and oxazolone-induced AD models,[21] displaying increased AMP levels. Interestingly, an upregulation of hBD-2 and hBD-3 has been reported in human acute AD lesions compared with healthy skin[47] or to chronic AD lesions.[8] This indicates that both AD models represent the acute phase of the disease.

In vivo analyses of skin microbiome have been mostly performed in genetically modified animal models to investigate the influence of induced mutations on microbial communities[4,48,49] or have focused on certain microorganisms, primarily *S. aureus*, as a hallmark of disease progression.[50,51] Here, we used a different approach, investigating the entire skin microbiome of wild-type mice in relation to AD development, given the importance of various contributors of the dysbiotic flora in affecting the course of the disease.[4,52] It is known that AD patients have a reduced microbiome diversity, while the number of representatives of the *Staphylococcus* group that colonize AD skin is increased.[53,54] We thus compared the lesional skin microbiome from atopic patients with our AD mice models. The oxazolone group exhibited remarkable similarities with human AD hallmarks, namely the decrease in α-diversity and expansion of *Staphylococci*. *S. aureus* was absent from mouse skin regardless of the treatment, due to specific pathogen-free conditions met in our animal facility, although it has been reported to spontaneously colonize the skin of a genetically modified AD mouse model under SPF regulations allowing its presence.[4] It is well established that *S. aureus* plays an important role in AD physiopathology particularly in its severe forms. Nevertheless, this pathogen was only detected in 24%

of affected AD children (n = 59) and in about 20% of adults patients (n = 69).[55] The overgrowth of other *Staphylococci* has also been linked to AD severity, particularly *S. epidermidis*.[56,57] Similarly, *S. xylosus* was detected in moderate-to-severe forms of children AD as reported in the MPAACH study[58] and other cohorts.[54,59] This strain has also been largely documented to colonise mouse skin under inflammatory conditions.[59,60] The expansion of *S. xylosus*, paralleled by a drop of microbial diversity and correlation with AD key markers including IL-4, TSLP and IL-33, supports the conclusion that the oxazolone model mimics an AD-like dysbiosis. Besides, the observed increase in *S. epidermidis* on oxazolone-induced lesions is in line with findings showing its overgrowth on human AD lesions.[53,56,61] Conversely, the decrease in *C. acnes* relative abundance reported for patients with atopic dermatitis[62,63] was not observed in our models.

Members of the *Lachnospiraceae* (*Fusimonas*) and *Muribaculaceae* groups were particularly increased upon calcipotriol treatment. These bacteria are among the most abundant taxa of the human and animal gut microbiota.[64] Their overgrowth on the calcipotriol-treated skin could be due to possible loss of antagonistic bacteria, occurring in the dysbiotic shift.

A limitation of our study was that the vehicle (EtOH) application also modulated skin microbiota. This partly derives from the disinfecting and degreasing properties of EtOH, leading to disturbance of the local microenvironment. Nevertheless, the observed differences of taxa relative abundances and distinct microbiome profiles between groups indicate that EtOH is not the main driver of the observed microbiome changes. Moreover, the application of EtOH neither reduced the α-diversity nor induced tissue inflammation. Further limitations are the incapacity of animal models in general to reflect the complete picture of the human condition and the merely descriptive approach we have employed. In light of our obtained results, we conclude that only oxazolone treatment mediates a mixed Th1-Th2 immune response reshaping the skin microbiome in favour of *Staphylococci* expansion, as seen on human AD lesions.[65] Therefore, this model is suitable for translational research on AD if alterations of the microbiome are in the focus of the investigation. Based on the similarity between murine and human microbiome,[66] the use of chosen AD models may detect aspects of skin dysbiosis involved in the onset, development and aggravation of disease in humans.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Histological and immunohistochemical analysis of murine ear tissue
Fig. S2. Linear correlations between AD markers in calcipotriol- and oxazolone treated mice
Fig. S3. Analysis of barrier gene relative expression in ear tissue lysates
Fig. S4. Analysis of cytokines relative expression in ear tissue lysates
Fig. S5. Diversity variations among experimental groups
Fig. S6. Taxonomy analysis of taxa relative abundances displayed at genus level
Fig. S7. Taxa with major shifts following ethanol application or unaffected by any of the treatments
Fig. S8. Linear discriminant analysis (LDA) displaying OTUs markers with major shifts among experimental groups
Table S1. Murine primer sequences used for real-time polymerase chain reaction
Supplementary Material. Supplementary methods