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Topical Mupirocin Treatment Reduces Interferon and Myeloid Signatures in Cutaneous Lupus Erythematous Lesions Through Targeting of *Staphylococcus* Species

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Objective. Cutaneous lupus erythematosus (CLE) is an inflammatory skin manifestation of systemic lupus erythematosus. Type I interferons (IFNs) promote inflammatory responses and are elevated in CLE lesions. We recently reported that CLE lesions are frequently colonized with *Staphylococcus aureus*. Here, we follow up via a proof-of-concept study to investigate whether type I IFN and inflammatory gene signatures in CLE lesions can be modulated with mupirocin, a topical antibiotic treatment against *S aureus*–mediated skin infections.

Methods. Participants with active CLE lesions (n = 12) were recruited and randomized into a week of topical treatment with either 2% mupirocin or petroleum jelly vehicle. Paired samples were collected before and after seven days of treatment to assess microbial lesional skin responses. Microbial samples from nares and lesional skin were used to determine baseline and posttreatment *Staphylococcus* abundance and microbial community profiles by 16S ribosomal RNA gene sequencing. Inflammatory responses were evaluated by bulk RNA sequencing of lesional skin biopsies.

Results. We identified 173 differentially expressed genes in CLE lesions after topical mupirocin treatment. Decreased lesional *Staphylococcus* burden correlated with decreased IFN pathway signaling and inflammatory gene expression and barrier dysfunction. Interestingly, mupirocin treatment lowered skin monocyte levels, and this mupirocin-associated depletion of monocytes correlated with decreased inflammatory gene expression.

Conclusion. Mupirocin treatment decreased lesional *Staphylococcus*, and this correlated with decreased IFN signaling and inflammatory gene expression. This study suggests a topical antibiotic could be employed to decrease lupus skin inflammation and type I IFN responses by reducing *Staphylococcus* colonization.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe autoimmune disease with pleiotropic manifestations, including disfiguring skin disease, nephritis, and an increased risk of mortality.[1] A total of 70% of patients with SLE experience specific cutaneous eruptions grouped under the category of cutaneous lupus erythematosus (CLE).[2,3] CLE can also exist in the absence of SLE. No US Food and Drug Administration–approved therapies specifically for CLE exist, and CLE can be difficult to treat, leading to use of treatments with difficult side effects or immunosuppressive features.[4] Thus, there is a critical need to uncover effective and less toxic therapies to improve patient outcomes.

Activation of interferon (IFN) signaling in the skin drives chemokine production which recruits monocytes and T cells to skin lesions and promotes the inflammatory process.[5–7] Recent trials that block type I IFN signaling have identified type I IFNs as a central contributor to cutaneous inflammation in lupus skin[8];

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[Correction added on 28 January 2025, after first online publication: In the article title, Staphyloccal was changed to *Staphylococcus*.]

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however, global type I IFN blockade increased the rates of herpes zoster and influenza infections. Cutaneous production of type I IFNs in CLE may occur by several mechanisms, including secretion by invading inflammatory cells[9] and epidermal production.[10–12] Cytosolic nucleic acid sensors, such as the cGAS/stimulator of type I IFN gene pathway, also contribute to type I IFN up-regulation in SLE.[13–15]

Human data support a link between *Staphylococcus aureus* and SLE. *S aureus* is the most frequent cause of bacteremia in lupus patients, implying clinically significant exposure.[16] Colonization data support increased intestinal and nasal carriage of S *aureus* in patients with lupus.[17] Importantly, *S aureus* nasal carriage may be associated with risk of disease flare and development of lupus nephritis.[18] We and others have recently reported that CLE lesions are frequently colonized by *S aureus*.[19,20] Furthermore, type I IFNs contribute to barrier disruption, leading to increased *S aureus* adherence in SLE versus healthy control keratinocytes.[19] *S aureus* is also known to produce mediators that can induce inflammatory signaling and type I IFN production. Therefore, we hypothesized that *Staphylococcus* colonization contributes to a feed forward loop in which type I IFNs permit colonization and colonization increases IFN production. We thus tested this in patients with SLE by disrupting *S aureus* colonization with topical antibiotic application.

MATERIALS AND METHODS

Study design and patients. Twelve patients with SLE with active CLE lesions were recruited from the Michigan Lupus Cohort for this study. Patients were randomly assigned to receive topical 2% mupirocin treatment (equivalent to 0.2 mM) or a vehicle control (petroleum jelly) three times per day for seven days. Lupus is a female-biased disease with a 9:1 female to male ratio. Our study sought to identify patients with SLE with active skin disease and did not discriminate based on sex. As a result, we had 1 male and 11 female patients in the study, in accordance with predicted ratios (See Supplemental Table 1). This study was approved by the University of Michigan Institutional Review Board, and all patients were provided with written informed consent.

CLE lesion analysis. This study was not designed to measure treatment effect. However, we did score the pre- and posttreatment CLE lesions for scale (0–2 points) and erythema (0–3 points) based on the Cutaneous Lupus Erythematosus Disease Activity and Severity Index measures for disease activity.[21] Scoring was performed by JMK in an anonymized method using photographs obtained of designated lesions at the day 0 and day 7 research visits.

Bulk RNA sequencing and gene expression analysis. Skin biopsies were flash-frozen and stored at −80°C until processing. RNA was isolated using Qiagen RNeasy kits. Libraries were generated with the assistance of the University of Michigan Advanced Genomics Core, and RNA sequencing was performed on NovaSeq 6000. Samples passing quality controls were mapped and analyzed on a transcript level using the tuxedo suite (tophat/cufflinks/cuffdiff). Further analyses including batch correction based on grouped samples, principal component analysis, variance stabilizing transformation (VST) normalization, and differential gene expression were conducted using the DESeq2 package.

Fast gene set enrichment analysis. Starting with 34,839 genes tested for differential gene expression analysis, removal of genes with null adjusted *P* values and mapping hgnc gene symbols to entrez gene identifiers resulted in 15,109 genes remaining for gene set enrichment analysis. These genes were sorted according to *t*-statistic, and the fgsea package (v1.16.0) was used to run gene set enrichment analysis on the Reactome pathways with a maximum size of 500 genes, resulting in 185 pathways with Bonferroni-corrected $P < 0.05/2{,}341$.[22] A similar process with differential gene expression analysis comparing petroleum jelly–treated samples with baseline yielded 11 pathways with Bonferroni-corrected $P < 0.05/2{,}341$.

Pathway gene score calculations. Genes from the relevant pathway (IFN signaling, dectin-1 signaling, and keratinization) were extracted from the Reactome pathways in the fsgea package, resulting in 118, 68, and 60 genes, respectively. Scores were calculated by taking the VST-normalized expression of each gene and subtracting the mean of that gene's expression across all mupirocin- or vehicle-treated samples before dividing by the SD of the VST-normalized expression of that gene in the same samples. Scores for each individual gene were then summed across all genes in the pathway.

Quantitative polymerase chain reaction of keratinocytes treated with mupirocin or *S aureus*. The immortalized human keratinocyte cell line (N/TERTs)[23] was used to evaluate type I IFN epidermal responses to mupirocin and *S aureus*. Keratinocytes were treated in vitro with 0.1 mM or 0.2 mM mupirocin or 10^7 CFU of *S aureus* strain USA300 or vehicle as previously described for 24 hours.[11,19,24] RNA was isolated from keratinocytes and converted to complementary DNA (cDNA) (iScript cDNA Synthesis Kit, BioRad), and quantitative real-time reverse transcriptase polymerase chain reaction (PCR) was performed using SyBR Green PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific) and 7900HT Fast Real-Time PCR system (Applied Biosystems) with the support of the University of University of Michigan Advanced Genomics Core. Primers used for quantification of gene expression are as follows (all listed 5′→3′): *IFNK* GTGGCTTGAGATCCTTATGGGT (forward), CAGATTTTGCCAGGTGACTCTT (reverse); *IFNB* GCTTGGATTCCTACAAAGAAGCA (forward) and TAGATGGTCAATGCGGCGTA (reverse), *MX1* TACCAGGACTACGAGATTG (forward), TGCCAGGAAGGTCTATTAG (reverse).

16S ribosomal RNA gene sequencing and analysis. Microbes colonizing CLE lesions were profiled with 16S ribosomal RNA (rRNA) gene sequencing. One skin lesion per patient was swabbed before treatment (day 0) and immediately following one week of treatment (day 7). Genomic DNA was extracted from lesional swab samples using MagAttract PowerMicrobiome kit (Qiagen), and the V4 hypervariable region of the 16S rRNA gene was amplified as previously described.[25] Libraries were cleaned and normalized and underwent quality control before a library was prepared for sequencing by the Illumina MiSeq platform using paired end reads, and sequences were processed using mothur (v.1.44.1).[25,26] Operational taxonomic units were curated and converted to relative abundance. Shannon diversity index was used to show microbial community diversity changes in CLE lesions after treatment with topical mupirocin or vehicle control.

Digital cytometry. CIBERSORTx was used to infer the abundance of various immune cell types using bulk skin transcriptomes generated from CLE lesions.[27] A matrix of bulk gene expression for all 24 samples was given to the CIBERSORTx web interface, and the impute cell fractions function was run mapping to the LM22 database, which contains 547 genes from 22 human hematopoietic cell phenotypes.

Correlation analysis. Pearson correlation coefficient analysis was performed to measure the strength and direction of the relationship between *Staphylococcus* abundance and CLE lesional skin signaling pathways. Correlations between mupirocin-induced changes in skin monocyte levels and cutaneous gene expression were also evaluated.

Immunofluorescence. Immunofluorescence staining of frozen tissue sections from CLE lesions was performed to probe for monocytes. CLE lesional biopsies embedded in OCT media were sectioned and stained with anti-CD14 antibody (B365.1 [B-A8], Invitrogen) and mounted with ProLong Gold Antifade medium containing DAPI (Invitrogen). $CD14^+$ cells present before and after mupirocin treatment in a representative CLE lesion were quantified using ImageJ software (National Institutes of Health) and normalized to total cell number. Cells with nuclei too dense to enumerate were excluded.

Statistical analyses. Statistical analyses of pre- and posttreatment comparisons between *Staphylococcus* abundance or changes in monocyte levels generated using a one-tailed paired *t*-test (for normally distributed data) or Wilcoxon signed-rank test (for nonparametric data) with GraphPad Prism software version 10.0.3 unless otherwise noted; $P < 0.05$ was considered statistically significant. The Shapiro-Wilk test was used to test for normal distribution. The Pearson correlation coefficient was calculated to measure correlations, and statistically significant relationships were determined by $P < 0.05$.

Study approval and Data Sharing. The study was reviewed and approved by the University of Michigan Institutional Review Boards of the University of Michigan Medical School under approval number HUM00136167. All patients underwent written, informed consent according to the Declaration of Helsinki. Clinical data information can be found in Supplemental Table 1. Sequence data from this study will be deposited in the GEO database and will be accessed using the accession code 14173587.

RESULTS

Mupirocin treatment reverses lupus-associated cutaneous gene expression. Patients with SLE with active CLE lesions were randomly assigned to either 2% mupirocin or petroleum jelly as a vehicle control. A single, accessible lesion was selected by investigators for treatment (CLE subtype listed in Supplemental Table 1). Lesions were assessed after seven days of topical treatment. No significant change in lesion erythema or scale was noted for either petroleum jelly or mupirocin-treated lesions (Supplemental Table 2). Skin biopsies were collected from the target lesion, and RNA sequencing was performed. Principle component analysis of these data revealed transcriptional differences between pre- and posttreatment samples in participants randomized to 2% mupirocin, whereas no such differences were observed in participants randomized to receive vehicle (Figure 1A). We observed 173 genes differentially expressed between paired pretreatment samples and mupirocin-treated lesions (Supplemental Table 3); importantly, vehicle treatment induced no significant gene expression changes (Figure 1B). Differential gene set enrichment analysis revealed 617 pathways that were significantly altered in lesional skin after mupirocin treatment. Examination of the top 20 pathways showing greatest effect sizes revealed that mupirocin decreased the expression of genes involved in IFN signaling and active cellular division in CLE lesions, whereas genes involved in epithelial barrier function and lipid metabolism were up-regulated (Figure 1C). In contrast, only 11 pathways were detected to be significantly modulated in CLE lesions treated with vehicle alone, and these responses were characterized by up-regulated homeostatic mitochondrial signaling pathways involved in metabolism and down-regulation of genes involved in smooth muscle contraction (Figure 1D).

As *S aureus* has been reported to promote type I IFN production,[28] we then tested the ability of *S aureus* to induce keratinocyte relevant IFN genes in human keratinocytes.[23] Indeed, treatment with heat-killed *S aureus* significantly up-regulated the type I IFN genes *IFNB* ($P < 0.0001$) and *IFNK* ($P = 0.0067$) and the IFN-stimulated gene *MX1* ($P < 0.0001$), whereas mupirocin alone had no effect on *IFNB*, *IFNK*, or *MX1* gene expression (Figure 2). Taken together, these data show that *S aureus* induces type I IFN production and that mupirocin treatment of CLE lesions

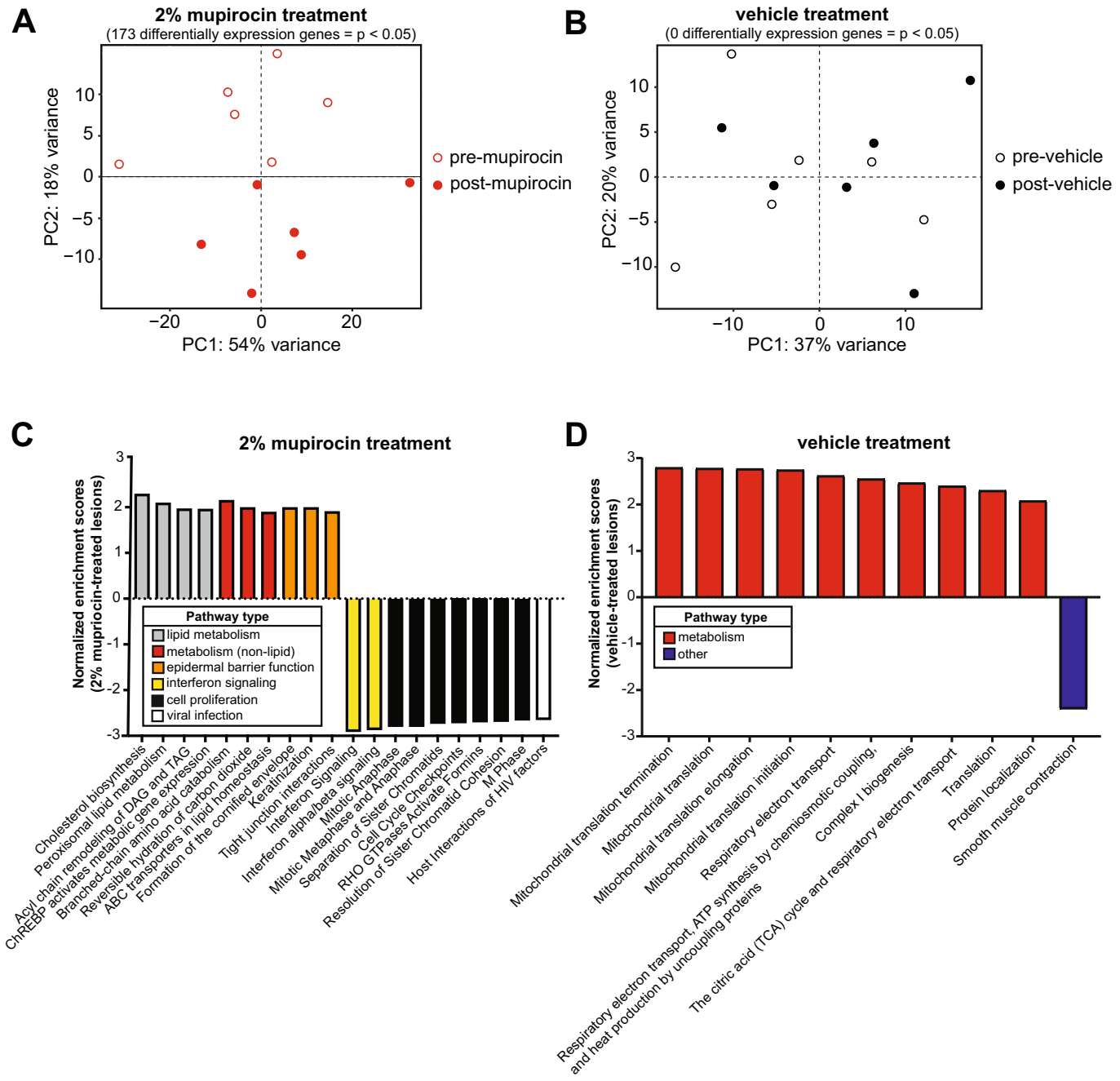

Figure 1. Mupirocin modifies skin gene expression in cutaneous lupus erythematosus lesions. PC analysis plots of (A) mupirocin-treated samples (n = 6) and (B) controls (n = 6). (C) Normalized gene enrichment scores calculated by fast gene set enrichment analysis (fgsea) showing the top 10 up-regulated and down-regulated pathways following mupirocin treatment, from a total of 185 pathways that were statistically significant by a Bonferroni-corrected $P < 0.05/2{,}341$. Petrolatum jelly (vehicle) treatment for 7 days specifically modulates homeostatic mitochondrial pathways and genes involved in smooth muscle contraction. (D) All 11 significantly up-regulated and down-regulated pathways associated with petroleum jelly vehicle control treatment are shown (Bonferonni-corrected $P < 0.05$). PC, principle component.

represses IFN signatures, likely through killing of bacteria sensitive to mupirocin and not through direct effects on keratinocytes.

Mupirocin treatment lowers *Staphylococcus* burden on CLE lesional skin. We next determined the impact of mupirocin or vehicle treatment on the microbial diversity and abundance of *Staphylococcus* on CLE lesions. Before treatment, the targeted lesion was swabbed, and a second swab was collected in the same location after one week of vehicle or mupirocin treatment. Paired nasal swabs were also collected. *Staphylococcus* abundance and cutaneous and nasal microbial diversity were assessed before and after topical treatment of the active CLE

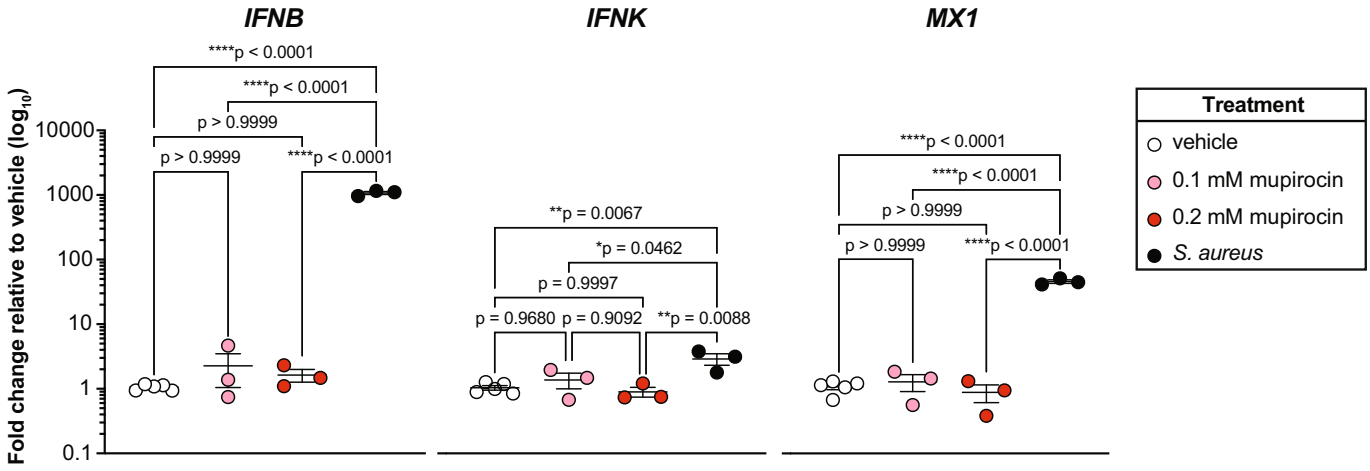

Figure 2. *Staphylococcus aureus* stimulates type I IFN responses in keratinocytes. Keratinocytes were incubated with 0.1 mM or 0.2 mM mupirocin (1% or 2%, respectively), live *S aureus* (10^7 colony-forming units), or vehicle for 24 hours and quantitative polymerase chain reaction was performed to evaluate induction of IFN-stimulated genes encoding IFN-κ, IFN-β, and MX1. Each dot represents the average of technical triplicates. Data were analyzed by one-way analysis of variance. *$P < 0.05$; **$P < 0.05$; ****$P < 0.0001$. IFN, interferon.

lesion. CLE lesional skin and nasal swab samples were assayed by 16S rRNA sequencing. The relative abundance of bacterial genera colonizing CLE lesions were largely unaltered by mupirocin treatment, with the exception of *Staphylococcus*, whereas vehicle treatment did not significantly change lesional microbiota composition (Figure 3A depicts the pre- and posttreatment mean alpha diversities of each patient; intraindividual diversity is shown in Supplemental Figure 1A). Mupirocin reduced *Staphylococcus* burden on CLE lesions ($P = 0.0352$; Figure 3B) without altering overall microbial diversity ($P = 0.2812$; Figure 3C) or levels of other Gram-positive bacteria, such as species within the *Streptococcus* genus ($P = 0.1095$; Supplemental Figure 1B). Taken together, these data demonstrate compliance with topical therapy and the desired specificity of *S aureus* as the target microbe for treatment.

Microbial profiling of nasal swabs demonstrated that topical skin application of mupirocin to CLE lesions also resulted in a significant decrease of *Staphylococcus* carriage in the nares ($P = 0.0165$; Supplemental Figure 2B), suggesting possible transfer of mupirocin by participants to the nares as well. As expected, no change was observed in the alpha diversity of microbes detected in nasal samples from participants receiving either treatment (Supplemental Figure 2C).

Staphylococcus burden on CLE lesions correlates with cutaneous inflammation and barrier dysfunction. Gene enrichment analysis was performed on CLE lesions to evaluate treatment-induced changes and determine whether they are associated with abundance in *Staphylococcus* species. Mupirocin treatment, but not vehicle control, resulted in decreased expression of genes involved in IFN signaling, the predominant dysregulated pathway in CLE lesions[29] (Figure 4A left panel; Supplemental Table 3). Similarly, mupirocin treatment also resulted in reduced expression of genes associated with signaling of dectin-1 (Figure 4A, center panel), a receptor expressed on monocytes, macrophages, and dendritic cells, all important cell populations in CLE lesions.[30,31] Genes involved in keratinization were enriched in CLE lesions after topical mupirocin (Figure 4A right). Critically, a reduction in *Staphylococcus* abundance was associated with decreased IFN and dectin-1 signaling and improved keratinization pathway scores (Figure 4B). No relationship was observed between the relative abundance of *Staphylococcus* nasal colonization of patients with CLE and alterations in these lesional skin pathways (Supplemental Figure 3A). Similarly, there was no relationship noted between *Staphylococcus* abundance and IFN or dectin-1 signaling before treatment (Supplemental Figure 3B). These data indicate that mupirocin reduces CLE lesional *Staphylococcus* colonization burden, and this reduction correlates with reduced signaling in CLE-associated IFN and dectin-1 signaling.

Mupirocin treatment lowers skin monocyte and activated dendritic cell levels in CLE lesions. Given that we consistently observed a reduction in signaling pathways commonly associated with immune cells following 2% mupirocin treatment, we then used CIBERSORTx to deconvolute the RNA sequencing data into predicted cell populations impacted by treatment. Mupirocin treatment specifically reduced genes predicted to represent monocyte contributions to CLE lesions ($P = 0.0104$; Figure 5A upper panels), whereas significant shifts in other major immune cell populations were not observed after seven days of application (Figure 5B). We then validated this using immunofluorescent staining against the monocyte marker CD14. In vivo comparison in a single patient revealed a five–percentage point decrease in the proportion of lesional cells expressing the

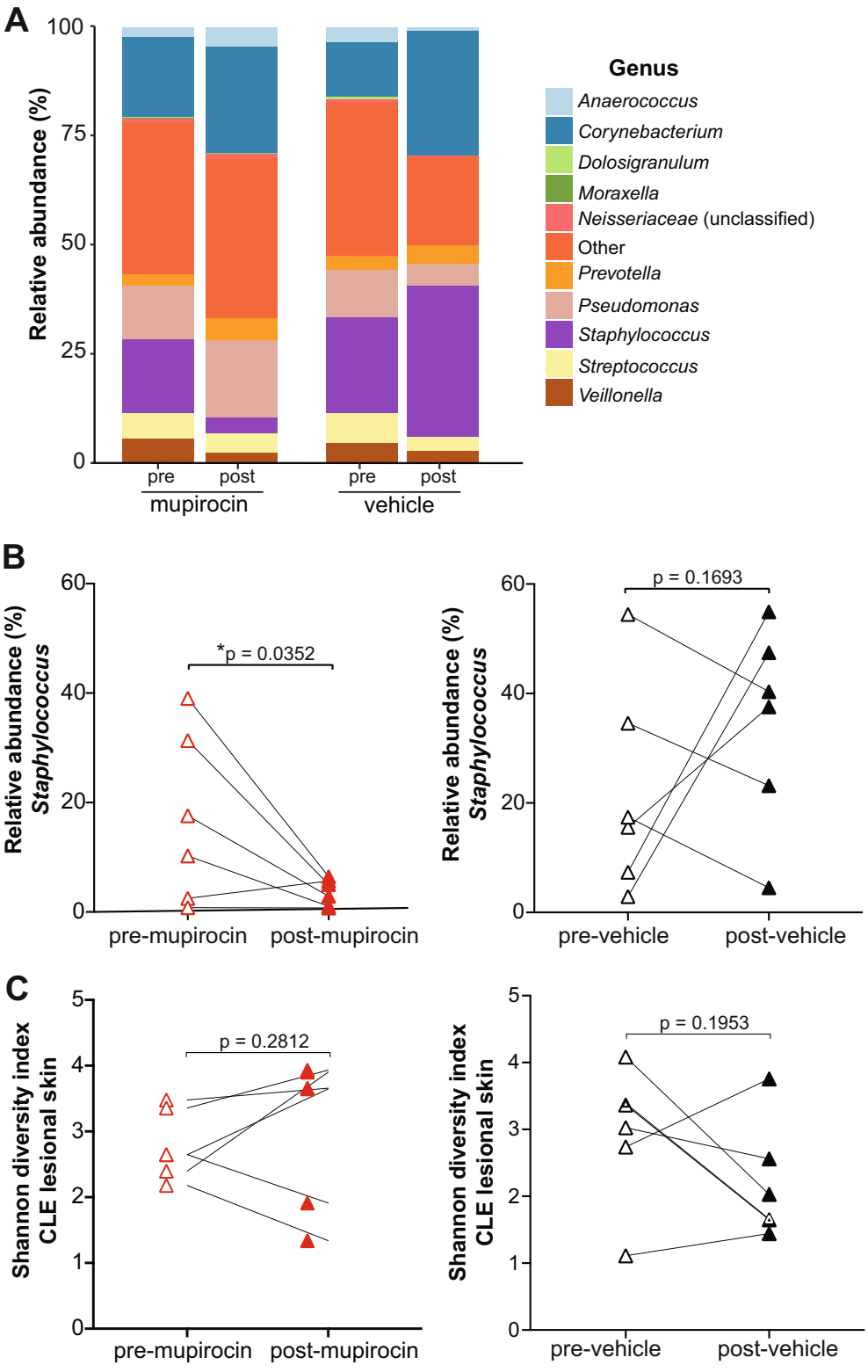

Figure 3. Mupirocin treatment of CLE lesions reduces *Staphylococcus* burden without altering overall skin microbial diversity. (A) Relative abundances of bacterial taxa by treatment group from lesional skin and nares (n = 6 per treatment). (B) Relative abundance plots of *Staphylococcus* and (C) Shannon diversity of bacteria colonizing CLE lesions pre- and posttreatment. Data were analyzed by one-tailed paired *t*-test (n = 6 per treatment condition). *$P < 0.05$. CLE, cutaneous lupus erythematosus.

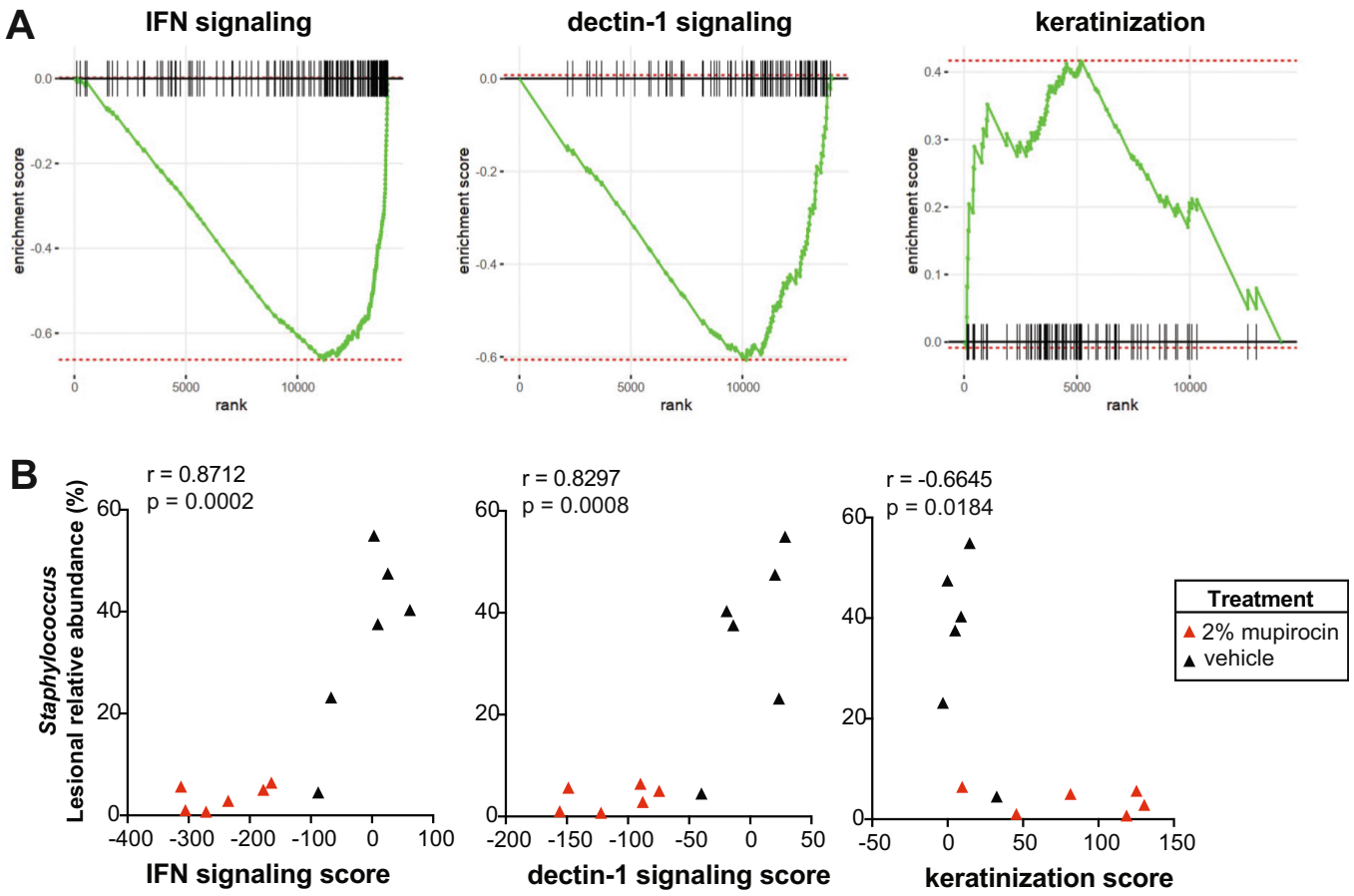

Figure 4. Mupirocin treatment modulates skin signaling pathways, and this correlates with *Staphylococcus* levels. (A) Relevant enrichment scores calculated for cutaneous lupus erythematosus lesions treated with mupirocin as determined by gene set enrichment analysis. (B) The relationship between *Staphylococcus* relative abundance and IFN signaling, dectin-1 signaling, or keratinization pathway scores after treatment was measured using the Pearson correlation coefficient (n = 6 samples per treatment). IFN, interferon.

monocyte marker CD14 after mupirocin treatment (Supplemental Figure 4). Genes predicted to represent activated dendritic cells in CLE lesions were also significantly decreased by mupirocin treatment ($P = 0.0443$; Figure 5A lower panels). These data show topical mupirocin treatment is associated with a reduction of monocytes and activated dendritic cells in CLE lesions.

Mupirocin-associated loss of monocytes and activated dendritic cells correlates with decreased expression of genes involved in cutaneous inflammation and skin homeostasis. We then correlated predicted monocyte and activated dendritic cell abundance based on digital cytometry with all genes differentially expressed after mupirocin treatment. Of 174 differentially expressed genes, 7 genes were significantly associated with a change in monocytes, and 4 genes were significantly associated with a change in activated dendritic cells (Table 1). Reduced monocyte scores were associated with diminished expression of *PLSCR1*, a gene encoding the IFN-inducible protein phospholipid scramblase 1 (PLSCR1) ($r = 0.8368$; $P = 0.0380$) associated with lipid metabolism. *CASP7* and *PLEKHA4* expression also decreased with larger reductions in monocyte scores. Decreased activated dendritic cell scores were associated with several type I IFN–responsive dendritic cell genes including *AQP7* ($r = 0.4956$; $P = 0.3174$), *CILP* ($r = 0.4078$; $P = 0.4222$), *IFIT3* ($r = 0.2020$; $P = 0.7011$), and *THY1* ($r = 0.1122$; $P = 0.8323$). No relationship was observed between predicted gene expression in vehicle-treated CLE lesions and monocyte or activated dendritic cell scores (Supplemental Figure 5).

Taken together, our data indicate that reductions in *Staphylococcus* burden on cutaneous lupus lesions result in decreased IFN signaling and a reduction in innate inflammatory cell infiltrates.

DISCUSSION

S aureus is a dynamic colonizer of 30% of the US population[32] and has been shown to promote SLE-like autoimmune inflammation.[33] Patients with SLE have a distinct skin microbiota compared with healthy controls,[34] and recent studies have reported increased *S aureus* colonization on CLE lesions, which

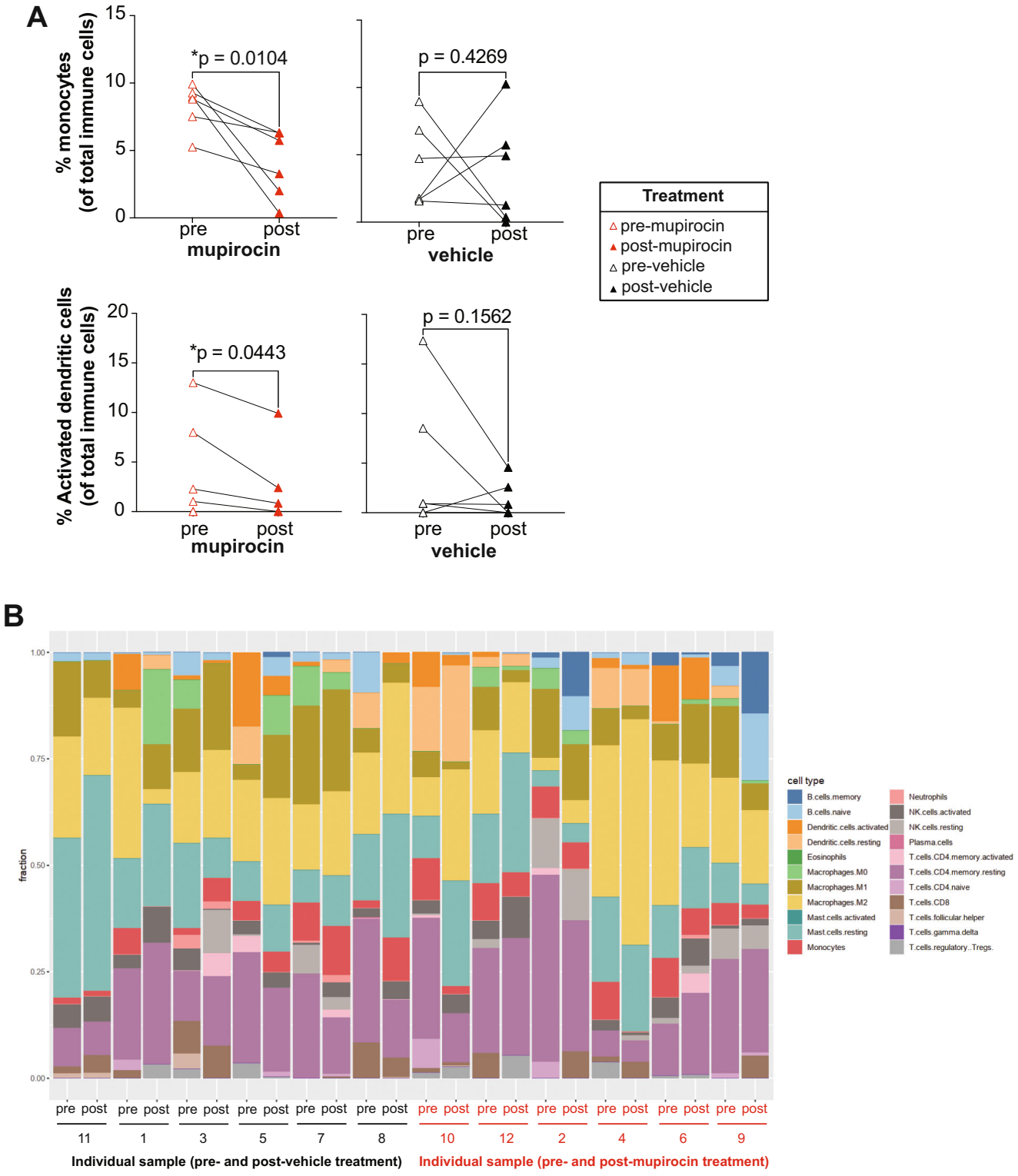

Figure 5. Mupirocin decreases monocytes in CLE lesional skin. (A) Percentage of monocytes and activated dendritic cells in CLE lesions pre- and posttreatment (n = 6 lesions), as inferred by digital cytometry. Data were analyzed by two-tailed paired *t*-test (B) Immune cell populations in CLE lesions pre- and posttreatment with mupirocin (red) or vehicle (black) (n = 6 paired samples per treatment group). Data were analyzed by one-tailed paired *t*-test or Wilcoxon signed-rank test. *$P < 0.05$. CLE, cutaneous lupus erythematosus.

is facilitated by the high type I IFN environment of SLE skin.[19,20] Here, we have now shown that elimination of *S aureus* through the use of a topical antibiotic provides a targeted reduction in staphylococcal burden and attenuates the inflammatory signature within CLE lesions, including a reduction in type I IFN and innate immune signaling profiles and an increase in skin barrier genes.

Table 1. Mupirocin-induced monocyte and activated DC depletion is associated with the expression of 11 genes in cutaneous lupus erythematosus lesions*

Differentially expressed gene	Pearson correlation (*r*)	*P* value
Δmonocytes		
IGLV3–19	0.9885	0.0002
TEAD4	0.9722	0.0011
PLEKHA4	0.96	0.0024
CASP7	0.8997	0.0146
AP001528.3	0.8733	0.023
PLSCR1	0.8363	0.038
DDX21	−0.8399	0.0364
Δactivated DCs		
AQP7	−0.8307	0.0406
CILP	−0.8750	0.0225
IFIT3	−0.8985	0.0149
THY1	−0.8699	0.0243

* Significant correlations between the change in predicted monocyte and activated DC scores and differentially expressed genes observed after mupirocin treatment are shown. Relationships were measured by Pearson correlation coefficient. All statistically significant correlations of the 173 differentially expressed genes from Supplemental Table 3 are shown. DC, dendritic cell.

The links between SLE and *S aureus* have been suggested for more than a decade. Prolonged exposure to *S aureus* via repeated injection of *S aureus* proteins induces development of lupus-like disease in wild-type mice.[35] Colonization of murine bladder catheters with *S aureus* results in sterile inflammatory cell infiltration of organs as diverse as the kidney and lung in the absence of bacteremia.[36] Intriguingly, recent data have suggested that penetration of the epidermis by *S aureus* regularly occurs, even in normal skin,[37] and this is exaggerated in the presence of dysfunctional or absent skin barrier proteins, such as filaggrin.[38] This suggests that ongoing colonization may provide exposure to *S aureus* inflammatory triggers. Intriguingly, known CLE triggers such as UV light[39] and smoking[40] also have negative effects on skin barrier function.[41,42]

Further study on the relationship between CLE triggers and *S aureus* colonization is warranted. Similarly, understanding CLE lesional factors influencing colonization by (or selection for) staphylococcal species may be required to develop precise and effective CLE-specific treatments.

Topical mupirocin treatment up-regulated the expression of key skin barrier pathways. This effect cannot be sufficiently explained by moisturization from treatment as use of petrolatum as a vehicle did not modify skin barrier gene expression. This is in contrast to a previous report in which treatment with petrolatum for 48 hours resulted in up-regulation of barrier genes and antimicrobial peptides.[43] This discrepancy may be related to differences in treatment protocol as we used petroleum jelly applied twice daily with nonocclusive bandages for 7 days, whereas the study by Czarnowicki et al used Finn chambers for full occlusion for 48 hours followed by 24 hours of no treatment before biopsy.[43] Petrolatum also did not result in major shifts in microbial composition in our study.

Treatment with mupirocin resulted in down-regulation of pathways involved in mitosis and cell division. This could reflect normalization of keratinocyte proliferation. The presence of epidermal scale is considered an indicator of disease in CLE. Discoid lupus erythematosus lesions in particular show hyperproliferation and abnormal differentiation of keratinocytes, manifesting clinically as hyperkeratosis and follicular plugging from keratin.[44] Here, treatment with mupirocin resulted in down-regulation of pathways involved in mitosis and cell division and increased keratinization, which could reflect normalization of keratinocyte maturation. Thus, mupirocin treatment may offer benefit in this regard. Longer-term studies are needed to assess the clinical response.

Type I IFN–related gene changes were observed to be correlated with decreased monocytes and activated dendritic cells after mupirocin treatment. PLSCR1 is an IFN-inducible phospholipid scramblase family member known to be involved in immune responses and antiviral activity.[45] Monocytes in patients with SLE have enhanced *PLSCR1* gene expression.[46] PLSCRs are important for lipid metabolism and contribute to inflammation, which is also modulated by mupirocin treatment. PLSCR1 negatively regulates Fc receptor–mediated phagocytosis during macrophage differentiation from precursors such as monocytes.[47] Furthermore, PLSCR1 may play a role in the antiviral response of IFN by amplifying and enhancing the IFN response through increased expression of select subset of potent antiviral genes.[48] Similarly, we also found repression of IFN-activated genes associated with an activated epidermal dendritic cell signature (*AQP7*, *CILP*, *IFIT3*, *THY1*).[49] Thus, mupirocin treatment may promote a switch from interferonogenic to tolerogenic myeloid phenotypes in the skin.

Thus, the data reported here raise the question of whether mupirocin, or other medications that target *S aureus* on the skin, could benefit CLE either as a primary treatment or as an adjunct therapy. A case report of lupus profundus showed benefit of mupirocin in combination with hydroxychloroquine and prednisone, but the effects of mupirocin alone were not assessed.[50] Typically, few side effects from mupirocin use are reported; however, the risk of antibacterial resistance remains a concern, with up to 80% resistance reported in countries where mupirocin is available over the counter.[51] Thus, application of mupirocin in clinical practice would likely need defined periods of use and a strong risk-to-benefit ratio. Larger trials with clinical response datapoints are needed to understand whether mupirocin should be incorporated into clinical practice.

Limitations to our study include the low number of study participants and the short time frame of treatment. We did not see a change in clinical phenotype of the lesions; however, one week of treatment is unlikely to provide significant clinical change given drugs with successful track record for CLE, such as anifrolumab,

can take up to 12 weeks to show improvement in lesions.[52] In addition, secondary to a tragic freezer accident that left us with only one paired pre- and postmupirocin biopsy sample available for confirmatory immunostaining, we were limited in our ability to validate the changes identified by RNA sequencing. These limitations notwithstanding, this study showed that in addition to direct antimicrobial properties, mupirocin improved CLE lesional inflammatory phenotypes. Lowering the burden of *Staphylococcus* was associated with reductions in IFN signaling and decreased monocyte infiltration. Thus, targeting staphylococcal skin colonization is an approach worth additional consideration and study for optimal management of CLE flares.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Kahlenberg confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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