**Staphylococcus aureus** Colonization Is Increased on Lupus Skin Lesions and Is Promoted by IFN-Mediated Barrier Disruption

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Cutaneous inflammation is recurrent in systemic lupus erythematosus (SLE), yet mechanisms that drive cutaneous inflammation in SLE are not well defined. Type I IFNs are elevated in nonlesional SLE skin and promote inflammatory responses. *Staphylococcus aureus*, known to induce IFN production, could play a role in cutaneous inflammation in SLE. We show here that active cutaneous lupus erythematosus lesions are highly colonized (~50%) by *S. aureus*. To define the impact of IFNs on *S. aureus* colonization, we examined the effects of type I and type II IFNs on *S. aureus* adherence and invasion. An increase in adherent *S. aureus* was observed after exposure to both IFN-α and -γ, whereas IFN-γ appeared to inhibit invasion of *S. aureus*. Cutaneous lupus erythematosus lesional skin microarray data and RNA sequencing data from SLE keratinocytes identified repression of barrier gene expression, such as filaggrin and loricrin, and SLE keratinocytes exhibited increased *S. aureus*-binding integrins. These SLE-associated changes could be replicated by IFN treatment of keratinocytes. Further, SLE keratinocytes exhibited increased binding to *S. aureus*. Together, these data suggest that chronic exposure to IFNs induces barrier disruption that allows for higher *S. aureus* colonization in SLE skin.


### INTRODUCTION

Cutaneous inflammation is a frequent and recurrent manifestation of systemic lupus erythematosus (SLE), a devastating autoimmune disease. Triggers for skin flares include exposure to UVB (Chasset and Arnaud, 2018; Muskardin and Niewold, 2018; Stannard and Kahlenberg, 2016), which raises type I IFNs in the skin. Keratinocytes are an important source of IFNs, both at baseline and after UVB exposure, which promotes infiltration of inflammatory cells in affected skin (Meller et al., 2005; Sarkar et al., 2018; Stannard et al., 2017).

One underexplored cause of cutaneous IFN production is microbial dysbiosis. The skin is home to many commensals that provide a living obstruction to colonization by harmful organisms (Byrd et al., 2018; Kong, 2011). *Staphylococcus aureus*, unlike other members of the genus *Staphylococcus*, is a relatively minor colonizer of the skin and is involved in the pathogenesis of skin-associated diseases such as atopic dermatitis (AD) (Kong, 2011; Nakatsuji et al., 2016; Williams and Gallo, 2017). *S. aureus* colonization precedes clinical onset of AD and contributes to the severity of the disease (Meylan et al., 2017; Nakatsuji et al., 2016; Williams and Gallo, 2017). We have demonstrated that production of IFNs follows colonization of mice by *S. aureus* and that *S. aureus* peptidoglycan induces production of IFN-κ in keratinocytes (Stannard et al., 2017; Syed et al., 2015). In addition, disruption of the epithelial barrier, which is important in promotion of *S. aureus* colonization (Wanke et al., 2013), is able to drive lupus disease activity (Clark et al., 2015). Moreover, *S. aureus* is the leading cause of bacteremia in patients with lupus, and its carriage may be associated with disease flares and development of lupus nephritis (Chen et al., 2008; Conti et al., 2016; Hajialilo et al., 2015). Reflecting the importance of *S. aureus*-driven immune activation, repeated injections of *S. aureus* super-antigen in wild type mice results in the development of a disease that mimics lupus (Chowdhary et al., 2012). Overall, investigations into *S. aureus* colonization in patients with SLE have been limited, and whether IFNs impact colonization by *S. aureus* is unknown.

In this paper, we investigate the role of IFNs in the regulation of colonization by *S. aureus*. We show that patients with SLE are frequently colonized by *S. aureus* on their rashes. We also demonstrate that exposure to type I IFNs increases *S. aureus* adherence and that SLE keratinocytes exhibit greater barrier disruption and *S. aureus* adhesion when compared with matched healthy controls (HCs). This suggests that dysregulation of type I IFNs in SLE could lead to...
a feed-forward loop resulting in greater \emph{S. aureus} colonization that in turn leads to inflammation and additional production of IFNs.

**RESULTS**

**SLE-associated skin lesions are colonized with \emph{S. aureus} at a high rate**

We wished to investigate the colonization frequency of members of the lupus cohort at the University of Michigan (see Supplementary Table S1 for demographics). Patients (n = 54) were tested for \emph{S. aureus} in their nares, on their chest, and on any lupus-related skin lesions. Patients with SLE were colonized by \emph{S. aureus} at a rate higher (~40%) than that reported in healthy adults (~30%), and the rate increased further when active skin lesions were sampled (50%) (Figure 1a). In comparison, psoriatic plaques, which are characterized by a mixed T helper type 1/T helper type 17 signature and low IFN levels (Baliwag et al., 2015), did not exhibit colonization on lesional skin. The colonization rates of SLE lesions and psoriasis thus differed significantly (Fisher’s Exact test; \( P < 0.0001 \)) (Figure 1b).

We next investigated the influence of \emph{S. aureus} colonization on disease activity. No association between rash-adjusted SLE Disease Activity Index scores and colonization by \emph{S. aureus} was noted (Figure 1c). The Cutaneous Lupus Disease Area and Severity Index (CLASI) is a validated score for severity of active cutaneous involvement in lupus (Robinson and Werth, 2015). CLASI activity scores trended higher among colonized individuals (\( q = 0.0586 \)). In addition, significant increases in CLASI scores were observed when uncolonized and rash-colonized patients were compared (\( q = 0.0016 \)). CLASI activity scores were also higher when patients with colonized skin lesions were compared with patients with SLE colonized in other locations.
besides the rashes ($q = 0.0458$) (Figure 1d). These data suggest that either the presence of S. aureus on the rash contributes to higher cutaneous disease activity or the disease activity contributes to an environment conducive to colonization by S. aureus.

### SLE lesional skin demonstrates lower barrier gene expression

Loss-of-function mutations and misregulation of FLG result in impaired barrier integrity and are important in AD pathogenesis (Nakatsuji et al., 2016). However, barrier gene expression in cutaneous lupus erythematosus is not well understood. Thus, we compared normalized data from microarray analysis of SLE lesional skin versus HC skin (Gene Expression Omnibus accession number GSE81071) (Berthier et al., 2019). Expectedly, IFN-stimulated genes and IFNs including IFN-κ, a regulator in keratinocytes for type I responses, were elevated significantly in cutaneous lupus lesions (1.53-fold change; $q = 0.0006$). Importantly, barrier genes such as LOR, CLDN1, and TGM5 were downregulated (Table 1). This suggests that the high IFN exposure may downregulate the epidermal barrier, which could promote S. aureus colonization.

### Exposure to IFNs results in diminished barrier gene expression

We next chose to determine whether the expression of barrier proteins was modulated by IFN exposure in N/TERTs, a human keratinocyte cell line. Following exposure to IFN-α or IFN-γ for 6 hours, RNA was isolated and gene expression was quantified via real-time PCR. As expected, IFN-α treatment resulted in high expression of the transcriptional regulator MX1, and IFN-γ–treated cells showed high expression of ICAM1, demonstrating that IFNs utilized for treatment were functional (Figure 2). Numerous genes involved in the formation of the cornified envelope, including FLG, LOR, and ELN, were found to be significantly repressed, whereas tight junction proteins such as DSG1 and FLG2 trended toward decreased expression following IFN exposure (Figure 2 and Supplementary Figure S1). Other genes for extracellular matrix molecules such as FGG and FN1, which are known to interact with S. aureus, demonstrated reduced expression particularly in the presence of IFN-γ. However, ITGA5 was upregulated in IFN-α–treated (but not IFN-γ–treated) N/TERTs. Keratinocytes respond to microbial surface molecules by the production of antimicrobial peptides including β-defensins (DEFB1). IFN-α, but not IFN-γ, treatment increased DEFB1 expression (Figure 2). These data suggest that IFN treatment of keratinocytes represses production of proteins that contribute to the integrity of the cornified layer, thus compromising the epithelial barrier while leaving the defensive competences intact.

### IFN exposure increased S. aureus adherence in keratinocytes

Given that IFNs downregulate barrier proteins and upregulate integrins (involved in bacterial adherence) (Clarke and Foster, 2006), we next investigated whether IFNs modulated adherence of S. aureus to the N/TERT keratinocyte cell line. Confluent N/TERTs were exposed to washed, log phase S. aureus for increasing periods of time to determine the kinetics of S. aureus adhesion with N/TERTs. S. aureus adherence occurred rapidly with approximately $1.3 \times 10^4$ S. aureus colony-forming units (CFUs) recovered at 30 minutes. Time of exposure demonstrated a linear relationship with S. aureus adherence, with approximately $1.3 \times 10^5$ CFUs recovered after 90 minutes (Supplementary Figure S2a). We also performed assays with gentamicin exposure to determine invasion kinetics, because S. aureus is reported to invade keratinocytes and reside within for varying periods of time (Edwards et al., 2011; Garzoni and Kelley, 2009; Löffler et al., 2014). The results indicated the same trend observed in the adhesion assays, although the number of recovered CFUs were, expectedly, at least two orders of magnitude lower than that observed during adhesion assays (Supplementary Figure S2b). These trends were also observed with stationary phase S. aureus (data not shown).

We then determined whether exposure to IFN-α or IFN-γ influenced S. aureus–keratinocyte interactions. Confluent N/TERTs were treated with 1,000 U/ml of either IFN-α or IFN-γ for 24 hours and then exposed to S. aureus. Consistent with a role for type I IFNs in promoting S. aureus colonization, greater numbers of CFUs were recovered from N/TERTs exposed to IFN-α, as shown in Figure 3a ($P = 0.0397$). IFN-γ treatment did not promote a significant increase in adherence in comparison with the untreated N/TERTs. Paired analysis performed on data from multiple experiments also revealed a significant increase in adhesion to N/TERTs after exposure to IFN-α ($P = 0.0056$). These trends were replicated when stationary phase S. aureus was utilized (Supplementary Figure S3).

To confirm that the effect on adhesion was due to IFNs, we used baricitinib, a small molecule inhibitor of Janus kinase that blocks signaling of type I and type II IFN receptors (Howell and Fitzsimons, 2018; Kontzias et al., 2012).  

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### Table 1. Genes Misregulated in CLE Lesional Skin Obtained from Microarray Data Analysis

<table>
<thead>
<tr>
<th>Function</th>
<th>Symbol</th>
<th>Description</th>
<th>FC</th>
<th>$q$-value</th>
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<tr>
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<td>loricrin</td>
<td>0.79</td>
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<td></td>
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<td></td>
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<td></td>
<td>TGM5</td>
<td>Transglutaminase 5</td>
<td>0.50</td>
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<td>kallikrein 11</td>
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</table>

Abbreviations: CLE, cutaneous lupus erythematosus; FC, fold change. Red genes are upregulated and blue genes are downregulated. $q$-value < 0.05 considered significant.
Preincubation of N/TERTs with baricitinib (10 μM) did not change basal rates of *S. aureus* adherence but blocked increased adhesion induced by IFN exposure (Figure 3a). To confirm our data from counts, N/TERTs/C6 IFNs and/C6 baricitinib exposed to fluorescently labeled *S. aureus* were evaluated by fluorescence microscopy. As shown in Figure 3b, an increase in *S. aureus* adhesion was noted with IFN-α treatment, and this was blocked by the addition of baricitinib. Together, these data indicate that IFNs, and type I IFNs in particular, promote adhesion of *S. aureus* to keratinocytes.

**S. aureus** invasion is not affected by IFN-α and is actively inhibited by IFN-γ

Keratinocytes are nonprofessional phagocytes that allow *S. aureus* entry by a fibronectin, integrin, and cytoskeleton rearrangement—mediated mechanism (Edwards et al., 2011; Löfler et al., 2014). We next examined whether IFN exposure also resulted in higher invasion into N/TERT monolayers. Confluent N/TERTs were exposed to IFNs and invasion assays were performed. IFN-α treatment did not result in a higher number of recovered CFUs. In stark contrast, IFN-γ strongly repressed *S. aureus* invasion (*P* < 0.0001), indicating a protective function for type II IFNs in N/TERTs (Figure 3c). Surprisingly, treatment with baricitinib alone also resulted in a significant decrease in *S. aureus* invasion. To confirm the invasion data, confocal microscopy was performed on N/TERTs exposed to fluorescent *S. aureus*. Representative images at the z-planes (0.5-μm sections) of the DAPI-labeled nuclei of N/TERTs demonstrate identifiable organisms within the cell, as evidenced by the presence of fluorescent bacteria in close proximity to nuclei (Figure 3d) and by counts that reflect the data from the invasion assays.

Keratinocytes from patients with SLE demonstrate low expression of barrier-related genes and increased adherence to *S. aureus*

Given that IFNs downregulate barrier genes and nonlesional keratinocytes from patients with SLE express elevated type I IFNs (IFN-κ) at baseline (Sarkar et al., 2018), we next explored whether barrier genes were differentially expressed in nonlesional SLE and control keratinocytes (HCs). To this
end, keratinocytes were obtained from nonlesional skin and subjected to RNA sequencing analysis as reported previously (Tsoi et al., 2019). Barrier genes such as FLG, LOR, IVL, and CLDN1, as well as antimicrobial peptides (DEFB), were repressed in SLE keratinocytes in comparison with HC keratinocytes (Figure 4). In contrast, genes involved in adhesion such as FN1, ITGA5, and ITGB1 were elevated in SLE keratinocytes and even more so after IFN-α treatment. Thus, these
data indicate that SLE keratinocytes exhibit impaired barrier formation at baseline and may be more prone to bacterial adhesion.

We then utilized nonlesional primary keratinocyte cultures to determine if *S. aureus* adhesion was increased in patients with SLE. Six sets of age- and sex-matched combinations of SLE and HC keratinocytes were used for adhesion and invasion assays. As demonstrated in Figure 5a, *S. aureus* demonstrated significantly greater adherence to most (5/6) SLE keratinocytes in comparison with HC keratinocytes. In addition, a higher number of adhered *S. aureus* CFUs were recovered from both SLE and HC keratinocytes exposed to IFN-α (Supplementary Figure S4). Some, but not all, SLE keratinocytes demonstrated increased invasion (Figure 5b). Together, these data suggest that higher colonization of patients with SLE likely is due in part to diminished barrier functions and chronic type I IFNs that drive increased adherence.

**DISCUSSION**

Factors that contribute to a propensity for skin inflammation in patients with SLE are not well defined. Here, we demonstrate that cutaneous lesions of patients with SLE are characterized by depressed barrier proteins and increased colonization by *S. aureus*. We also show that type I IFNs can repress barrier gene expression and increase adherence of *S. aureus*, which serves as a mechanism for our observation that SLE keratinocytes have increased adherence to *S. aureus* when compared with HCs.

Our study identified colonization rates among patients with SLE similar to previous reports by Conti et al. (2016) (~21%) and Hajialilo et al. (2015) (~48%). When their strategies for *S. aureus* identification were examined, it was noted that the latter group utilized mannitol salt agar for growth and did not employ secondary confirmatory strategies. This could have yielded false positive results from mannitol fermentation positive staphylococci (Sirobhushanam et al., 2019). In our study, we utilized CHROMagar to identify potentially positive colonies, followed by both mannitol salt and PCR confirmation of strains. Despite our rigorous methodology, we determined that SLE lesional skin samples were highly colonized (50%) and demonstrated association with disease activity as evidenced by the higher CLASI scores in colonized patients. These results are in line with AD results, which demonstrate higher colonization on affected skin (Gong et al., 2006). We did not find similarly high rates of colonization on psoriasis rashes, which suggests that specific skin lesions may attract and interact with *S. aureus*. One caveat to consider here is that patients with SLE were typically on immunosuppressive and antimalarial medications, which potentially may influence colonization. Further studies will help to delineate this.
Cutaneous IFNs Promote S. aureus Colonization

Analysis of adhesion and invasion kinetics revealed a linear relationship with time, although we did not observe threshold values for adherent S. aureus as had been reported by other groups. This could be due to differences in properties unique to cell lines that are currently unknown (HaCaT vs. N/TERTs) (Edwards et al., 2011). Our inquiry into the impact of IFNs on S. aureus colonization showed that N/TERTs exposed to IFN-α exhibited increased S. aureus adhesion. Higher expression of ITGA5, a component of the integrin α5β1 known to be involved in S. aureus adhesion and invasion, could be a likely mechanism for promotion of adherence by type I IFNs (Foster et al., 2014). Defense capabilities of N/TERTs (DEFB1 expression) were not compromised by exposure to IFN-α, suggesting that colonization does not necessarily imply infection.

The effects of IFNs on barrier proteins are likely multifaceted. Components of the cornified envelope (FLG, FLG2, LOR, and IVL) and tight junction components (DSG1) showed lower expression following IFN exposure, indicating a negative impact to the epithelial barrier. Also, barrier genes were repressed both at baseline and in the presence of IFNs in SLE keratinocytes. LOR, IVL, DSG1, and FLG2 are also necessary in the formation of corneocytes and stacked layers of lipid lamellae, critical to the formation of an intact barrier. Formation of an impaired barrier is known to result in penetration of S. aureus into the deeper layers of the skin in AD (Nakatsuji et al., 2016). Furthermore, the protective function of commensal microbiota is reversed upon damage to the epidermal barrier (Burian et al., 2017). Loss of filaggrin may have several impacts. Natural moisturizing factor is formed via breakdown of filaggrin and lowers skin pH, and S. aureus can proliferate rapidly with small increases in pH because of low filaggrin (Cabanillas and Novak, 2016; Mijašlovic et al., 2010). Also, filaggrin loss has been reported to result in higher photosensitivity (Mildner et al., 2010). It could thus be hypothesized that, in addition to promoting S. aureus colonization, barrier compromise because of prolonged IFN exposure leads to inflammatory responses to commensals or other triggers such as UV light.

Treatment with IFN-γ also resulted in downregulation of barrier components. Similar results were obtained when neonatal foreskin keratinocytes were exposed to IFN-γ, leading to significant damage to the epidermal barrier (Banno et al., 2003). Lower FLG expression upon exposure to IFN-γ has been reported previously, whereas Noh et al. (2010) also report the increase in filaggrin protein content upon such exposure (Hvid et al., 2011; Noh et al., 2010). IFN-γ exposure leading to low invasion of S. aureus likely is due to the significantly lower expression of surface molecules, thus reducing interaction with S. aureus. Also, higher expression of ICAM1 upon exposure to IFN-γ likely leads to infiltration into the epidermis by neutrophils, contributing to damage (Dustin et al., 1988). Further study of the protective effects of IFN-γ are warranted.

SLE keratinocytes, when compared with HC keratinocytes, show higher S. aureus adhesion, supporting our colonization data. Our studies on S. aureus invasion into keratinocytes demonstrated significance in a subset of data, but this was not a defining feature of type I IFN exposure. IFN-γ exposure protected against invasion; given that IFN-γ did not result in
upregulation of ITGA5, these data potentially identify an important target for prevention of invasion. Colonization and disease activity data gathered from longitudinal studies could yield a clearer picture of the role of the skin microbiome in disease progression, including whether there are links to atopy presenting in patients with SLE. We hypothesize that S. aureus colonization could be a part of a feed-forward loop where chronic IFN dysregulation in SLE promotes colonization by S. aureus, which then induces the production of inflammatory cytokines by keratinocytes that further increase colonization. This could not only lead to long term colonization but also may impact systemic disease development. Addressing skin inflammation by further investigations into barrier restoration and its role in S. aureus colonization and skin could illuminate novel treatment strategies.

In summary, we demonstrate increased colonization with S. aureus in cutaneous lupus erythematosus lesions in patients with SLE and an increased propensity for SLE keratinocytes to adhere to S. aureus. This is promoted by chronic type I (and possibly type II) IFN exposure through dysregulation of barrier proteins and upregulation of adhesion molecules.

MATERIALS AND METHODS

Human subjects
All patients and HC subjects gave written informed consent according to the guidelines of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the University of Michigan Medical School. Patients with SLE fulfilled the criteria for diagnosis (American College of Rheumatology) (Hochberg, 1997) and were recruited from the University of Michigan Lupus Cohort. Skin biopsies for keratinocyte isolation were obtained from nonlesional skin of the upper thigh. SLE Disease Activity Index and CLASI scores of the subjects were also recorded for each subject. To avoid skewing of the SLE Disease Activity Index in patients with active rashes (which would overestimate the significance of rash colonization), we adjusted the SLE Disease Activity Index scores to remove the rash-related scores in all subjects.

Cell culture
Primary keratinocytes were isolated and cultured from biopsies as previously described (Aasen and Izpisúa Belmonte, 2010; Stannard et al., 2017). In brief, keratinocytes were grown in serum-free growth media (Epilife, Cascade Biologics, Portland, OR) supplemented with 1% penicillin-streptomycin (100 U/ml penicillin and 100 μg/ml of streptomycin; Gibco, Thermo Fisher Scientific, Waltham, MA) and 0.25 μg/ml of amphotericin B (Fungizone; Gibco) and human keratinocyte grown serum containing bovine pituitary extract, bovine insulin, hydrocortisone, bovine transferrin, human epidermal growth factor, and 0.1 mM Ca²⁺. For N/TERTs, see Supplementary Materials and Methods.

S. aureus colonization analysis
Patients were swabbed in their nares and on their chest and any lupus-related lesions present with sterile FLOQSwabs (Copan Diagnostics, Murrieta, CA) moistened with sterile phosphate buffered saline. Demographic data as well as associated serological information on the subjects are summarized in Supplementary Table S1. Vortexed sample (100 μl) was plated on CHROMagar (BD-BBL, Franklin Lakes, NJ) and incubated at 35 ± 2 °C for 24 hours. Mauve-colored colonies, indicating growth of S. aureus, were picked for further analysis. See Supplementary Materials and Methods for S. aureus confirmatory strategies.

Adhesion and invasion assays
Cell adhesion and invasion assays were performed as outlined previously (Edwards et al., 2011). Briefly, N/TERTs were seeded into 24-well plates and grown to confluence. N/TERTs were treated with IFNs at 1,000 U/ml for 24 hours ± baricitinib (10 μM) or DMSO as control in DMEM supplemented with 2% fetal bovine serum (to promote keratinocyte differentiation) (Kontzias et al., 2012). Dilutions of overnight cultures of S. aureus (1:100) were grown to mid log phase in tryptic soy broth (~2.5 hours) and washed before use. N/TERTs were washed in sterile phosphate buffered saline before adding S. aureus (~20–30 multiplicity of infection) suspended in keratinocyte growth medium containing 200 μg/ml gentamicin for 60 minutes to kill surface-associated S. aureus, followed by washing with phosphate buffered saline and CFU quantification.

See Supplementary Materials and Methods for other methods.

Data availability statement
Datasets related to the article can be found at Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). RNA sequencing data is available in Gene Expression Omnibus accession number GSE124939; Microarray data from cutaneous lupus erythematosus lesional skin is available in Gene Expression Omnibus accession number GSE81071.

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CONFLICT OF INTEREST
JEG received research funding from AbbVie, SunPharma, Celgene, and Genentech and serves on advisory boards for Novartis, AbbVie, and MiRagen. JMK received research funding from Celgene and serves on advisory boards for AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, and Eli Lilly. The other authors state no conflicts of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: SS, NP, JEG, JMK; Data Curation: SS, CCB, LCT; Formal Analysis: SS, CCB, LCT, JMK; Funding Acquisition: JMK; Investigation: SS, NP; www.jidonline.org

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.11.016.

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SUPPLEMENTARY MATERIALS AND METHODS

Bacterial strains and growth conditions

*Staphylococcus aureus* strain USA300, a community-acquired methicillin-resistant *S. aureus* kindly provided by Dr Alexander R. Horswill, was utilized for the adhesion and invasion assays involving keratinocytes and N/TERTs. *S. aureus* USA300 containing the plasmid pHCl48 (pCM29-dsRed) and expressing red fluorescent protein, also provided by Dr Horswill, was utilized for fluorescence and confocal microscopy. Chloramphenicol was supplemented at 10 μg/ml for growth of *S. aureus* USA300 expressing dsRed. *S. aureus* was grown in tryptic soy broth at 37 °C with shaking at 250 rpm. *S. aureus* was heat killed by incubation of log phase culture at 70 °C for 1 h for utilization in studies requiring killed *S. aureus*.

Confirmation of isolated *S. aureus* colonies

Mauve-colored colonies identified as *S. aureus* were restreaked on mannitol salt agar for confirmation of mannitol metabolism and grown in tryptic soy broth at 37 °C for colony PCR using primers targeting a region specific to *S. aureus* thermocinase (SaNuc forward 5'-GGGATTGTGATTGA TACGTTT-3' and reverse 5'-AGCCAAGCCTTTGACGAC TAAAGC-3' to confirm the presence of *S. aureus* as described previously (Zhang et al., 2004).

Cell culture

A human keratinocyte cell line (N/TERTs), immortalized by expression of human telomerase catalytic subunit hTERT, was employed for the in vitro assays. This cell line was utilized because of its ability to replicate for numerous passages while retaining the ability to differentiate (Dickson et al., 2000; Sarkar et al., 2018). All cells were cultured in serum-free keratinocyte growth medium supplemented with 30 μg/ml bovine pituitary extract, 0.2 ng/ml epidermal growth factor, and 0.3 mM calcium chloride as previously described (Gibco, Thermo Fisher Scientific, Waltham, MA) (Sarkar et al., 2018).

RNA isolation and real-time quantitative PCR

Total RNA was isolated from keratinocytes treated with different conditions using Direct-Zol RNA MiniPrep kit (Zymo Research, Irvine, CA) following manufacturer’s instructions. cDNA from 0.5 μg of isolated RNA was prepared using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Waltham, MA) and oligo (dT)12-18 primer. Quantitative real-time reverse transcriptase—PCR was performed using SYBRGreen PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific) and 7900HT Fast Real-time PCR system (Applied Biosystems) with the support of the University of Michigan DNA sequencing core. All samples were assessed in triplicate with controls utilized as appropriate.

Primers utilized for quantification of gene expression were ITGA5 forward 5'-GGGATTGTGATTGA TACGTTT-3' and reverse 5'-ATTCCGGTGAAGATCATTCTGTG-3', DEFB81 forward 5'-GGGATTGTGATTGA TACGTTT-3' and reverse 5'-GGGATTGTGATTGA TACGTTT-3', FLG forward 5'-GGGATTGTGATTGA TACGTTT-3', and reverse 5'-ACCAGGCCTATGCTTCT-3'.

RNA sequencing data analysis and gene expression heatmap

RNA sequencing data was obtained from keratinocytes isolated from nonlesional control and systemic lupus erythematosus skin as previously reported (RNA sequencing data is available in Gene Expression Omnibus; accession number GSE124939) (Tsoi et al., 2019). RNA sequencing log2 expression values were averaged for each selected gene in each condition. A heatmap was generated using the module HeatMap Viewer version 13.9 from GenePattern at https://cloud.genepattern.org/gp (Ref: Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP, GenePattern 2.0 Nature Genetics 38 no. 5 (2006): pp500-501) with the row relative color scheme, meaning that the values were converted to colors using the mean, minimum, and maximum values in each row.

Fluorescence and confocal microscopy

Glass cover slips (13-mm diameter) were coated with 0.1% gelatin (Sigma, St. Louis, MO) and seeded with N/TERTs and grown to confluence. IFN treatments were followed by treatment with Hoescht stain (1:100) for 10 minutes, washing with phosphate buffered saline to remove the excess and treatment with Prolong Gold to prevent fading (Thermo Fisher Scientific). Fluorescent bacteria and labeled nuclei were visualized with an Axio Observer Z1 Inverted Microscope (Zeiss, Oberkochen, Germany) using the appropriate filters to obtain images. N/TERTs and adherent *S. aureus* were also visualized by confocal microscopy using the Nikon A1 confocal laser microscope (60× objective, 1.4 na) made available by the University of Michigan microscopy core facility. N/TERT monolayer exposed to *S. aureus* for 90 minutes was utilized for automatically capturing sets of confocal images (1,024 pixels/inch) in the z-axis with a slice thickness of 0.2 μm. Images were also obtained at the position on the z-axis corresponding to the nuclear plane (0.5-μm section) to observe labeled nuclei (DAPI) as well as fluorescently labeled *S. aureus* (DsRed).
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SUPPLEMENTARY REFERENCES


Supplementary Figure S1. IFN exposure leads to inhibition of several barrier-related genes. N/TERTs were treated with 1,000 U/ml of IFN-α and IFN-γ for 6 hours followed by expression analysis via qRT-PCR. Graphs represent relative expression to β-actin. Results are from four individual experiments ± SEM. Data were analyzed by ANOVA in Prism and the corresponding P-values are reported. Ctrl, control; qRT-PCR, quantitative real-time reverse transcriptase–PCR; SEM, standard error of the mean.

Supplementary Figure S2. Staphylococcus aureus adhesion to WT N/TERTs increases with time of exposure. (a) N/TERTs were grown to confluence and treated for 24 hours with DMEM +2% fetal bovine serum. S. aureus adhesion assays were performed as described in Materials and Methods and results were presented as S. aureus CFUs recovered per 10,000 N/TERTs. Data presented are from at least four replicates from three independent experiments ± SEM. (b) S. aureus invasion to WT N/TERTs increases with time of exposure. N/TERTs were grown to confluence and treated for 24 hours with DMEM +2% FBS. S. aureus invasion assays were performed as described in methods and results were presented as S. aureus CFUs recovered per 10,000 N/TERTs. Data presented are from at least four replicates from three independent experiments ± SEM. CFU, colony-forming unit; SEM, standard error of the mean; WT, wild type.
Supplementary Figure S3. N/TERTs exposed to IFN-α allow greater adherence of stationary phase *Staphylococcus aureus*. *S. aureus* was grown overnight and washed cells were allowed to adhere to washed N/TERTs grown to confluence. Data were analyzed by ANOVA using Prism and *P*-values were reported as obtained from post hoc testing. ANOVA, analysis of variance; Ctrl, control.

Supplementary Figure S4. HC and SLE keratinocytes demonstrate greater adherence to *Staphylococcus aureus* when exposed to IFN-α. Keratinocytes were grown to confluence with or without 1,000 U/ml of indicated IFNs. *S. aureus* adhesion assays were performed as described in Materials and Methods, and results are presented as *S. aureus* CFUs recovered per 10,000 keratinocytes. Blue represents HC and green represents SLE keratinocytes. Graphs represent means of three independent experiments for four SLE and three HC keratinocyte cultures. CFU, colony-forming unit; HC, healthy control; SLE, systemic lupus erythematosus; Tx, treatment.
**Supplementary Table S1. Demographic Data on SLE Patient Cohort**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with SLE (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at sampling (SD)</td>
<td>38.96 (12.4)</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (5.9)</td>
</tr>
<tr>
<td>Female</td>
<td>48 (94.1)</td>
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<tr>
<td>Race (%)</td>
<td></td>
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<tr>
<td>White</td>
<td>36 (70.6)</td>
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<tr>
<td>Black</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (7.8)</td>
</tr>
<tr>
<td>Serology (% positive)</td>
<td></td>
</tr>
<tr>
<td>dsDNA</td>
<td>24 (51.1)</td>
</tr>
<tr>
<td>SS-a</td>
<td>22 (47.8)</td>
</tr>
<tr>
<td>SS-b</td>
<td>10 (21.7)</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>21 (45.7)</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>22 (47.8)</td>
</tr>
<tr>
<td>Cardiolipin IgG/IgM</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>B2GP IgG/IgM</td>
<td>6 (15.4)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Low C3</td>
<td>8 (17)</td>
</tr>
<tr>
<td>Low C4</td>
<td>9 (19.1)</td>
</tr>
<tr>
<td>Drugs currently taken (%)</td>
<td></td>
</tr>
<tr>
<td>Prednisone ≤ 10</td>
<td>31 (60.8)</td>
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<tr>
<td>Prednisone &gt;10</td>
<td>4 (7.8)</td>
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<tr>
<td>Mycophenolate mofetil</td>
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<tr>
<td>Methotrexate</td>
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<tr>
<td>Rituximab</td>
<td>10 (19.6)</td>
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<tr>
<td>Azathioprine</td>
<td>10 (19.6)</td>
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<tr>
<td>Cyclophosphamide</td>
<td>12 (23.5)</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>46 (90.2)</td>
</tr>
</tbody>
</table>

Abbreviations: dsDNA, double-stranded DNA; SD, standard deviation; SLE, systemic lupus erythematosus.