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Staphylococcus aureus colonization is increased on lupus skin lesions and is promoted by interferon-mediated barrier disruption

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Abstract

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 non-lesional 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 (CLE) 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

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exposure to both IFNα and γ whereas IFNγ appeared to inhibit invasion of *S. aureus*. CLE lesional skin microarray data and RNA-seq 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 ultraviolet light (UVB) (Chasset and Arnaud 2018; Muskardin and Niewold 2018; Stannard and Kahlenberg 2016), which raises type I interferons (IFNs) in the skin. Keratinocytes are an important source of IFNs, and SLE patients exhibit elevated production of type I IFNs, both at baseline and after UVB exposure, that 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 (Grice and Segre 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 IFNκ 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 lupus patients 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* superantigen 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 SLE patients have been limited, and whether IFNs impact colonization by *S. aureus* is unknown.

In this paper we investigate the role of IFNs in regulation of the colonization by *S. aureus*. We show that SLE patients 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 to matched healthy controls (HC). This suggests that dysregulation of type I IFNs in SLE could

lead to a feed-forward loop resulting in greater *S. aureus* colonization that in turn leads to inflammation and additional production of IFNs.

RESULTS

SLE-associated skin lesions are colonized with *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 Table S1 for demographics). Patients (n=54) were tested for *S. aureus* in their nares, on their chest and on any lupus-related skin lesions. SLE patients were colonized by *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%) (Fig. 1A). In comparison, psoriatic plaques, which are characterized by a mixed Th1/Th17 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; $p<0.0001$) (Fig. 1B). We next investigated the influence of *S. aureus* colonization on disease activity. No association between rash-adjusted SLE Disease Activity Index (SLEDAI) scores and colonization by *S. aureus* were noted (Fig. 1C). CLASI (Cutaneous Lupus Disease Area and Severity Index) 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 the CLASI scores were observed when uncolonized and rash colonized patients were compared ($q=0.0016$). Importantly, CLASI activity scores were also higher when patients with colonized skin lesions were compared to SLE patients colonized in other locations besides the rashes ($q=0.0458$) (Fig. 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 filaggrin (*FLG*) result in impaired barrier integrity and are important in AD pathogenesis (Nakatsuji *et al.* 2016). However, barrier gene expression in CLE is not well understood. Thus, we compared normalized data from microarray analysis of SLE lesional skin vs. HC skin (Gene Expression Omnibus (GEO); accession number GSE81071) (Berthier *et al.* 2019). Expectedly, IFN stimulated genes and IFNs including IFNκ, a regulator in keratinocytes for type I responses, were significantly elevated in cutaneous lupus lesions (1.53-fold change; $q=0.0006$). Importantly, barrier genes such as loricrin (*LOR*) and claudin *(CLDN1)* and transglutaminase (*TGM5*) were downregulated (Table 1). This suggests that the high IFN environment may downregulate the epidermal barrier, which could promote *S. aureus* colonization.

Exposure to IFNs results in diminished barrier gene expression

We thus 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 IFNα or IFNγ for 6 hours, RNA was isolated and gene expression was quantified via real-time PCR. As expected, IFNα treatment resulted in high expression of the transcriptional regulator *MX1,* and IFNγ treated cells showed high expression of the intercellular adhesion

molecule (*ICAM1*) demonstrating that IFNs utilized for treatment were functional (Fig. 2). Numerous genes involved in the formation of the cornified envelope including *FLG*, loricrin (*LOR),* and elastin (*ELN*), were found to be significantly repressed while tight junction proteins such as desmoglein (*DSG1*) and *FLG2* trended towards decreased expression following IFN exposure (Fig. 2; Fig. S3). Other extracellular matrix molecules such as fibrinogen (*FGG*) and fibronectin (*FN1*), which are known to interact with *S. aureus,* demonstrated reduced expression particularly in the presence of IFNγ. However, α-integrin (*ITGA5*) was upregulated in IFNα treated (but not IFNγ treated) N/TERTs. Keratinocytes respond to microbial surface molecules (MAMPs) by the production of antimicrobial peptides including β-defensins (*DEFB1*). IFNα treatment, but not IFNγ, increased *DEFB1* expression (Fig. 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 ~1.3 X 10^4 *S. aureus* CFUs recovered at 30 min. Time of exposure demonstrated a linear relationship with *S. aureus* adherence with ~1.3 X 10^5 CFUs recovered after 90 min (Fig. S1A). We also performed assays with gentamicin exposure to determine invasion kinetics since *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 (Fig. S1B). These trends were also observed with stationary phase *S. aureus* (data not shown).

We then determined whether exposure to IFNα or IFNγ influenced *S. aureus*-keratinocyte interactions. Confluent N/TERTs were treated with 1000 U/ml of either IFNα or IFNγ 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 IFNα as observed in Fig. 3A ($p=0.0397$). IFNγ treatment did not promote a significant increase in adherence in comparison to the untreated N/TERTs. Secondly, paired analysis performed on data from multiple experiments also revealed a significant increase in adhesion to N/TERTs post exposure to IFNα ($p=0.0056$). These trends were replicated when stationary phase *S. aureus* was utilized (Fig. S2).

To confirm that the effects 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 *et al.* 2018; Kontzias *et al.* 2012). Preincubation of N/TERTs with baricitinib (10μM) did not change basal rates of *S. aureus* adherence but blocked increased adhesion induced by IFN exposure (Fig. 3A). To confirm our data from counts, N/TERTs ± IFNs and

± 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 IFNα 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 IFNα and is actively inhibited by IFNγ

Keratinocytes are non-professional phagocytes that allow *S. aureus* entry by a fibronectin, integrin and cytoskeleton rearrangement mediated mechanism (Edwards *et al.* 2011; Löffler *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. IFNα treatment did not result in higher recovered CFUs. In stark contrast, IFNγ strongly repressed *S. aureus* invasion ($p<0.0001$) indicating a protective function for type II IFNs in N/TERTs (Fig. 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. R*epresentative images at the z-planes (0.5 μm sections) of the DAPI labeled nuclei of N/TERT demonstrate identifiable organisms within the cell as evidenced by the presence of fluorescent bacteria in close proximity to nuclei (Fig. 3D) and by counts which reflect the data from the invasion assays.

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

Given that IFNs downregulate barrier genes and non-lesional keratinocytes from SLE patients express elevated type I IFNs (IFNκ) at baseline (Sarkar *et al.* 2018), we next explored whether barrier genes were differentially expressed in non-lesional SLE vs. control keratinocytes (HC). To this end, keratinocytes were obtained from non-lesional skin and subjected to RNA-seq analysis as reported previously (Tsoi *et al.* 2019). Barrier genes such as *FLG*, *LOR*, *IVL, CLDN1* as well as antimicrobial peptides (DEFB) were repressed in SLE keratinocytes in comparison to HC keratinocytes (Fig. 4). In contrast, genes involved in adhesion such as *FN1*, *ITGA5* and *ITGB1* were elevated in SLE keratinocytes and even more-so after IFNα 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 non-lesional primary keratinocyte cultures to determine if *S. aureus* adhesion was increased in SLE patients. 6 sets of age and gender-matched combinations of SLE and HC keratinocytes were used for adhesion and invasion assays. As demonstrated in Fig. 5A, *S. aureus* demonstrated significantly greater adherence to the majority (5/6) of SLE keratinocytes in comparison to HC keratinocytes. In addition, higher adhered *S. aureus* CFUs were recovered from both SLE and HC keratinocytes exposed to IFNα (Fig. S4). Some, but not all, SLE keratinocytes demonstrated increased invasion (Fig. 5B). Together, these data suggest that higher colonization of SLE patients is likely 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 SLE patients are not well-defined. Here, we demonstrate that cutaneous lesions of SLE patients 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 to HCs.

Our study identified colonization rates among SLE patients similar to previous reports by Conti *et al.* (~21 %) and Hajialilo *et al.* (~48%) (Conti *et al.* 2016; Hajialilo *et al.* 2015). 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 similar 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 SLE patients were typically on immunosuppressive and antimalarial medications, which potentially may influence colonization. Further studies will help to delineate this.

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 IFNα exhibited increased *S. aureus* adhesion. Higher expression of *ITGA5,* a component of the integrin $\alpha_5\beta_1$ 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.* 2013). Defense capabilities of N/TERTs (*DEFB1* expression) were not compromised by exposure to IFNα suggesting that colonization does not necessarily imply infection.

The effects of IFNs on barrier proteins are likely multi-faceted. Components of the cornified envelope (*FLG, FLG2, LOR, 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, 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; *S. aureus* can proliferate rapidly with small increases in pH due to low filaggrin (Cabanillas and Novak 2016; Miajlovic *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 due to prolonged IFN exposure leads to inflammatory responses to commensals or other triggers such as UV light.

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

SLE keratinocytes, when compared to 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. IFNγ exposure protected against invasion; given that IFNγ 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 lead not only to long term colonization but may also 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 in CLE lesions in SLE patients with *S. aureus* 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 healthy control 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) (Marc C. Hochberg 1997) and were recruited from the University of Michigan Lupus Cohort. Skin biopsies for

keratinocyte isolation were obtained from non-lesional skin of the upper thigh. SLEDAI and CLASI scores of the subjects were also recorded for each subject. In order to avoid skewing of the SLEDAI in patients with active rashes (which would overestimate the significance of rash colonization), we adjusted the SLEDAI 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 Belmonte 2010; Stannard *et al.* 2017). In brief, keratinocytes were grown in serum free growth media (Epilife, Cascade Biologics) supplemented with 1% pen-strep (100 U/mL penicillin and 100 μg/mL of streptomycin; Gibco, MD) and 0.25 μg/mL of amphotericin B (Fungizone; Gibco, MD) and human keratinocyte grown serum containing BPE, bovine insulin, hydrocortisone, bovine transferrin, human epidermal growth factor, and 0.1 mM Ca^{2+}. For N/TERTs, see supplementary information.

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 PBS. Demographic data as well as associated serological information on the subjects are summarized in Table. S1. 100 μl of vortexed sample 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 information 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 1000U/mL for 24 hours +/− Baricitinib (10μM) or DMSO as control in DMEM supplemented with 2% FBS (to promote keratinocyte differentiation) (Kontzias *et al.* 2012). 1:100 dilutions of overnight cultures of *S. aureus* were grown to mid log phase in tryptic soy broth (~2.5 hours) and washed prior to use. N/TERTs were washed in sterile PBS before adding *S. aureus* (~20 −30 MOI) suspended in KGM for 90 min. Nonspecifically adhered *S. aureus* were removed by washing and N/TERTs liberated with 0.25% trypsin. N/TERTs were then quantified in each well via hemocytometer, lysed with 1% Triton X100, serially diluted, and plated on tryptic soy agar (TSA) to determine CFUs. For invasion assays, keratinocytes were treated with KGM containing 200 μg/ml Gentamicin for 60 min in order to kill surface associated *S. aureus* followed by washing with PBS and CFUs quantification.

See supplementary information for other methods.

Data Availability

Datasets related to the article can be found at GEO (https://www.ncbi.nlm.nih.gov/geo/). RNA-seq data is available in GEO accession number GSE124939; Microarray data from CLE lesional skin is available in GEO accession number GSE81071.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CONFLICT OF INTEREST

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ABBREVIATIONS USED

IFN	Interferon
SLE	Systemic Lupus Erythematosus
CLE	Cutaneous Lupus Erythematosus
HC	Healthy Control

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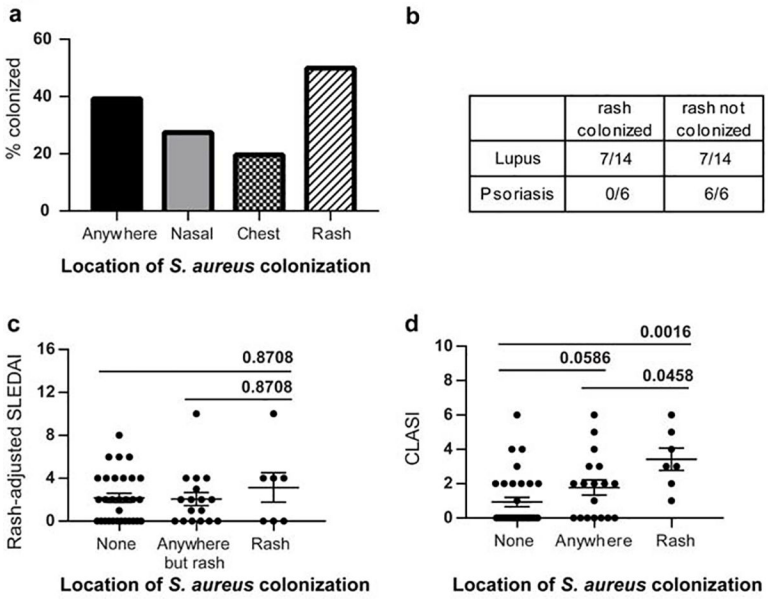

Fig.1. *S. aureus* colonization in SLE patients.

A. Graph represents percent of SLE patients (n=54) with positive colonization by *S. aureus* at indicated locations. Positive colonization was determined by mauve colonies on Chromagar after 24 hrs incubation followed by confirmation by PCR as outlined in Methods. **B. *S. aureus* colonization rate in SLE is significantly different from the same in psoriasis.** Lesions in SLE and psoriasis were swabbed and cultured and checked for *S. aureus* colonization. Number of samples (rashes) identified as *S. aureus* positive are presented as a fraction of the total number of samples that were tested. **C. Colonization with *S. aureus* on rashes with respect to systemic disease activity.** SLEDAI scores were obtained from all patients who were sampled for *S. aureus* colonization. Data represents the rash-adjusted SLEDAI scores (see methods) plotted against the colonization status of patients ± SEM. **D. Colonization with *S. aureus* is associated with higher cutaneous disease activity.** CLASI scores of patients were obtained for all the patients sampled for *S. aureus* colonization. Data represents CLASI scores graphed based on their colonization status ± SEM. Data were analyzed by ANOVA on ranks and post-hoc testing using Kruskal-Wallis test was performed using Prism and the computed q values are presented.

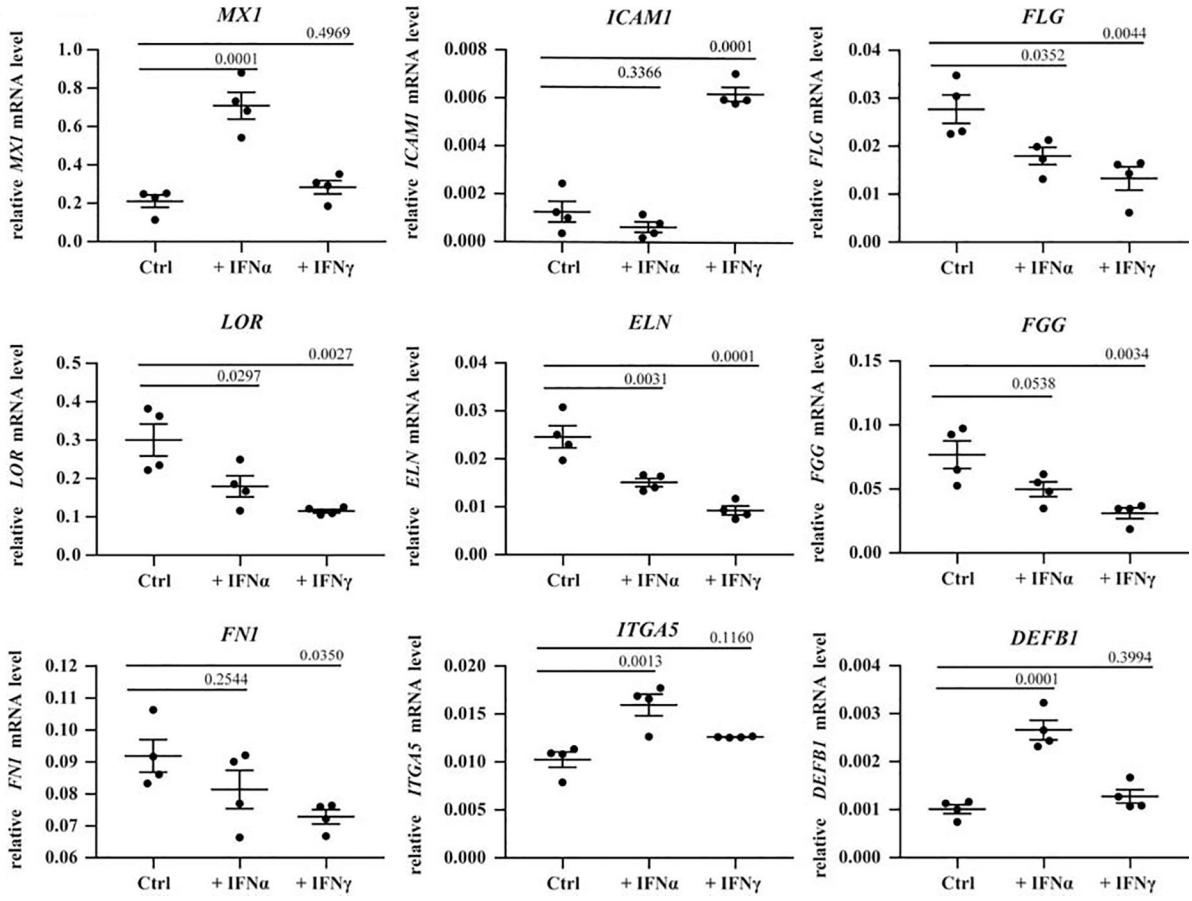

Fig. 2. Interferon exposure leads to inhibition of several barrier-related genes.

N/TERTs were treated with 1000 U/ml of IFNα and IFNγ for 6 hours followed by expression analysis via qRT-PCR. Graphs represent relative expression to β-actin. Results are from 4 individual experiments ± SEM. Data were analyzed by ANOVA in Prism and the corresponding p values are reported.

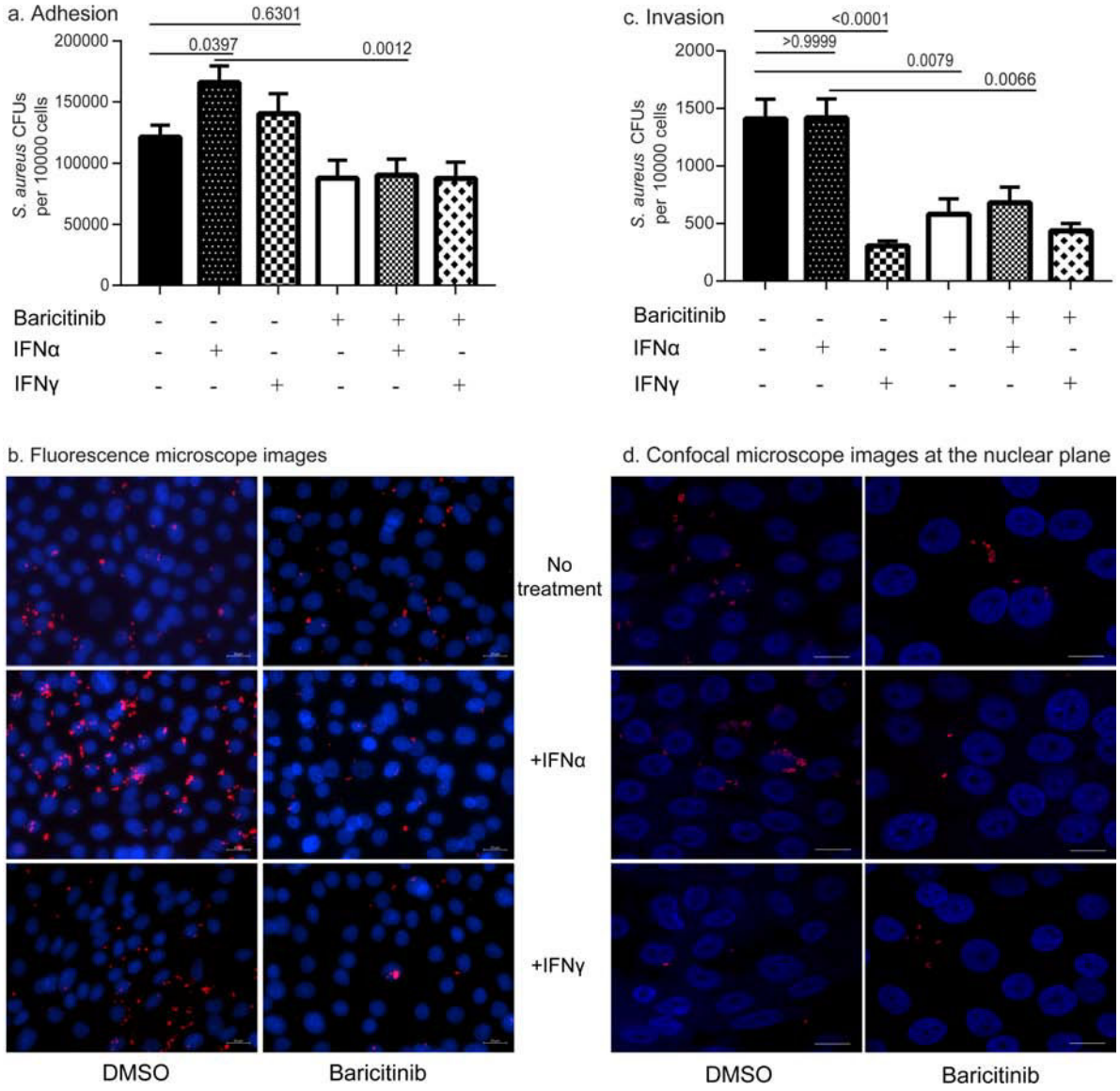

Fig 3. IFNα increases adhesion of *S. aureus* to WT N/TERTs.

A. N/TERTs were grown to confluence with or without 1,000 U/ml of indicated IFNs. In some instances, N/TERTs were exposed to 10 μM Baricitinib for 60 min before the addition of IFNs. *S. aureus* adhesion assays were performed as described in methods, and results are presented as *S. aureus* CFUs recovered per 10,000 N/TERTs. Data presented are from at least three independent experiments ±SEM. Data were analyzed by ANOVA in Prism and significance was reported as computed p values. **B. Fluorescence microscopy demonstrating higher *S. aureus* adhesion among IFN treated N/TERTs.** Confluent N/TERTs ± Baricitinib and ± IFNs were treated with *S. aureus* USA300 expressing dsRed. Hoescht stain was utilized for staining nuclei and representative images from at least three individual experiments are presented. Scale bar-20 μm. **C. Exposure to IFNs does not lead to an increase in invasive *S. aureus* CFUs.** N/TERTs were grown to confluence with or without 1,000 U/ml of indicated IFNs. In some instances, N/TERTs were exposed to 10 μM

Baricitinib for 60 min before the addition of IFNs. *S. aureus* invasion assays were performed as described in methods, and results are presented as *S. aureus* CFUs recovered per 10,000 N/TERTs. Data presented are from at least three independent experiments ±SEM. Data were analyzed by ANOVA in Prism and significance was reported as computed p values. **D. Confocal microscopy displaying invasion of *S. aureus* within N/TERTs.** Confluent N/TERTs ± Baricitinib and ± IFNs were treated with *S. aureus* USA300 expressing dsRed. Hoescht stain was utilized for staining nuclei and representative images obtained in the nuclear plane (0.5 μm sections) from at least three individual experiments are presented. Scale bar-60 μm.

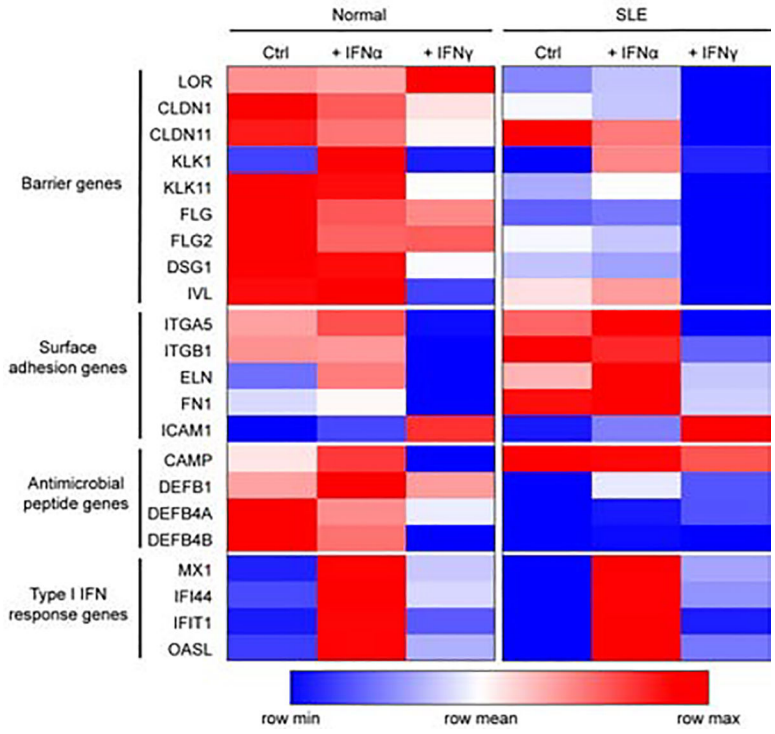

Fig 4. SLE keratinocytes exhibit lower barrier gene expression.

Heatmap of selected genes from primary control and SLE keratinocytes treated for 6 hours with indicated IFNs. Gene expression was measured via RNAseq. The largest values are displayed as the reddest, the smallest values are displayed as the bluest, and intermediate values are a lighter color of either blue or red.

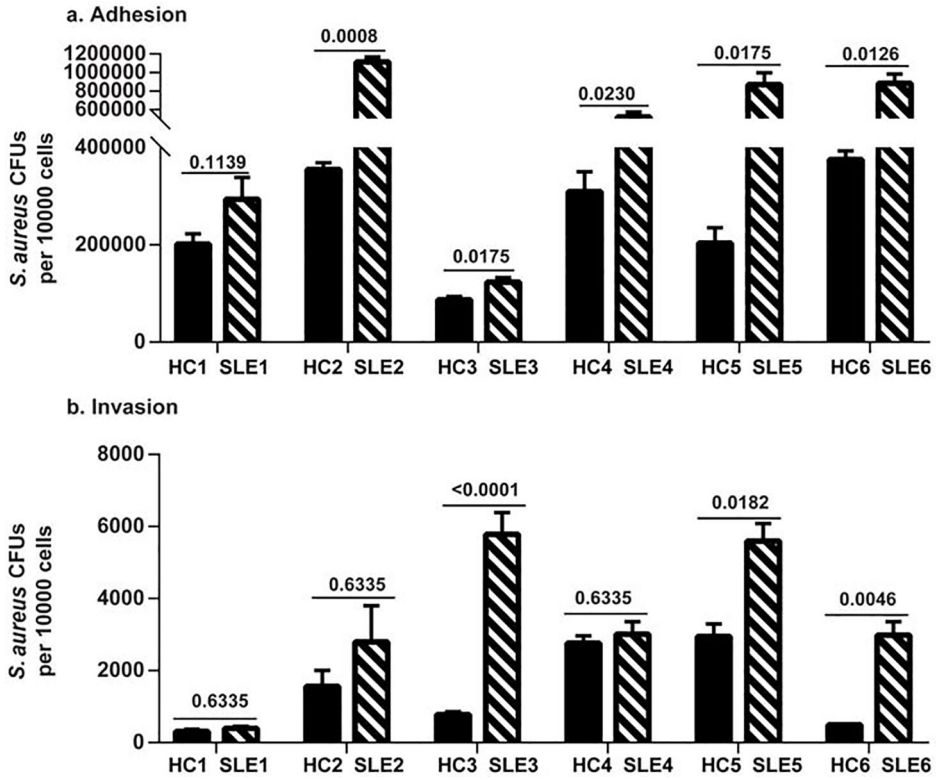

Fig 5. *S. aureus* demonstrated greater adherence to SLE keratinocytes compared to HC keratinocytes.

Keratinocytes from non-lesional skin from SLE patients and matched healthy controls (HC) were grown to confluence and exposed for 90 min to washed log phase *S. aureus* AH1263. *S. aureus* adhesion (A) and invasion (B) assays were performed as described in methods, and results are presented as *S. aureus* CFUs recovered per 10,000 keratinocytes. Results are presented as means from experiments performed at least in triplicate from each patient and paired control ± SEM (SLE n=6, HC n=6). Student's t test was performed on each matched pair and the p values are denoted.

Table 1.

Genes misregulated in CLE lesional skin obtained from microarray data analysis.

Function	Symbol	Description	FC	q-value
Barrier genes	LOR	loricrin	0.79	0.0401
	CLDN1	claudin 1	0.57	0.0007
	CLDN11	claudin 11	0.78	0.0401
	TGM5	Transglutaminase 5	0.50	0.0000
	KLK1	kallikrein 1	0.66	0.0011
	KLK11	kallikrein 11	0.55	0.0000
	FLG	*filaggrin*	*0.95*	*0.3028*
	FLG2	*filaggrin family member 2*	*0.77*	*0.2067*
	IVL	involucrin	2.09	0.0121
	ITGA5	*integrin subunit alpha 5*	*1.02*	*0.5746*
	ITGB1	*integrin subunit beta 1*	*1.04*	*0.5433*
Interferon response genes	IFI44	interferon induced protein 44	17.23	0.0000
	IFIT1	interferon induced protein with tetratricopeptide repeats 1	5.9	0.0000
Interferon genes	MX1	MX dynamin like GTPase 1	8.67	0.0000
	OASL	2'–5'-oligoadenylate synthetase like	2.35	0.0000
	IFNA10	interferon alpha 10	1.7	0.0149
	IFNG	interferon gamma	1.36	0.0488
	IFNK	interferon kappa	1.53	0.0006

Red - Upregulation; Blue - Downregulation; q value <0.05 considered significant