role of NETs in *S. aureus* colonization in skin microbiota or dysbiosis. Reevaluation of key findings in alternative ways would consolidate these proposals on the roles of NETs in skin homeostasis of bacterial colonization.

Second, it is not certain that all of the findings indicate physiologically relevant roles for NETs in vitro and in vivo and whether they can be attributed to identical phenomena in neutrophils. There are many controversies regarding the feasibility and reliability of the NET evaluation. PAD4 is a critical player in NET formation. PAD4 citrullinates histones, and histone citrullination is often used as a marker to detect NETosis. PAD4 inhibitors therefore have been utilized in NET evaluation in vitro and in vivo. However, PAD4 does not just regulate NETs but is involved in immune cell function in multiple ways, including hematopoiesis, cytokine production, apoptosis, and regulation of reactive oxygen species generation (Zhou et al., 2018). A reduction of skin bacteria after DNase treatment may also be caused by biofilm breakdown or disruption of NET-mediated immune evasion mechanisms of *S. aureus* via macrophage killing (Thammavongsa et al., 2013).

The new insights into the role of NETs in skin microbiota provided here raise further questions: (i) What is the precise molecular mechanism of the contact-free role of NETs? (ii) Which components (or secondary products) are essential for *S. aureus* survival? (iii) How are live keratinocytes involved? (iv) Do specific types of NETs have unique roles? and (v) Do NETs have different roles in skin infection versus colonization with microbes? These questions will stimulate further studies to address physiological roles of NETs in the delicate control of homeostasis of host defense, which are not addressed in this study, in the skin and other organs. Moreover, the argument raised here tempts us to consider unique roles for NETs in *S. aureus*—associated dysbiosis in specific pathological conditions, as well as in the interplay between NETs and microbiota in disease development and propagation in atopic dermatitis, diabetic foot ulcers, and beyond the skin.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**REFERENCES**


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**A Spectrum of Skin Disease:**

*How Staphylococcus aureus Colonization, Barrier Dysfunction, and Cytokines Shape the Skin*

Mary C. Moran\(^1,2\), Lisa A. Beck\(^1\) and Christopher T. Richardson\(^1\)

Cytokines are key mediators of skin homeostasis and disease through their effects on keratinocytes, skin barrier integrity, immune activation, and microbial ecology. Sirobhushanam et al. (2020) suggest that the IFN signature in lupus erythematosus (LE) alters expression of epithelial barrier and adhesin genes, which, in turn, promotes *Staphylococcus aureus* colonization. This work highlights the need to better understand both barrier function and *S. aureus* colonization in LE, two new potential therapeutic targets for the treatment of LE.

**COMMENTARY**

Psoriasis (PS), atopic dermatitis (AD), and cutaneous lupus erythematosus (LE) represent a unique spectrum of skin diseases based on the characteristics of barrier dysfunction, *Staphylococcus aureus* colonization, and cutaneous cytokine profiles. These features are thought to contribute to disease onset and exacerbations in a feed-forward loop, but without real clarity on which is primary or their relative importance. Sirobhushanam et al. (2020) investigate the effects of type I IFNs on *S. aureus* colonization and epithelial barrier gene...
**Clinical Implications**

- Patients with lupus are more commonly colonized with *Staphylococcus aureus* than patients with psoriasis.
- The epithelium of patients with lupus has alterations in gene expression, which may affect *S. aureus* adhesion, barrier function, and innate immune responsiveness.
- Some of these epithelial abnormalities may be explained by the actions of type I or type II IFNs.

expression in LE. This study, together with our current understanding of epidermal barrier function and *S. aureus* colonization in PS and AD, places LE in the center of this barrier disruption and *S. aureus* colonization continuum (Table 1).

*S. aureus* skin colonization

*S. aureus* skin colonization can play a significant role in the onset, progression, and severity of skin disease. Numerous studies have shown that >90% of patients with AD are colonized with *S. aureus*, which contrasts with ~20% of healthy controls and rare individuals with PS (Otto, 2010). We recently demonstrated that subjects with AD colonized with *S. aureus* have more severe disease, as measured by the Eczema Area and Severity Index; greater skin barrier disruption, as measured by increased transepidermal water loss (TEWL); and more type 2 immune deviation, as measured by elevations in serum total IgE, CCL17 levels, and absolute eosinophil counts (Simpson et al., 2018). Sirobhushanam et al. (2020) evaluate the frequency of *S. aureus* skin colonization in a small cohort of patients with PS and a much larger sample of patients with LE using routine culture techniques and PCR validation. They observed a modest increase in the percentage of patients with LE who were colonized with *S. aureus*, an enhanced epithelial expression of *S. aureus* adhesins (IGA5 and FN1), and a greater *S. aureus* adhesion to LE keratinocytes.

**Psoriasis**

PS, in contrast to AD and LE, is a largely IL-17–driven disease. Although this IL-17 skewing is relevant for the pathogenesis of a number of autoimmune and inflammatory disorders, it is also recognized as an important component of host defense and repair following infections with *S. aureus* (Otto, 2010). IL-17 is thought to have a number of protective roles in the skin, including enhancing production of antimicrobial peptides, such as lipocalin 2 and β-defensin, as well as neutrophil recruitment (Guttmann-Yassky and Krueger, 2017). More recently, IL-17A has also been shown to enhance tight junction (TJ) barrier function in primary human keratinocytes (Brewer et al., 2019). Collectively, these actions likely contribute to the observation made by Sirobhushanam et al. (2020) that none of their six subjects with PS were colonized with *S. aureus*.

**Atopic Dermatitis**

AD is characterized by type 2 immunity, epidermal barrier disruption, and *S. aureus* colonization (Weidinger et al., 2018). Barrier dysfunction is

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**Table 1. Unifying and Distinguishing Features of Three Common Inflammatory Skin Disorders**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Skin <em>S. aureus</em> (culture)</th>
<th>Skin <em>S. aureus</em> Adhesin Expression</th>
<th>Reduced Skin Barrier Gene Expression</th>
<th>NL Skin Barrier Dysfunction (TEWL)</th>
<th>Skin Cytokines</th>
<th>Skin Lesion Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>L &gt; NL2 (90%)</td>
<td>Yes6,7 (FGG &amp; FN1)</td>
<td>FLG, FLG2, CLDN1, CLDN4, CLDN8, CLDN2, IL-4, IL-13, IL-22, IFNγ</td>
<td>Flexors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus</td>
<td>L (50%)1, NL (0%)</td>
<td>Yes3 (ITGAS)</td>
<td>DSG1, CLDN1, CLDN11, FLG, FLG2, IL-12, IL-17, IL-23</td>
<td>UV exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psoriasis</td>
<td>L (0%)3</td>
<td>ND</td>
<td>Not well studied7</td>
<td>Normal19</td>
<td>TNFα, IFNγ, IL-12, IL-17, IL-22, IL-23</td>
<td>Extensors, palms &amp; soles</td>
</tr>
</tbody>
</table>

**Abbreviations:** CLDN, claudin; DSG, desmoglein; FGG, fibrinogen; FLG, filaggrin; FN1, fibronectin; ITGAS, integrin subunit alpha S; L, lesional; ND, not done; NL, nonlesional; *S. aureus*, *Staphylococcus aureus*; TEWL, transepidermal water loss.

Red text indicates unifying features.

1We have included only epithelial genes whose reduced expression has been shown to affect barrier function.

2Weidinger et al., 2018.

3Sirobhushanam et al., 2020.

4Cho et al., 2001.


6Brewer et al., 2019.

7De Benedetto et al., 2011.

8Darlenski et al., 2018.

9Visconti et al., 2015.

10Guttmann-Yassky et al., 2011.

11Polivka et al., 2018.

12Table 1.

13Figure 4.
thought to be the consequence of reduced expression of stratum corneum (SC) and TJ structural proteins, an imbalance of proteases and protease inhibitors, and altered lipid composition and organization. Epidermal barrier disruption promotes the release of alarmins such as TSLP, IL-25, and IL-33 that activate innate lymphoid cell type 2 cells and promote the recruitment of T helper type 2 (Th2) cells by inducing the release of the chemokines CCL17 and CCL20. The type 2 cytokines IL-4 and IL-13 have been shown to increase keratinocyte sensitivity to S. aureus toxins, suppress antimicrobial peptides, and promote S. aureus attachment by enhancing expression of fibrinogen and fibronectin (Cho et al., 2001; Weidinger et al., 2018). Collectively, these effects are thought to explain the high rates of S. aureus colonization in this disease, which arguably fuels a vicious cycle of barrier disruption and inflammation, ultimately leading to greater disease severity.

**Lupus Erythematosus**

Whereas PS is characterized by increased skin barrier function with decreased bacterial colonization and AD by the opposite, little was known about skin barrier proteins and bacterial colonization in the skin of patients with lupus. LE is characterized by an elevated type 1 IFN signature, both in the skin and systemically. Sirobhushanam et al. (2020) suggest that, similar to AD, patients with LE may have a feedback loop whereby type 1 IFNs alter the expression of epidermal barrier genes and S. aureus adhesins, thereby promoting S. aureus skin colonization, which drives further cytokine expression. This hypothesis would suggest that patients with LE with S. aureus colonization would have more severe systemic disease, which should be addressed in future studies. The effect of the cytokine milieu on barrier function in LE appears to be complex. As in AD, FN1 expression is increased and β-defensins are decreased in LE skin lesions as compared with controls (Figure 4), which would increase the likelihood of chronic S. aureus colonization. These expression differences are further enhanced in AD by the actions of Th2 cytokines (IL-4 and IL-13). However, the opposite effect was observed in IFNα-stimulated keratinocytes (Figures 2 and 4). This suggests that other cytokines that are present in LE skin are likely to be responsible for S. aureus colonization. IL-13, which has been shown to be elevated in LE, may contribute to the observed gene expression changes and ultimately S. aureus skin colonization observed in half of patients with LE (Morimoto et al., 2001).

The epidermal barrier has been studied in AD at the genetic, mRNA, protein, and functional levels. Null mutations in SC barrier genes such as FLG and FLG2 predispose individuals to development of AD, but their precise effects on barrier function and S. aureus colonization are still disputed (Hansmann et al., 2012; Kawasaki et al., 2012; Weidinger et al., 2018). The allergen sensitization that is characteristic of patients with AD is thought to be due to disruption of both SC and TJ barriers, which is supported by the reduced expression of a number of TJ proteins (CLDN-1, -4, -8, and -23). Similar observations have been made in canine AD (Altunbulakli et al., 2018; De Benedetto et al., 2011; Kim et al., 2016). Sirobhushanam et al. (2020) show that mRNA expression of several SC and TJ barrier proteins is decreased in skin biopsies or LE keratinocytes, but only a few of these proteins have been shown to affect barrier function (Table 1 and Figure 4; FLG, CLDN-1, CLDN-11, DSG1) (Tamura and Tsukita, 2014). Unfortunately, this study did not determine if these alterations in barrier gene expression were reflected in physiological measures of epidermal barrier function in patients with LE (in vivo) or in their keratinocytes (in vitro). Future studies will need to evaluate whether reductions in these barrier genes increase TEWL measurements in patients with LE or diminish transepithelial electrical resistance in LE keratinocytes propagated ex vivo. Such studies may highlight the potential importance of addressing skin barrier disturbance as a new therapeutic strategy for patients with LE.

**Future therapeutic approaches**

The authors previously demonstrated the importance of IFNγ in cutaneous LE pathogenesis (Sarkar et al., 2018). The three IFN types, type I IFN (e.g., IFNα and IFNκ), type II (IFNγ), and type III (IFNλ), should be explored to define their relative importance in epithelial barrier integrity, epithelial innate immune responses, expression of S. aureus adhesins, and ultimately in S. aureus colonization. Baricitinib, which is an oral selective Janus kinase (Jak) 1 and Jak2 inhibitor that improves the signs and symptoms of LE, was shown to reverse the increased S. aureus adhesion observed in IFNα-stimulated keratinocytes (Figure 3) (Wallace et al., 2018). A more targeted therapy to directly address the effect of type 1 IFNs on barrier function and S. aureus colonization in patients with LE would be anifrolumab, which is a fully human monoclonal antibody that binds to subunit 1 of the type I IFN receptor. Anifrolumab has recently been shown to be effective in LE (Morand et al., 2019).

Ustekinumab, a monoclonal antibody targeting IL-12 and IL-23 that is FDA-approved to treat PS, has also shown some benefit for the treatment of LE (van Vollenhoven et al., 2018). This might seem surprising based on its inhibition of the IL-17 pathway, which one might predict would further aggravate skin barrier dysfunction and promote S. aureus colonization in LE (Brewer et al., 2019). Although IL-17 has been shown to be modestly elevated in LE, there is not much evidence that this is relevant for LE pathogenesis (Martin et al., 2014). Therefore, one might assume that ustekinumab is improving LE primarily via its actions on IL-12 blockade and the inhibitory effect this has on the T helper type 1 response.

New therapeutic approaches to LE, both cutaneous and systemic, are needed as only one medication has been FDA-approved for LE in the past 60 years. By comparison, great strides have been made in the development of targeted and safe treatments for PS and recently for AD as well. This paper highlights a new hypothesis, namely that cutaneous colonization with S. aureus may play a role in LE pathogenesis. The importance of S. aureus colonization in systemic LE as either a driver of type 1 IFNs or as a consequence of skin barrier disruption (or both) needs to be established. This
study also suggests that there is much more to be done to understand the complex relationship between inflammatory skin diseases and cutaneous microbial ecology.

CONFLICT OF INTEREST
LAB is a consultant for Abbvie, Allakos, Astra-Zeneca, Connect Biopharma, LEO Pharma, Lilly, Novartis, Pfizer, Regeneron, Sanofi, UCB, and Vimalan. She has an investigator for Abbvie, LEO Pharma, Pfizer, and Regeneron, and she owns Pfizer and Medtronics stock. CTR is an investigator for LEO Pharma. MCM has no conflict of interest.

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