Cutaneous T-Cell Lymphoma Skin Microbiome Is Characterized by Shifts in Certain Commensal Bacteria but not Viruses when Compared with Healthy Controls


TO THE EDITOR
Cutaneous T-cell lymphomas (CTCLs) are a heterogeneous group of lymphoid malignancies derived from skin-homing T cells. Mycosis fungoides (MF) is the most common form of CTCL, and Sezary syndrome (SS) is an aggressive variant with varying levels of clonal lymphocytes in the blood. The variable presentation and lack of definitive diagnostic markers make CTCL diagnosis challenging. Although the biology of these malignancies is not fully understood, some microbes, particularly viruses, have been hypothesized to play roles in malignant T-cell transformation in CTCL (Berger et al., 2002; Mirvish et al., 2013; van der Loo et al., 1979). However, high throughput sequencing

SUPPLEMENTARY MATERIAL
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approaches have failed to consistently detect viral sequences in the skin or peripheral blood of patients with CTCL (Anderson et al., 2018; Dereure et al., 2013). Infections are common in patients with advanced stage, and antibiotic treatment results in skin improvement and decreased disease activity (Lindahl et al., 2019). Hence, to better understand the spectrum of microbial involvement in CTCL, we performed a comprehensive evaluation of the skin microbiome in a cohort of patients with MF and SS as compared with healthy controls.

In this pilot study, we used shotgun metagenomic sequencing to investigate microbial communities at predetermined, matched skin sites in 4 patients with MF (stages IA—IIB), 2 patients with SS (stage IVA1), and 10 age- and sex-matched healthy volunteers (HVs) (Supplementary Table S1). The study was approved by the Institutional Review Boards of Johns Hopkins (Baltimore, MD) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH (Bethesda, MD). Subjects provided written informed consent and underwent skin preparatory regimens. Premoistened swabs were used to collect samples from the nares, lower back, and thigh skin (sites of CTCL predilection) and air controls (see Supplementary Materials and Methods for details on patient recruitment and sampling). DNA was isolated, and libraries were created for metagenomic sequencing (Oh et al., 2014).

Bacterial, fungal, and viral communities were investigated by mapping microbial reads to a multikingdom reference database (Supplementary Table S2). Analyses focused on comparing the microbiomes between lesional patient skin and HV skin from the lower back and thigh. Bacteria predominated microbial communities at all sites (Figure 1a, Supplementary Figures S1a and S2a, and Supplementary Table S3). Of the most abundant taxa across kingdoms (Figure 1b and Supplementary Figures S1b and S2b), <0.5% of metagenomic reads mapped to eukaryotic viruses (predominantly Papillomaviridae and Polyomaviridae) in MF and/or SS and HV lower backs (0.09% ± 0.1% vs. 0.05% ± 0.05%) and thighs (0.08% ± 0.1% vs. 0.07% ± 0.08%) with no discernible differences regardless of sampling area (Figure 1c, Supplementary Figures S1c and S2c, and Supplementary Table S4). Similarly, fungal abundances did not differ significantly between HVs and patients with MF and/or SS (Figure 1b, Supplementary Figure S1b, and Supplementary Table S5); Shannon diversity was comparable (Supplementary Figure S3a and b).

Given the low viral and fungal relative abundances, we focused on bacterial communities in patients with MF and/or SS and HVs. We performed principle coordinates analysis using Bray-Curtis dissimilarity index, which demonstrated separation of HV and MF and/or SS bacterial skin communities on both lower backs and thighs (Figure 1d and Supplementary Figure S1d). Superimposing MF and/or SS stages on the principle coordinates analysis showed greatest separation between HVs and patients with stage IVA1, suggesting that skin microbiomes of patients with stage IV are the most distinct from controls.

We then further investigated specific taxa contributing to differences in bacterial communities among MF, SS, and HV skin. Given the association with Staphylococcus aureus colonization and infection in CTCL (Krejsgaard et al., 2014; Lindahl et al., 2019) and reported staphylococcal-corynebacterial interactions (Ramsey et al., 2016), we compared these and other common cutaneous bacteria. S. aureus abundances were low in most MF, SS, and HV skin samples (Supplementary Figure S4a and b). One HV and one patient with MF had higher S. aureus relative abundances on the skin. Commensal staphylococci (S. capitis, S. epidermidis, and S. hominis) trended higher in MF (3.8% ± 3.9%, 2.7% ± 2.1%, and 1.8% ± 2.4%, respectively) versus HV lower back skin (0.6% ± 0.6%, 1.4% ± 1.1%, and 0.8% ± 1.2%, respectively) (Supplementary Figure S4). Two Corynebacterium species (C. tuberculosis and C. simulans) were increased on MF and SS skin, with highest mean relative abundances in patients with SS (C. tuberculosis on lower back: 25.6% ± 24.3% [SS] vs. 4.4% ± 5.8% [HV]; C. simulans on lower back: 6.5% ± 5.5% [SS] vs. 0.3% ± 0.5% [HV]) (Figure 1e and Supplementary Figure S1e). MF and SS skin also displayed lower relative abundances of Cutibacterium acnes and Cutibacterium nannetense than HV skin. These bacterial shifts were not statistically significant, likely because of the small number of patients. However, comparing HV to MF to SS skin, we observed increasing trends in the mean relative abundances of Corynebacterium species and decreasing trends in Cutibacterium species, suggesting that bacterial shifts may correlate with disease stage or treatment status (Figure 1e and Supplementary Figure S1e).

Our findings suggest that eukaryotic DNA viruses are negligible components of the skin microbiome in our MF and/or SS cohort. These results corroborate and extend previous reports suggesting that CTCL is unlikely to originate from infection by a directly oncogenic DNA virus (Dulmage et al., 2015). However, other mechanisms by which viral pathogenesis can affect neoplastic transformation must be further explored including (i) indirect viral tumorigenesis by transient exposure to viral genomes (hit-and-run oncogenesis) (Niller et al., 2011), (ii) integration of retroviruses or DNA viral elements into human host DNA, and (iii) antigenic stimulation of T cells in the peripheral blood.

Our patients with MF and/or SS showed no marked differences in skin viral or fungal communities as compared with age-matched HVs sampled at consistent sites. Nonetheless, we observed bacterial community shifts including higher relative abundances of Corynebacterium species and lower relative abundances of Cutibacterium species in MF and/or SS skin. Several staphylococcal and corynebacterial species tended higher in MF and/or SS skin and would be important to examine in larger studies. Relative abundances of C. tuberculoastericum were high (>25% on average) in patients with stage IVA1. Patients with advanced stage may be at increased risk of infection from impaired skin integrity and immune dysregulation (Axelrod et al., 1992), and C. tuberculoastericum has been shown to upregulate proinflammatory
Figure 1. Bacteria predominate in skin microbiomes of both patients with MF and/or SS and healthy adults, but bacterial communities differ between patients and controls. (a) Relative abundances of lower back skin microbes classified by kingdom in HVs (n = 10) and patients with MF and/or SS (n = 6). Boxplot with each dot corresponding to one subject and representing the mean relative abundance (%) of one lower back sample per subject (lesional skin for patients; healthy skin for HVs). For each box, the central line represents the median, lower and upper edges represent the first and third quartile, and whiskers represent values up to 1.5 times the interquartile range. Shotgun metagenomics microbial reads were mapped to a multikingdom reference database containing 2,356 bacterial, 395 fungal, 4,695 viral, and 67 archaeal reference genomes using Bowtie2. (b) Mean relative abundances (%) of the major microbial taxa detected across kingdoms in lower back skin samples of HVs compared with MF and/or SS. In the barplot, colors represent distinct taxa. Owing to the unusually high
responses in human skin cells, suggesting a potential link to cutaneous inflammation (Altonsy et al., 2020).

Recently, Salava et al. (2020) reported some differences in bacterial communities between clinically unaffected and affected patient skin without including HV controls. Because of the potential absence of uninvolved contralateral skin in patients with advanced stage, reports of lymphoma involvement in normally appearing skin in patients with CTCL (Pujol et al., 2000), and different skin microbiomes in anatomically distinct but adjacent sites, we studied control samples from age-matched HVs sampled at comparable anatomical sites.

The separation of bacterial communities between patients with stage IVA1 and HVs is notable, and it is intriguing that bacterial shifts appear to correlate with disease stage. Whether these findings are driven by disease severity or other unrelated variables, including systemic treatments, is unknown. Further investigation with larger and ideally multicenter CTCL cohorts is warranted to validate these findings and to evaluate the relationship between disease stage and the skin microbiome.

Data availability statement
All sequencing data were deposited and are available at the National Center for Biotechnology Information Sequence Read Archive under BioProject number PRJNA642893.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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Corynebacterium spp. and Xanthomonas campestris on the lower back skin of HV4, this subject was removed from downstream analyses. (c) Relative abundances (%) of eukaryotic viruses on the skin of HV4 and patients with MF and/or SS. (d) PCoA of lower back skin bacterial communities in HVs and patients with MF and/or SS. The distance between samples was measured using Bray-Curtis dissimilarity index. Each dot corresponds to one lower back sample per subject. HVs (n = 9) are represented by gray dots. Patients with MF and/or SS are color-coded by clinical stage IA (n = 1), IB (n = 1), IIB (n = 1), IIA (n = 1), IVA1 (n = 2). Clustering of dots in the PCoA indicates higher similarity between skin bacterial communities. (e) Mean relative abundances (%) of common cutaneous bacteria (Corynebacterium spp. and Cutibacterium spp.) on the lower back skin of HVs (n = 9) compared with patients with MF (n = 4) and SS (n = 2). Each dot corresponds to one subject and represents the relative abundance in one representative lower back sample. Dots are color-coded as in (d). Black horizontal lines represent the mean. HV, healthy volunteer; MF, mycosis fungoides; PC, principle coordinate; PCoA, principle coordinates analysis; spp., species; SS, Sézary syndrome.
Topical Fluoxetine as a Potential Nonantibiotic Adjunctive Therapy for Infected Wounds


TO THE EDITOR

Chronic wounds remain a significant clinical challenge, with annual estimated expenditures in the United States alone being $28.1 billion (Sen, 2019), which is expected to increase with the projected 165% jump in diabetes by 2050 (Boyle et al., 2010). Recent studies have identified microbial infection as an important factor driving wound chronicity (Williams et al., 2018; Zhao et al., 2016). In these wounds, host antimicrobial and inflammatory defenses cannot eradicate pathogens, which then continue to recruit neutrophils and monocytes resulting in persistent proinflammatory signals that impair healing. Bacterial virulence factors are also linked to impaired healing. Staphylococcus aureus species are known to upregulate various proteases allowing for penetration into wound tissue (Kolar et al., 2013). Biofilm formation is a nearly universal ability shared by highly virulent pathogens and documented to be associated with wound chronicity (Kalan et al., 2019). Wound biofilms are difficult to eradicate owing to their resistance to penetration by neutrophils and their poor response to systemic antibiotics, which brings attendant risks, including bacterial resistance. With studies indicating that an estimated 65% and 80% of all microbial infections in humans are attributed to biofilms (Lewis, 2007), biofilm eradication remains a top challenge, and this underscores the need to develop nonantibiotic wound infection therapies that can also target the biofilm.

Although the selective serotonin reuptake inhibitor, fluoxetine (FLX), has been reported to have antimicrobial activity against some gram-positive bacteria in vitro (Munoz-Bellido et al., 2000) and diminish in vitro biofilm formation by Proteus mirabilis (Nzakizwanayo et al., 2017), these findings have not been translated to mammalian wounds or examined in clinically relevant models. The known effects of FLX on improving keratinocyte migration speeds and promoting a shift of the wound environment from proinflammatory to anti-inflammatory (Nguyen et al., 2019) combined with potential antimicrobial and antibiofilm activities present a potential for a multipronged therapeutic to improve healing in chronic wounds. To test this possibility, we used in vitro as well as a clinically translational human skin ex vivo and murine models to examine the potential of FLX to modulate factors contributing to wound chronicity—epithelialization, persistent inflammation, inflammatory mediators, and biofilm formation—within the context of wound infection.

Planktonic cultures of S. aureus clinical isolate UAMS-1 cultured with the minimum inhibitory concentration 100 µM FLX (Supplementary Figure S1) exhibited significantly decreased growth compared with the medium control (Figure 1a). UAMS-1 biofilms cultured with 100 µM FLX similarly exhibited a decrease in mass (Figure 1b). The expression of virulence factors implicated in biofilm formation, including fib, sarA, and hla, were significantly downregulated (Figure 1c) in planktonic cultures. For clinically relevant translation, we used a human skin ex vivo wound infection model (Yoon et al., 2019) wherein human skin was collected following written informed consent under a University of California Davis Institutional Review Board–approved protocol. When S. aureus–infected human skin wound explants were treated with daily application of topical 0.2% FLX, scanning electron microscope images of the wound surface resembled those of the uninected controls with the absence of detectable biofilm, which was clearly detectable in the untreated, infected wounds (Figure 1d). Treated wounds exhibited a greater than 10^5-fold reduction in viable bacteria compared with the infected control (1.6 × 10^9 vs. 6.6 × 10^10 colony-forming units) (Figure 1e). Pre reparative innate responses were observed in the FLX-treated skin samples, including upregulation of proinflammatory cytokines IL-6 and TNF-α (Figure 1f).

The synergism of host response with topical FLX was examined using an in vivo murine wound infection model...