Vgll3 causes discoid lupus-like fibrosis in a mouse model of lupus
M Ghareh-Kermani1, AC Billi, JM Kahnemberg2 and JE Gadjillosson3 1Department of Dermatology, University of Michigan, Ann Arbor, Michigan, United States
Fibrosis is an abnormal wound healing process characterized by collagen deposition, myo-fibroblast accumulation and extracellular matrix remodelling. Fibrosis arises as a consequence of many autoimmune diseases, where it may be widespread and affect organs beyond the skin including lungs and kidneys. Skin and organ fibrosis is often associated with high morbidity and even mortality, and there is no effective treatment. Recent work from our laboratory has shown that epidermal overexpression of murine Vgll3 causes severe lupus-like skin lesions reminiscent of discoid lupus erythematosus (DLE), as well as systemic autoimmune disease with end-organ damage. Given the apparent fibrotic nature of the skin lesions in transgenic (TG) Vgll3 mice, we wanted to determine whether Vgll3 induced fibrosis. We analyzed male and female TG and wild-type (WT) mice aged 2-3 months, comparing fibrotic biomarkers of human DLE and scleroderma. Here, we demonstrate that epidermal Vgll3 overexpression causes development of not only cutaneous inflammation but also severe fibrosis. Changes include increased infiltration of granulocytes/monocytes accompanied by significant expression of fibrotic biomarkers (Acta2, Col1, Tgb1, and Ccn2, also known as connective tissue growth factor (Ctgf) and pro-fibrotic cytokines (I4 and I31) in TG mice. The detection of high expression of Ctgf and Tgb1 as well as Col1 mRNA and protein in the skin of TG mice, as is seen in skin of human scleroderma and DLE patients, suggests that skin-directed overexpression of Vgll3 may implicate fibrosis development, and there may be a role for targeting Ctgf, Tgb1 and other pro-fibrotic cytokines in fibrosis. Further studies will need to elucidate the specific mechanisms that may be at play.

Multidimensional in situ immune profiling of discoid and subacute cutaneous lupus erythematosus
T Vazquez1, J Patel2, E Keyes2, D Yan3, D Diaz3, M Bashir3, R Feng4, M Grinnell2, VM Billi1, FM Wurth1, O Plazyo1, R Wasikowski1, M Gharaee-Kermani1,3, A Hurst3, C Dobry1, L Ortolan2, N Henderson2 and A Jabbari3,2,1
1Dermatology, University of Michigan, Ann Arbor, Michigan, United States and 2 Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States
Cutaneous lupus erythematosus (CLE) can be subdivided into acute cutaneous (ACLE), sub-acute cutaneous (SCLE), and chronic cutaneous LE (CCL), and the predominant subtype of discoid lupus erythematosus (DLE) is the predominant subtype). Previous studies using RNA extracts or traditional immunostaining have demonstrated subtle differences between the subtypes; however, no multiplexed, single-cell analyses have been conducted. We profiled the immune infiltrate of DLE and SCLE using Imaging Mass Cytometry, an unbiased, high-plexed, cell analyses have been conducted. We profiled the immune infiltrate of DLE and SCLE using Imaging Mass Cytometry, an unbiased, high-plexed, imaging technique for cellular level analysis. 19 CLE and 18 DLE, treatment-naive FFPE biopsies were stained with 37 metal-conjugated antibodies that were selected on the Hyperion Imaging System (Fluidigm). Cells were segmented using a nuclear based algorithm on Visipharm and imported into histocAT where cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm based on cell markers. Significance was determined by the Mann-Whitney test, bivariate correlations were determined by Pearson’s r. We found 9 unique populations consisting of dermal CD T4, CD T8, CD14+CD16+ macrophages, CD68+ macrophages, B cells, CD56+ Cells, Tregs, conventional dendritic cells (cDC), and plasmacytoid dendritic cells (pDC) with similar percentages between DLE and CLE (p>0.05). 16 cytokines and phosphorlated inflammatory signaling pathways were included and the data revealed higher pTBK1 in DLE compared to CLE (p<0.05). At the cell type level, the data showed increased pIRF3 in DLE pDCs compared to CLE (p<0.05). Overall, these results suggest substantial overlap between DLE and CLE, with a potential role for pTBK1 and pIRF3 in DLE. Future studies are needed to investigate the potential suitability of these pathways as targeted ther- apies for DLE.

Single-cell composition and architecture of cutaneous lupus
AC Billi, MA O'Plazyo, W Wasikowski, M Ghareh-Kermani1,2, A Hurst1, C Dobry1, LT Stron, MP Mellrin1, R Modlin1, JG Jodjilsson1 and JM Kahnemberg2 11Dermatology, University of Michigan, Ann Arbor, Michigan, United States, 2 Molecular, Cell, and Developmental Biology, University of California Los Angeles, Los Angeles, California, United States and 3J Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, Michigan, United States
Cutaneous lupus erythematosus (CLE) is an incompletely understood autoimmune disease that can occur in isolation or in the context of systemic lupus erythematosus (SLE). Lupus is often disfiguring, and no FDA-approved therapies for CLE exist. Further, evidence suggests skin inflammation in CLE can provoke systemic autoimmune disease, including precipitating dangerous kidney inflammation. Thus, understanding CLE pathogenesis has great potential to alleviate patient suffering and even mortality. We employed single-cell RNA-sequencing (scRNA-seq) and spatial sequencing to investigate the transcriptionsmes and arrangement of the cellular players in CLE. 7 patients with active CLE were enrolled. 5/7 carried a diagnosis of SLE. Single-cell RNA sequencing and spatial gene expression data was performed on dermal and epidermal skin biopsies and peripheral blood mononuclear cells (PBMCs) were subjected to scRNA-seq on the 10x platform. Comparison to control cells derived from 14 healthy skin biopsies and PBMCs from 4 healthy donors revealed dramatic transcriptionsal differences between healthy, nonlesional CLE and lesional CLE keratinocytes, fibroblasts, and immune cell subsets. Additionally, subclustering of skin biopsy-derived immune cells and PBMCs identified potential circulating precursors to the immune cells that infiltrate the skin and give rise to CLE lesions. Finally, integration of the scRNA-seq data with spatial gene expression data enabled prediction of T cell populations that are differentially regulated in tolerance due to decreased Tregs in patients refractory to antiinflammatories. Our results show that activated T cell populations in CLE may be responsive to TGF-β1, CD40L, and IL-23, and may therefore be a potential target for therapeutic intervention.

Microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials
J Patel1, T Vazquez1, D Yan2, E Keyes2, D Diaz3, Y Li1, M Grinnell1,2, R Feng4 and V Wurth1 1Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States
Lupus erythematosus (LE) is a systemic autoimmune disease with a variety of cutaneous manifestations. Antimalarials are first-line systemic therapy, yet not all patients respond to chloroquine (CQ) or hydroxychloroquine (HCQ), quinacrine, or either (NC). Our group has previously shown that CQ responders demonstrate increased conventional dendritic cells (cDC) and TNFα relative to HCQ responders. Here, we investigated the differences between these patients using Imaging Mass Cytometry (IMC), an unbiased multiplexed technique. 12 HCQ, 11 CQ, and 20 NC patients were enrolled, and single-cell FFPE samples were stained with 37 metal conjugated antibodies and ablated on the Hyperion Imaging System (Fluidigm). Images were segmented using a nuclear app-based algorithm in Visipharm and imported into histocAT where cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm. One-way ANOVA, Kruskal-Wallis, and post-hoc Tukey’s tests (p data normality) were performed. Correlations were determined by Pearson’s r. NC patients were found to have a higher expression of pSTING and pIFNα compared to CQ responders (p<0.05). CQ responders had a higher expression of pSTING and IFNα compared to HCQ responders (p<0.05). The total expression of pSTING and IFNα was found to positively correlate and colocalize in skin (p<0.001), r=0.676). CD14+CD16+CD68+ macrophages and cDCs were the predominant cell type cluster identified in all patients and were present in increased numbers in response to antimalarials in CQ responders. Our results show that activated STING correlated with IFNα, suggesting co-regulation in macrophages and cDCs. This may lead to further discovery of biomarkers that may predict patient response to therapy and direct target- ed treatment.