AWARD NUMBER: W81XWH-20-1-0482

TITLE: Immunologic Mechanisms for Heterogeneity of Cutaneous Lupus Erythematosus

PRINCIPAL INVESTIGATOR: Victoria Werth

CONTRACTING ORGANIZATION: Philadelphia Research and Education Foundation
Philadelphia, PA

REPORT DATE: October 2023

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Final	01Jul2020-30Jun2023

4. TITLE AND SUBTITLE

Immunologic Mechanisms for Heterogeneity of Cutaneous Lupus Erythematosus

5a. CONTRACT NUMBER
LR190107

5b. GRANT NUMBER
W81XWH-20-1-0482

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Victoria Werth

E- Mail:werth@pennmedicine.upenn.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Philadelphia Research and Education Foundation
3900 Woodland Ave
Philadelphia, PA 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our goal is to understand the biologic determinants in newly diagnosed patients with discoid lupus erythematosus who then do not respond to antimalarials. Understanding the molecular and cellular basis for the heterogeneity of responses to treatment will aid in the appropriate selection of patients for a given treatment and potentially indicate new therapeutic targets. Phosphorylated STING and NFκB are higher in HCQ+QC-responders, and we have performed sub-analyses demonstrating a correlation between pSTING and IFNκ, as well as increased cDC-labeled pSTING and IFNκ, further verified by in situ hybridization studies. In addition, the numbers of T regulatory were lower in antimalarial-refractory than QC-responsive patients. With the advent of new therapies such as STING inhibitors and other therapies that boost T regulatory cells, the studies will aid in subsetting patients for evaluation for future therapeutic

15. SUBJECT TERMS
Cutaneous lupus erythematosus, immune cells, pathways

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	24	USAMRDC
Unclassified	Unclassified	Unclassified			19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Introduction:
Approximately 50% of patients with discoid lupus erythematosus (DLE) respond to antimalarials, the primary first-line therapy for DLE. Because of a two-month delay in onset of action of antimalarials, however, the other half of these patients continue with disease activity and progressive scarring, only to find they are not responding. Antimalarials treatment includes hydroxychloroquine (HCQ), yet not all patients respond to hydroxychloroquine (HCQ), quinacrine (QC), and many are refractory to antimalarials (NR). Our group has previously shown that QC responders demonstrate increased conventional dendritic cells (cDC) and TNFα relative to HCQ responders. We proposed to investigate the differences between these patients using imaging mass cytometry (IMC), an unbiased multiplexed technique. The molecular and cellular basis for this heterogeneity of responses to treatment remains uncharacterized, leading to treatment delays and then the use of toxic therapies and multiple therapeutic regimens. Our goal is to understand the biologic determinants in newly diagnosed DLE patients who then do not respond to antimalarials. We hypothesize that, before treatment, antimalarial non-responders will have differentially expressed T cell subsets and unique pathway markers relative to antimalarial responders.

Keywords: Discoid lupus erythematosus, immune cells, CyTOF, pathways

Accomplishments:

Major goals: In Aim 1, immunologic analyses of the cellular infiltrate in lesional DLE skin from well-characterized patients at baseline who subsequently were determined to be antimalarial responders or antimalarial non-responders will be examined using CyTOF.

In aim 2, analysis of cells and corresponding signaling pathways together in lesional DLE skin from well-characterized patients at baseline who subsequently were determined to be antimalarial nonresponders or antimalarial responders. In addition, lesional skin mRNA expression for key pathways will be examined.

What was accomplished under these goals?

Patients were carefully selected and their response to antimalarials characterized. Skin punch biopsies (4mm) from lesional DLE skin from 20 patients (11 antimalarial responsive and 9 antimalarial refractory) were obtained at baseline prior to treatment (Table 1). These biopsies were formalin-fixed and paraffin-imbedded specimens. Many had to be requested from outside labs since they were done at the time of diagnosis, requiring data transfer agreements. Two panels of 39 antibodies each were designed for the study of cells and pathways. The antibodies were tested for appropriate

		DLE (n = 20)	HCQ (n=5)	QC (n=6)	NR (n=9)
Age – median (IQR)		35.5 (29.0 – 54.0)	35 (28.0-61.5)	36 (30.5-51.0)	38 (28.5 – 49.0)
SLE – n (%)		6 (30.0)	0 (0.0)	2 (33.3)	4 (44.4)
Sex – n (%)	Female	18 (90)	3 (60.0)	6 (100.0)	9 (100.0)
	Male	2 (10.0)	2 (40.0)	2 (15.4)	0 (0)
Race – n (%)	Caucasian	9 (45.0)	4 (80.0)	1 (16.7)	4 (44.4)
	African American	9 (45.0)	0 (0)	4 (66.7)	5 (55.6)
	Asian	2 (10.0)	1 (20.0)	1 (16.7)	0 (0)

Table 1. Demographics of patients

concentrations, labeled with metal halides selected based on staining intensity, and then used to stain the lesional DLE tissue sections. Slides were imaged using an Image Mass Cytometer (IMC). The final panels that were designed to evaluate pathways within specific cell types, as well as the types of cells present are as follows:

Panel 1: CD45, CD20, STAT3, pSTING, CLEC10A, CD14, IFNκ, CD16, STAT5A, CLE9A, CCR4, JAK3, CD31, CD25, IFNb, STAT1, FoxP3, CD4, CCR7, CD68, CD69, BDCA2, CD8, IRF3, CD56, TBK1, CD45RA, IFNγ, IL4, IL17, CD3, pERK1/2, IFNα, CD11c, HLA-DR, TNFα, and NFκB.
Panel 2: CD45, CD20, STAT3, STAT2, CCD14, STAT4, CD16, STAT5A, MPO, IRF5, JAK3, CD31, STAT6, IFNβ, STAT1, FoxP3, CD4, JAK2, CD68, TYK2, BDCA2, CD8, granzyme B, TCRγδ, CCR6, CD45RA, IFNγ, IL4, IL17, CD3, JAK1, IFNα, CD11c, HLA-DR, MAC387, CXCR3.

For visualization of cell clusters, dimensional reduction was performed using a t-SNE algorithm in histoCAT. The t-SNE plot highlighted cell clusters produced using PhenoGraph. Expression heatmap for cell clusters identified were then used to demonstrate differential marker expression in histoCAT. Significance was determined by the Mann-Whitney test, bivariate correlations were determined by Pearson's r.

Results are as follows:
We found 9 unique populations consisting of dermal CD4 T, CD8 T, CD14+CD16+ macrophages, CD14+CD16- macrophages, CD68+ macrophages, B cells, CD56+ cells, Tregs, conventional dendritic cells (cDC), and plasmacytoid dendritic cells (pDC's), with results showing the numbers (Figure 1) and percent of cell types relative. We next split the results into the three responder groups (HCQ, HCQ+QC, antimalarial refractory), looking at cells and pathways.

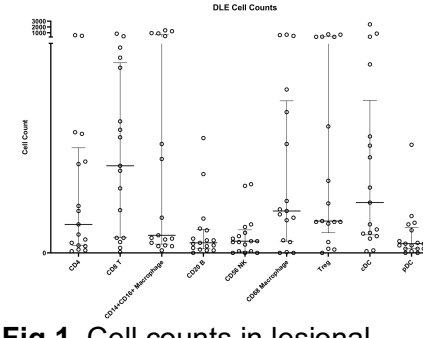

Fig 1. Cell counts in lesional DLE skin.

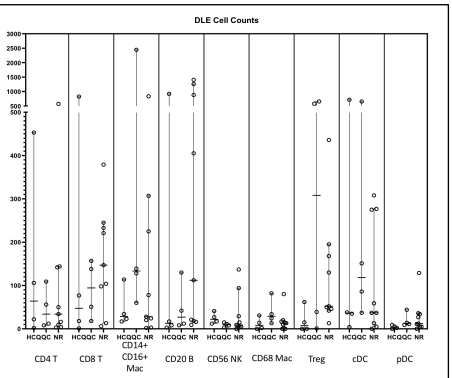

Fig 2. DLE cell counts according to antimalarial response.

As can be seen the cell counts varied between DLE samples, with the biggest range in CD4, CD8, $CD14^{+}CD16^{+}$ macrophages, CD68 macrophages, T regs, and cDCs. The nonresponders had a trend to more CD8 cells and more CD20 B cells. The numbers of T regs were lower in antimalarial-refractory than QC-responsive patients (Fig 2). Our results confirmed that cDCs were more prevalent in HCQ+QC responders. Phosphorylated STING and NFκB are higher in HCQ+QC-responders, and we have performed sub-analyses demonstrating a correlation between pSTING and IFNκ, as well as increased cDC-labeled pSTING and IFNκ (Figure 3). Biopsy level (i.e. marker

expression across all segmented cells) pathway median pixel intensity (MPI) of inflammatory pathways was evaluated between DLE and SCLE revealing increased expression of TBK1 in DLE compared to SCLE ($p<0.05$).

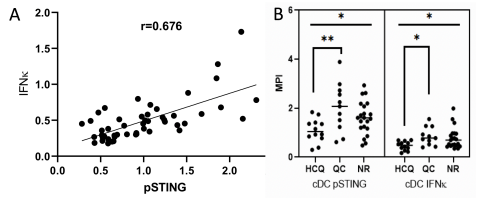

Fig. 3. Strong correlation between pSTING and IFNκ.

Hierarchical clustering revealed smoking status' association with CLASI-A, neutrophils, and GZMB, and direct analysis was conducted on this subgroup. Smokers had higher CLASI-A compared to non-smokers. Smokers had increased neutrophil percentages compared to non-smokers ($p<0.05$). GZMB expression within endothelial cells or within the vessel lumen was increased in smokers compared to non-smokers ($p<0.01$).

In situ hybridization using RNAscope techniques co-localized pSTING and IFNκ to cDCs (Figure 4). Additional studies with IMC derived heatmaps of single cell pathway expression in pDCs revealed a lack of type I IFN expression in DLE, with other cells serving as positive internal controls. mRNA *in situ* hybridization of 8 DLE subjects' skin and suspension CyTOF of 1 DLE subject's blood was performed to validate this pDC phenotype.

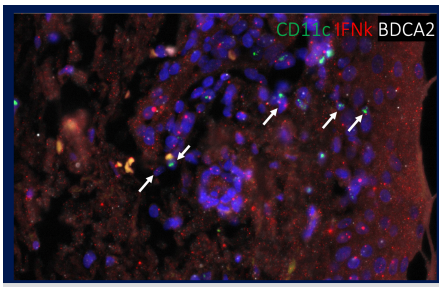

Fig. 4. Co-localization of IFNk mRNA to cDCs and not pDCs.

In situ hybridization revealed a lack of co-expression of IFNα1 and IFNβ mRNA with CLEC4C (BDCA2), a pDC marker, but overlap with ITGAX (CD11C), a cDC marker. A median of 4.5 IFN-1 negative and 0 IFN-1 positive pDCs were seen per high powered field. IMC visualization of skin pDCs highlighted overlap between BDCA2 and GZMB. Lesional pDCs were also visualized by BDCA2 with IFNα1/13 and IFNβ. Extensive IFN-1 staining was seen in the skin but minimal overlap with BDCA2. Further verification using a Z-axis overlay of intracellular markers on tSNE plots of immune cell clusters identified by CyTOF confirmed low expression of type I IFN and the interferogenic pathway, pSTING, in the pDCs.

We extracted total RNA from lesional DLE and SCLE biopsies for gene expression analysis by qPCR. The interferon inducible gene, Myxovirus resistance 1 (Mx1), was significantly increased in DLE (3.01) relative to SCLE (0.62, $p<0.01$). No significant differences were observed with IFNγ, IL31RA, IFNβ, TNF, IL16, or IL18. IL16 was nominally, but not significantly increased in SCLE with two high expression outliers in the DLE group.

How were the results disseminated to communities of interest?

Eight abstracts were submitted to the Society of Investigative Dermatology and the American College of Rheumatology (ACR). One was presented as a virtual oral poster presentation in 2021 (Vazquez T, Patel J, Keyes E, Yan D, Diaz D, Bashir M, Feng R,

Grinnell M, Werth VP. Multidimensional in situ immune profiling of discoid and subacute cutaneous lupus erythematosus. Journal of Investigative Dermatology (supplement) 141:#021, S4, 2021). Another was accepted for oral presentation at the ACR meeting in 2021.

Patel J, Vazquez T, Yan D, Keyes E, Diaz D, Li Y, Grinnell M, Feng R, Werth V. Immune microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials. Journal of Investigative Dermatology (supplement) 141:#024, S4, 2021.

Vazquez T, Patel J, Yan D, Keyes E, Diaz D, Li Y, Grinnell M, Feng R, Werth V. Multiplexed Profiling of Treatment Naïve Cutaneous Lupus Skin Stratified by Patient Response to Antimalarials [abstract]. *Arthritis Rheumatol.* 2021; 73 (suppl 10). Invited for oral presentation.

F Chin , T Vazquez , J Patel , R. Feng, V. Werth. Network analysis suggests Th1, Th2 and Th17 mechanisms to be linked together in pathogenesis of cutaneous lupus erythematosus. Society of Investigative Dermatology. Abstract # 807, 2022.

F. Chin , T Vazquez , J Patel , R. Feng, V. Werth. Unsupervised learning reveals different degrees of heterogeneity as well as cell involvement in cutaneous lupus erythematous antimalarial treatment response subgroups. Society of Investigative Dermatology. Abstract # 840, 2022.

T. Vazquez, D. Diaz, N. Kodali, J. Patel, E. Keyes, G. Sprow, M. Sharma, M. Ogawa-Momohara, M. Grinnell, J. Dan, V. Werth. Plasmacytoid dendritic cells are not major producers of type 1 interferons in cutaneous lupus. Society of Investigative Dermatology. Abstract # 66, 2022.

Chin F, Vazquez T, Dan J, Diaz D, Sprow G, Patel J, Kodali N, Feng R, Werth V. Partial Correlations Network Models Show Th1, Th2 and Th17 Responses to Be Interlinked in Dermal Pathogenesis of Cutaneous Lupus Erythematous [abstract]. *Arthritis Rheumatol.* 2022; 74 (suppl 9).

Chin F, Vazquez T, Dan J, Diaz D, Sprow G, Patel J, Kodali N, Feng R, Werth V. Correlation Matrices Visualize Differential Degree of Cell and Pathway Heterogeneity in Skin of Cutaneous Lupus Erythematosus Treatment Subgroups [abstract]. *Arthritis Rheumatol.* 2022; 74 (suppl 9).

Manuscripts:
Patel J, Vazquez T, Chin F, Keyes E, Yan D, Diaz D, Grinnell M, Sharma M, Li Y, Feng R, Sprow G, Dan J, Werth VP. Multidimensional immune profiling of cutaneous lupus erythematosus in vivo stratified by patient responses to antimalarials. Arthritis Rheumatol Arthritis Rheumatol. 2022 Oct;74(10):1687-1698.

Vazquez T, Patel J, Kodali N, Diaz D, Bashir MM, Chin F, Keyes E, Sharma M, Sprow G, Grinnell M, Dan J, Werth VP. Plasmacytoid dendritic cells are not major producers of type 1 interferon in cutaneous lupus: An in depth immunoprofile of subacute and discoid lupus. J Invest Dermatol, resubmitted.

4. Impact

What was the impact on the development of the principal discipline of the project. This work is beginning to define important pathways that predict response to therapy in cutaneous lupus erythematosus. It opens up understanding of interactions between cells and pathways.

What was the impact on other disciplines. The results are encouraging interactions between rheumatology and dermatology. Based on current results, a grant submission has just occurred to link centers together to expand prospective collection of samples and utilize evolving technology to address issues related to heterogeneity of response to therapy in lupus. The plan is to expand to studying acute cutaneous LE as well. This mechanism is the second part of the Accelerating Medicines Partnership (AMP), a public-private partnership between the National Institutes of Health (NIH), the U.S. Food and Drug Administration (FDA), multiple biopharmaceutical and life science companies, and non-profit organizations.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology? Nothing to report.

5. Changes/problems.

There have been no changes or problems. As suggested by the reviewers, we have focused on DLE and have added gene expression studies to our studies that are ongoing.

6. Products

Publications, conference papers, presentations

Vazquez T, Patel J, Keyes E, Yan D, Diaz D, Bashir M, Feng R, Grinnell M, Werth VP. Multidimensional in situ immune profiling of discoid and subacute cutaneous lupus erythematosus. Journal of Investigative Dermatology (supplement) 141:#021, S4, 2021.

Patel J, Vazquez T, Yan D, Keyes E, Diaz D, Li Y, Grinnell M, Feng R, Werth V. Immune microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials. Journal of Investigative Dermatology (supplement) 141:#024, S4, 2021.

7. Participants and other collaborating organizations

Name	Meena Sharma

Project Role:	Technician
Researcher identifier	
Nearest person month worked	2.4
Contribution to project	Labeling of antibodies, analysis
Funding Support	

Name	Rui Feng
Project Role:	Statistician
Researcher identifier	0000-0003-4151-7228
Nearest person month worked	0.6
Contribution to project	Labeling of antibodies, analysis
Funding Support	

Name	Thomas Vazquez
Project Role:	Fellow
Researcher identifier	0000-0003-1721-5617
Nearest person month worked	12
Contribution to project	Labeling of antibodies and tissue, analysis
Funding Support	Rheumatology Research Foundation

Name	Jay Patel
Project Role:	Fellow
Researcher identifier	0000-0003-4737-8740
Nearest person month worked	3
Contribution to project	Labeling of antibodies, analysis
Funding Support	

Name	Emily Keyes
Project Role:	Fellow
Researcher identifier	
Nearest person month worked	1
Contribution to project	Identifying lupus patients and response from database
Funding Support	

8. Special Reporting requirements: None

9. Appendix

ABSTRACTS | Adaptive and Auto-Immunity

019

Vgll3 causes discoid lupus-like fibrosis in a mouse model of lupus

M Gharaee-Kermani[1,2], AC Billi[1], JM Kahlenberg[2,1] and JE Gudjonsson[1] *1 Department of Dermatology, University of Michigan, Ann Arbor, Michigan, United States and 2 Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States*

Fibrosis is an abnormal wound healing process characterized by collagen deposition, myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can also be seen in 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-directed 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*, *Tgfb1*, and *Ccn2*, also known as connective tissue growth factor (*Ctgf*)) and pro-fibrotic cytokines (*Il4* and *Il13*) in TG mice. The detection of high expression of *Ccn2* and *Tgfb1* 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 impact fibrosis development, and there may be a role for targeting *CTGF* in early autoimmune fibrosis. Further studies will need to elucidate the specific mechanisms that may be at play.

020

Induction of hair loss by expanded CD4 T cells from previously affected AA mice

SJ Connell[3,2], S Crotts[2], P Christy[2], L Ortolan[2], N Henderson[2] and A Jabbari[3,2,1] *1 Iowa City VA Medical Center, Iowa City, Iowa, United States, 2 Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States and 3 Interdisciplinary Graduate Program in Immunology, The University of Iowa Graduate College, Iowa City, Iowa, United States*

Alopecia Areata (AA) is a common autoimmune disease characterized by infiltration of the hair follicle by T cells, resulting in nonscarring hair loss. Our published work suggested an increased representation of IFN-γ-producing, activated CD4 T cells in the skin-draining lymph nodes of AA mice when compared to unaffected (UA) controls. Our objective was to determine the contribution of CD4 T cells to AA pathogenesis. We adapted a recently described model of mouse AA induction whereby adoptive transfer of in vitro expanded bulk lymph node (LN) cells from previously affected AA mice induced disease in previously unaffected mice. To address the role CD4 T cells play in AA pathogenesis, we first sorted CD4 T cells and assessed their ability to induce AA. Mouse recipients of in vitro expanded CD4 T cells isolated from the LNs of AA mice developed AA at a substantially increased rate compared to mouse recipients of in vitro-expanded CD4 T cells from LNs of UA mice. CD4-mediated AA induction was found to be dose-dependent, with larger numbers of CD4 T cells inducing disease in recipient mice at a higher rate. Using congenic markers, we found that the transferred CD4 T cells were present in the skin draining LNs of recipient mice at three weeks following transfer but largely absent at 16 weeks, suggesting these cells may be conferring their effect early during disease development. Additionally, we found that the CD4 T cell population is critically dependent on the presence of endogenous CD8 T cells in order to transfer disease. Our data suggests that CD4 T-helper type 1 cells contribute to the activation of CD8 T cells to enable autoimmune attack on the hair follicle. Further studies are needed to further dissect how CD4 T cells, and IFN-γ, lead to AA.

021

Multidimensional *in situ* immune profiling of discoid and subacute cutaneous lupus erythematosus

T Vazquez[1,2], J Patel[1,2], E Keyes[1,2], D Yan[1,2], D Diaz[1,2], M Bashir[1,2], R Feng[2], M Grinnell[1,2] and VP Werth[1,2] *1 Dermatology, Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 Dermatology, Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

Cutaneous lupus erythematosus (CLE) can be subdivided into acute cutaneous (ACLE), subacute cutaneous (SCLE), and chronic cutaneous LE (of which discoid lupus [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, *in situ* technique for cellular level analysis. 19 SCLE and 18 DLE, treatment-naïve FFPE biopsies were stained with 37 metal-conjugated antibodies. Slides were ablated on the Hyperion Imaging System (Fluidigm). Cells were segmented using a nuclear based algorithm on Visiopharm 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 CD4 T, CD8 T, 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 SCLE ($p>0.05$). 16 cytokines and phosphorylated inflammatory signaling pathways were included and the data revealed higher pTBK1 in DLE compared to SCLE ($p<0.05$). At the cell type level, the data showed increased pIRF3 in DLE pDCs compared to SCLE ($p<0.05$). Overall, these results suggest substantial overlap between DLE and SCLE, with a potential role for pTKB1 and pIRF3 in DLE. Future studies are needed to investigate the potential suitability of these pathways as targeted therapies for DLE.

022

UHRF1 downregulation promotes T follicular helper cell differentiation by increasing BCL6 expression in SLE

Ml Liu, Yl Hu and M Zhao *Central South University, Changsha, Hunan, China*

Background: Transcription factor B cell lymphoma 6 (BCL6) is a master regulator of T follicular helper (Tfh) cells, which play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanisms by which BCL6 expression is regulated are poorly understood. Ubiquitin-like with PHD and RING finger domains 1 (UHRF1) is an important epigenetic factor that regulates DNA methylation and histone modifications. In the present study, we assessed whether UHRF1 can regulate BCL6 expression and influence the differentiation and proliferation of Tfh cells. Results: Compared to healthy controls, the mean fluorescence intensity of UHRF1 (UHRF1-MFI) in Tfh cells from SLE patients was significantly downregulated, whereas that of BCL6 (BCL6-MFI) was significantly upregulated. In vitro, UHRF1 knockdown led to BCL6 overexpression and promoted Tfh cell differentiation. In contrast, UHRF1 overexpression led to BCL6 downregulation and decreased Tfh cell differentiation. In vivo, conditional UHRF1 gene knockout (UHRF1-cKO) in mouse T cells revealed that UHRF1 depletion can enhance the proportion of Tfh cells and induce an augmented GC reaction in mice treated with NP-keyhole limpet hemocyanin (NP-KLH). Mechanistically, UHRF1 downregulation can decrease DNA methylation and H3K27 trimethylation (H3K27me3) levels in the *BCL6* promoter region of Tfh cells. Conclusions: Our results demonstrated that UHRF1 downregulation leads to increased BCL6 expression by decreasing DNA methylation and H3K27me3 levels, promoting Tfh cell differentiation in vitro and in vivo. This finding reveals the role of UHRF1 in regulating Tfh cell differentiation and provides a potential target for SLE therapy.

023

Single-cell composition and architecture of cutaneous lupus

AC Billi[1], F Ma[2], O Plazyo[1], R Wasikowski[1], M Gharaee-Kermani[1,3], A Hurst[3], C Dobry[1], L Tsoi[1], M Pellegrini[2], R Modlin[2], JE Gudjonsson[1] and JM Kahlenberg[1,3] *1 Dermatology, 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 3 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). CLE 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 lupus morbidity and even mortality. We employed single-cell RNA-sequencing (scRNA-seq) and spatial sequencing to investigate the transcriptomes and arrangement of the cellular players in CLE. 7 patients with active CLE were enrolled. 6/7 carried a diagnosis of SLE. Lesional and nonlesional sun-protected 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 transcriptomic 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 sequencing revealed a complex architecture of immune cells, stromal cells, and keratinocytes, with spatially distinct inflammatory responses. Collectively, these data provide deep characterization of skin alterations and inflammation in CLE and offer a resource for further interrogation of the roles of constituent cell types in CLE pathogenesis.

024

Immune microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials

J Patel[1,2], T Vazquez[1,2], D Yan[1,2], E Keyes[1,2], D Diaz[1,2], Y Li[1,2], M Grinnell[1,2], R Feng[2] and V Werth[1,2] *1 Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

Lupus erythematous (LE) is a systemic autoimmune disease with a variety of cutaneous manifestations. Antimalarials are first-line systemic therapy, yet not all patients respond to hydroxychloroquine (HCQ), quinacrine (QC), or either (NR). Our group has previously shown that QC responders demonstrate increased conventional dendritic cells (cDC) and TNFa relative to HCQ responders. Here, we investigated the differences between these patients using Imaging Mass Cytometry (IMC), an unbiased multiplexed technique. 12 HCQ, 11 QC, and 20 NR treatment-naïve 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 Visiopharm and imported into histoCAT where single cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm. One-way ANOVA, Kruskal-Wallis, and post-hoc Tukey/Dunn's tests (per data normality) were performed. Correlations were determined by Pearson's r. NR patients were found to have a decreased percentage of Tregs compared to QC responders ($p<0.05$). QC responders had a higher expression of pSTING and IFNκ compared to HCQ responders ($p<0.05$). The total expression of pSTING and IFNκ was found to positively correlate and colocalize in skin ($p<0.0001$, $r=0.676$). CD14+CD16+/CD68+ macrophages and cDCs were the predominant cell types found to express pSTING and IFNκ. These data may suggest a relative dysregulation in tolerance due to decreased Tregs in patients refractory to antimalarials. Our results show that activated STING correlated with IFNκ, suggesting co-regulation in macrophages and cDCs that may be responsive to QC. This analysis on treatment naïve biopsies may lead to further discovery of biomarkers that may predict patient response to therapy and direct targeted treatment.

019

Vgll3 causes discoid lupus-like fibrosis in a mouse model of lupus

M Gharaee-Kermani[1,2], AC Billi[1], JM Kahlenberg[2,1] and JE Gudjonsson[1] *1 Department of Dermatology, University of Michigan, Ann Arbor, Michigan, United States and 2 Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States*

Fibrosis is an abnormal wound healing process characterized by collagen deposition, myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can also be seen in 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-directed 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*, *Tgfb1*, and *Ccn2*, also known as connective tissue growth factor (*Ctgf*)) and pro-fibrotic cytokines (*Il4* and *Il13*) in TG mice. The detection of high expression of *Ccn2* and *Tgfb1* 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 impact fibrosis development, and there may be a role for targeting *CTGF* in early autoimmune fibrosis. Further studies will need to elucidate the specific mechanisms that may be at play.

020

Induction of hair loss by expanded CD4 T cells from previously affected AA mice

SJ Connell[1,2], S Crotts[2], P Christy[2], L Ortolan[2], N Henderson[2] and A Jabbari[3,2,1] *1 Iowa City VA Medical Center, Iowa City, Iowa, United States, 2 Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States and 3 Interdisciplinary Graduate Program in Immunology, The University of Iowa Graduate College, Iowa City, Iowa, United States*

Alopecia Areata (AA) is a common autoimmune disease characterized by infiltration of the hair follicle by T cells, resulting in nonscarring hair loss. Our published work suggested an increased representation of IFN-γ-producing, activated CD4 T cells in the skin-draining lymph nodes of AA mice when compared to unaffected (UA) controls. Our objective was to determine the contribution of CD4 T cells to AA pathogenesis. We adapted a recently described model of mouse AA induction whereby adoptive transfer of in vitro expanded bulk lymph node (LN) cells from previously affected AA mice induced disease in previously unaffected mice. To address the role CD4 T cells play in AA pathogenesis, we first sorted CD4 T cells and assessed their ability to induce AA. Mouse recipients of in vitro expanded CD4 T cells isolated from the LNs of AA mice developed AA at a substantially increased rate compared to mouse recipients of in vitro-expanded CD4 T cells from LNs of UA mice. CD4-mediated AA induction was found to be dose-dependent, with larger numbers of CD4 T cells inducing disease in recipient mice at a higher rate. Using congenic markers, we found that the transferred CD4 T cells were present in the skin draining LNs of recipient mice at three weeks following transfer but largely absent at 16 weeks, suggesting these cells may be conferring their effect early during disease development. Additionally, we found that the CD4 T cell population is critically dependent on the presence of endogenous CD8 T cells in order to transfer disease. Our data suggests that CD4 T-helper type 1 cells contribute to the activation of CD8 T cells to enable autoimmune attack on the hair follicle. Further studies are needed to further dissect how CD4 T cells, and IFN-γ, lead to AA.

021

Multidimensional *in situ* immune profiling of discoid and subacute cutaneous lupus erythematosus

T Vazquez[1,2], J Patel[1,2], E Keyes[1,2], D Yan[1,2], D Diaz[1,2], M Bashir[1,2], R Feng[2], M Grinnell[1,2] and VP Werth[1,2] *1 Dermatology, Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 Dermatology, Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

Cutaneous lupus erythematosus (CLE) can be subdivided into acute cutaneous (ACLE), subacute cutaneous (SCLE), and chronic cutaneous LE (of which discoid lupus [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, *in situ* technique for cellular level analysis. 19 SCLE and 18 DLE, treatment-naïve FFPE biopsies were stained with 37 metal-conjugated antibodies. Slides were ablated on the Hyperion Imaging System (Fluidigm). Cells were segmented using a nuclear based algorithm on Visiopharm 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 CD4 T, CD8 T, 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 SCLE ($p>0.05$). 16 cytokines and phosphorylated inflammatory signaling pathways were included and the data revealed higher pTBK1 in DLE compared to SCLE ($p<0.05$). At the cell type level, the data showed increased pIRF3 in DLE pDCs compared to SCLE ($p<0.05$). Overall, these results suggest substantial overlap between DLE and SCLE, with a potential role for pTKB1 and pIRF3 in DLE. Future studies are needed to investigate the potential suitability of these pathways as targeted therapies for DLE.

022

UHRF1 downregulation promotes T follicular helper cell differentiation by increasing BCL6 expression in SLE

Ml Liu, Yl Hu and M Zhao *Central South University, Changsha, Hunan, China*

Background: Transcription factor B cell lymphoma 6 (BCL6) is a master regulator of T follicular helper (Tfh) cells, which play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanisms by which BCL6 expression is regulated are poorly understood. Ubiquitin-like with PHD and RING finger domains 1 (UHRF1) is an important epigenetic factor that regulates DNA methylation and histone modifications. In the present study, we assessed whether UHRF1 can regulate BCL6 expression and influence the differentiation and proliferation of Tfh cells. Results: Compared to healthy controls, the mean fluorescence intensity of UHRF1 (UHRF1-MFI) in Tfh cells from SLE patients was significantly downregulated, whereas that of BCL6 (BCL6-MFI) was significantly upregulated. In vitro, UHRF1 knockdown led to BCL6 overexpression and promoted Tfh cell differentiation. In contrast, UHRF1 overexpression led to BCL6 downregulation and decreased Tfh cell differentiation. In vivo, conditional UHRF1 gene knockout (UHRF1-cKO) in mouse T cells revealed that UHRF1 depletion can enhance the proportion of Tfh cells and induce an augmented GC reaction in mice treated with NP-keyhole limpet hemocyanin (NP-KLH). Mechanistically, UHRF1 downregulation can decrease DNA methylation and H3K27 trimethylation (H3K27me3) levels in the *BCL6* promoter region of Tfh cells. Conclusions: Our results demonstrated that UHRF1 downregulation leads to increased BCL6 expression by decreasing DNA methylation and H3K27me3 levels, promoting Tfh cell differentiation in vitro and in vivo. This finding reveals the role of UHRF1 in regulating Tfh cell differentiation and provides a potential target for SLE therapy.

023

Single-cell composition and architecture of cutaneous lupus

AC Billi[1], F Ma[2], O Plazyo[1], R Wasikowski[1], M Gharaee-Kermani[1,3], A Hurst[3], C Dobry[1], L Tsoi[1], M Pellegrini[2], R Modlin[2], JE Gudjonsson[1] and JM Kahlenberg[1,3] *1 Dermatology, 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 3 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). CLE 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 lupus morbidity and even mortality. We employed single-cell RNA-sequencing (scRNA-seq) and spatial sequencing to investigate the transcriptomes and arrangement of the cellular players in CLE. 7 patients with active CLE were enrolled. 6/7 carried a diagnosis of SLE. Lesional and nonlesional sun-protected 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 transcriptomic 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 sequencing revealed a complex architecture of immune cells, stromal cells, and keratinocytes, with spatially distinct inflammatory responses. Collectively, these data provide deep characterization of skin alterations and inflammation in CLE and offer a resource for further interrogation of the roles of constituent cell types in CLE pathogenesis.

024

Immune microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials

J Patel[1,2], T Vazquez[1,2], D Yan[1,2], E Keyes[1,2], D Diaz[1,2], Y Li[1,2], M Grinnell[1,2], R Feng[2] and V Werth[1,2] *1 Corporal 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 hydroxychloroquine (HCQ), quinacrine (QC), or either (NR). Our group has previously shown that QC responders demonstrate increased conventional dendritic cells (cDC) and TNFa relative to HCQ responders. Here, we investigated the differences between these patients using Imaging Mass Cytometry (IMC), an unbiased multiplexed technique. 12 HCQ, 11 QC, and 20 NR treatment-naïve 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 Visiopharm and imported into histoCAT where single cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm. One-way ANOVA, Kruskal-Wallis, and post-hoc Tukey/Dunn's tests (per data normality) were performed. Correlations were determined by Pearson's r. NR patients were found to have a decreased percentage of Tregs compared to QC responders ($p<0.05$). QC responders had a higher expression of pSTING and IFNκ compared to HCQ responders ($p<0.05$). The total expression of pSTING and IFNκ was found to positively correlate and colocalize in skin ($p<0.0001$, $r=0.676$). CD14+CD16+/CD68+ macrophages and cDCs were the predominant cell types found to express pSTING and IFNκ. These data may suggest a relative dysregulation in tolerance due to decreased Tregs in patients refractory to antimalarials. Our results show that activated STING correlated with IFNκ, suggesting co-regulation in macrophages and cDCs that may be responsive to QC. This analysis on treatment naïve biopsies may lead to further discovery of biomarkers that may predict patient response to therapy and direct targeted treatment.

Arthritis & Rheumatology
Vol. 74, No. 10, October 2022, pp 1687–1698
DOI 10.1002/art.42235

American College of Rheumatology
Empowering Rheumatology Professionals

Multidimensional Immune Profiling of Cutaneous Lupus Erythematosus In Vivo Stratified by Patient Response to Antimalarials

Jay Patel,[1] Thomas Vazquez,[1] Felix Chin,[1] Emily Keyes,[1] Daisy Yan,[1] DeAnna Diaz,[1] Madison Grinnell,[1] Meena Sharma,[1] Yubin Li,[1] Rui Feng,[2] Grant Sprow,[1] Josh Dan,[1] and Victoria P. Werth[1]

Objective. The pathogenesis of cutaneous lupus erythematosus (CLE) is multifactorial, and CLE is difficult to treat due to the heterogeneity of inflammatory processes among patients. Antimalarials such as hydroxychloroquine (HCQ) and quinacrine (QC) have long been used as first-line systemic therapy; however, many patients do not respond to treatment with antimalarials and require systemic immunosuppressants that produce undesirable side effects. Given the complexity and the unpredictability of responses to antimalarial treatments in CLE patients, we sought to characterize the immunologic profile of patients with CLE stratified by subsequent treatment outcomes to identify potential biomarkers of inducible response.

Methods. We performed mass cytometry imaging of multiple immune cell types and inflammation markers in treatment-naive skin biopsy samples from 48 patients with CLE to identify baseline immunophenotypes that may predict the response to antimalarial therapy. Patients were stratified according to their response to treatment with antimalarials, as HCQ responders, QC responders, or nonresponders.

Results. HCQ responders demonstrated increased CD4+ T cells compared to the QC responder group. Patients in the nonresponder group were found to have decreased Treg cells compared to QC responders and increased central memory T cells compared to HCQ responders. QC responders expressed increased phosphorylated stimulator of interferon genes (pSTING) and interferon-κ (IFNκ) compared to HCQ responders. Phosphorylated STING and IFNκ were found to be localized to conventional dendritic cells (cDCs), and the intensity of pSTING and IFNκ staining was positively correlated with the number of cDCs on a tissue and cellular level. Neighborhood analysis revealed decreased regulatory cell interactions in nonresponder patients. Hierarchical clustering revealed that nonresponder patients could be further differentiated based on expression of pSTAT2, pSTAT3, pSTAT4, pSTAT5, phosphorylated interferon regulatory factor 3 (pIRF3), granzyme B, pJAK2, interleukin-4 (IL-4), IL-17, and IFNγ.

Conclusion. These findings indicate differential immune cell compositions between patients with CLE, offering guidance for future research on precision-based medicine and treatment response.

INTRODUCTION

Lupus erythematosus (LE) is a complex autoimmune disease with a variety of systemic and cutaneous manifestations. Cutaneous lupus erythematosus (CLE) can occur with or without concomitant systemic lupus erythematosus (SLE) and occurs in 75–85% of patients with lupus. CLE significantly impacts quality of life through the psychological and physical distress it causes

Supported by the United States Department of Defense, United States Department of Veterans Affairs Office of Research and Development, and Core A of the Penn Skin Biology and Diseases Resource-based Center (grant 1P30-AR-069589-01). Dr. Vazquez's work was supported by the Lupus Research Alliance Administrative Supplement to Promote Diversity in Research and by a Rheumatology Research Foundation Medical and Graduate Student Preceptorship. Dr. Werth's work was supported by a Lupus Research Alliance-Bristol Myers Squibb Accelerator Award, the Lupus Research Alliance Administrative Supplement to Promote Diversity in Research, and a Rheumatology Research Foundation Medical and Graduate Student Preceptorship.

Drs. Patel and Vazquez contributed equally to this work.

[1]Jay Patel, MD, Thomas Vazquez, MD, Felix Chin, BS, Emily Keyes, MD, Daisy Yan, MD, DeAnna Diaz, DO, Madison Grinnell, MD, Meena Sharma, PhD, Yubin Li, PhD, Grant Sprow, BA, Josh Dan, BA, Victoria P. Werth, MD: Corporal Michael J. Crescenz Veterans Affairs Medical Center and University of Pennsylvania, Philadelphia; [2]Rui Feng, PhD: University of Pennsylvania, Philadelphia.

Author disclosures are available at https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42235&file=art42235-sup-0001-Disclosureform.pdf.

Address correspondence to Victoria P. Werth, MD, Department of Dermatology, University of Pennsylvania, Perelman Center for Advanced Medicine, Suite 1-330A, 3400 Civic Center Boulevard, Philadelphia, PA 19104. Email: werth@pennmedicine.upenn.edu.

Submitted for publication November 19, 2021; accepted in revised form May 12, 2022.

(1–3). The pathogenesis of CLE is multifactorial, with a complex interaction between genetic and environmental factors leading to immune dysregulation. A multitude of mechanisms are thought to contribute to the pathogenesis of CLE, including ultraviolet radiation, smoking, NETosis, altered nucleic acid processing, cell death/apoptosis, type I interferon (IFN) production, JAK/STAT pathway activation, T cell dysregulation, myeloid and plasmacytoid dendritic cell stimulation, and autoantibody production, all of which contribute to dysregulation of immune tolerance (4).

Oral antimalarials such as hydroxychloroquine (HCQ) and quinacrine (QC) have been used to treat CLE since 1894 and have since become first-line systemic therapy for all types of CLE (5). While their exact mechanism of action is still incompletely understood, the therapeutic effects of HCQ and QC may be attributed to immunomodulatory properties including photoprotection, alteration of Toll-like receptor signaling, inhibition of dendritic cell antigen presentation, suppression of prostaglandins and cytokines, and lysosomal stabilization (6,7). In a previously published study, it was demonstrated that ~50% of CLE patients will respond to treatment with HCQ alone (3). Of those patients considered to be nonresponsive to HCQ, 66% will respond to the addition of QC. This leaves a sizeable number of nonresponding patients with CLE refractory to antimalarials, ultimately requiring immunosuppressive medications, which often carry an undesirable side-effect profile. These second- and third-line therapies encompass a broad range of medication classes and mechanisms of action that are incompletely understood, requiring lengthy periods of trial and error in which providers must determine the most effective therapy for their patients.

To our knowledge, there are few published studies in which the immune environment of CLE patients stratified by treatment response has been characterized. In a previous study by Zeidi et al, immunohistochemistry was used to identify increased CD11c+ dendritic cells and tumor necrosis factor (TNF) messenger RNA (mRNA) in QC-responding patients (8). The complexity of CLE pathogenesis requires immunologic analysis on a multiplexed scale to best identify differences between groups stratified by response to treatment. Given the diversity of responses to antimalarials and immunosuppressive drugs, we attempted to characterize the immunologic profile of treatment-naive CLE skin samples stratified by subsequent antimalarial response through the use of imaging mass cytometry (IMC). By understanding the differences in the immunologic profiles of CLE patients at baseline, we sought to identify potential biomarkers for the prediction of in vivo response to antimalarial treatment and to identify potential targets for precision-based medicine.

PATIENTS AND METHODS

Patients. Patients were retrospectively enrolled from a longitudinal, prospective CLE patient database at the University of Pennsylvania. All participants signed written informed consents and approval was obtained from the University of Pennsylvania and Philadelphia VA Medical Center Institutional Review Boards.

Patients were diagnosed as having CLE according to the Gilliam classification criteria (9) or investigator experience (VPW). SLE diagnosis was determined according to the 1997 American College of Rheumatology classification criteria (10). Archived treatment-naive, formalin-fixed paraffin-embedded lesioned biopsy samples were obtained. The CLE database and patient charts were reviewed to identify each patient's treatment course and response. Cutaneous Lupus Erythematosus Disease Area and Severity Index Activity (CLASI-A) scores (11) recorded at the time of biopsy were utilized.

Each patient was categorized according to their established response to antimalarials, which was ascertained by chart review and was verified by 3 investigators (DY, EK, and VPW). Patients whose symptoms improved with HCQ therapy and did not require the addition of any other drug were designated as HCQ responders. Patients who benefited from and required only HCQ and QC combination therapy were designated as QC responders. Patients who required the addition of other immunomodulatory agents (e.g., methotrexate, mycophenolate mofetil, lenalidomide, etc.) were designated as nonresponders. Treatment with topical medications and glucocorticoids was allowed in each group. Patients with other autoimmune connective tissue diseases were excluded from the study, with the exception of patients meeting criteria for Sjögren's syndrome. Patients whose responses to treatment with antimalarials were unclear were also excluded from the study. Patient demographics can be found in Table 1.

Data acquisition. A detailed description of IMC, in situ hybridization, and the statistical methods used in this study are included in the Supplementary Materials and Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42235.

Data availability. Raw data are available on Mendeley Data at doi: 10.17632/b7vr75sbdz.1.

RESULTS

Study population. In total, 48 CLE patients met the criteria for inclusion. Thirteen patients were classified as HCQ responders, 13 as QC responders, and 22 as nonresponders. Patient demographics are detailed in Table 1. Notably, the 2 most prevalent clinical CLE subtypes represented in our patient population were discoid lupus erythematosus and subacute CLE, and their composition in each response group was relatively similar. Most patients were White, middle-aged women. African American patients were well-represented, making up 22.9% of the cohort.

Table 1. Demographic and baseline clinical characteristics of CLE patients included in the cohort*

	All CLE patients (n = 48)	Treatment response group		
		HCQ responders (n = 13)	QC responders (n = 13)	Nonresponders (n = 22)
Age, median (IQR) years	52.5 (32.25–64)	52 (26–63.5)	55 (34.5–66.5)	48 (31.5–60.25)
SLE	12 (25.0)	2 (15.4)	4 (30.8)	9 (40.9)
CLASI-A score, median (IQR)†	14 (5–25)	7 (3–28)	17 (6–29)	16 (13–21)
Sex				
Female	41 (85.4)	8 (61.5)	11 (84.6)	22 (100)
Male	7 (14.6)	5 (38.5)	2 (15.4)	0
Race				
African American	11 (22.9)	0	4 (30.8)	7 (31.8)
Asian	3 (6.3)	2 (15.4)	1 (7.7)	0
White	34 (70.8)	11 (84.6)	8 (61.5)	15 (68.2)
Smoking history, yes	20 (41.7)	6 (46.2)	4 (30.8)	10 (45.5)
CLE subtype				
Acute CLE	1 (2.1)	0	0	1 (4.5)
Subacute CLE	19 (39.6)	5 (38.5)	5 (38.5)	9 (40.9)
DLE	19 (39.6)	4 (30.8)	6 (46.2)	9 (40.9)
Lupus tumidus	2 (4.2)	1 (7.7)	0	1 (4.5)
HLE	3 (6.3)	1 (7.7)	2 (15.4)	0
Chilblain lupus	1 (2.1)	0	1 (7.7)	0
Lupus panniculitis	1 (2.1)	0	0	1 (4.5)

* Except where indicated otherwise, values are the number (%) of patients. CLE = cutaneous lupus erythematosus; HCQ = hydroxychloroquine; QC = quinacrine; IQR = interquartile range; SLE = systemic lupus erythematosus; CLASI-A = Cutaneous Lupus Erythematosus Disease Area and Severity Index Activity; DLE = discoid lupus erythematosus; HLE = hypertrophic lupus erythematosus.
† CLASI-A score ranges are as follows: mild (0–9), moderate (10–20), and severe (21–70).

Inflammatory cell makeup. Based on the data obtained from 2 IMC panels (panel 1 and panel 2), 12 unique cell phenotypes were identified through the use of PhenoGraph and were displayed using a heatmap of cell marker expression (Figures 1A and B). The most common cell types observed across all treatment response groups included conventional dendritic cells (cDCs), CD8+ T cells, CD4+ T cells, and Treg cells (Figure 1C). There was marked heterogeneity of cell composition in each treatment response group (Figure 1D). Proportions of CD4+ T cells and Treg cells differed significantly between the treatment response groups ($P < 0.05$) (Figure 1D). CD4+ T cells were significantly increased in HCQ responders compared to QC responders ($P < 0.05$) (Figure 1D). Treg cells were significantly increased in QC responders compared to nonresponders ($P < 0.05$) (Figure 1C). Similar to the findings of a previous study by Zeidi et al, there was a trend toward increased cDCs in QC responders (8).

We also observed a trend toward an increase in CD8+ T cells in HCQ responders (Figure 1D). The percentage of plasmacytoid dendritic cells (pDCs) was notably similar in each treatment response group. When CD4+ T cells (Figure 1E) and CD8+ T cells (Figure 1F) were gated into T cell subpopulations, a significant difference in the numbers of CD4+ central memory T (Tcm) cells and numbers of CD8+ Tcm cells was observed ($P < 0.05$) with a trend toward increased numbers of Tcm cells in nonresponders compared to HCQ responders (Figures 1E and F). Among all groups, effector memory T (Tem) cells were the largest constituent of the T cell compartment for both CD4+ T cells and CD8+ T cells (Figures 1E and F).

With regard to cellular composition, we determined that the CLASI-A score was negatively correlated with the numbers of CD14+CD16+ macrophages in HCQ responders (r = –0.0683), but not in QC responders or nonresponders (Supplementary Figure 1, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Similarly, a negative correlation (r = –0.759) between CLASI-A and γδ T cells was observed in nonresponders but not in HCQ responders or QC responders (Supplementary Figure 1).

Cytokines and signaling pathways. Multiple cell types are activated in CLE, and heatmaps of each intracellular cytokine or signaling pathway included in panel 1 (Figure 2A) and panel 2 (Figure 2B) were used to visualize activation. We found that the most activated cell types in skin biopsy samples from patients with CLE included cDCs, CD56+ natural killer (NK) cells, CD14+CD16+ macrophages, CD4+ T cells, neutrophils, and endothelial cells. The major producers of both antiviral IFNα and IFNβ were found to be cDCs and CD14+CD16+ macrophages, although type I IFNs are expressed in a multitude of immune cells. Neutrophils, though lacking in numbers, also showed significantly increased expression of proinflammatory cytokines, including type I IFNs, pSTAT3, and interleukin-18 (IL-18). Similarly, γδ T cells showed highly elevated expression of IFNα on a per-cell basis. Endothelial cells were found to be positive for numerous markers of CLE, especially IL-4, a Th2 cytokine.

We plotted the identified mean pixel intensity (MPI) per cell of each intracellular marker for each patient stratified according to

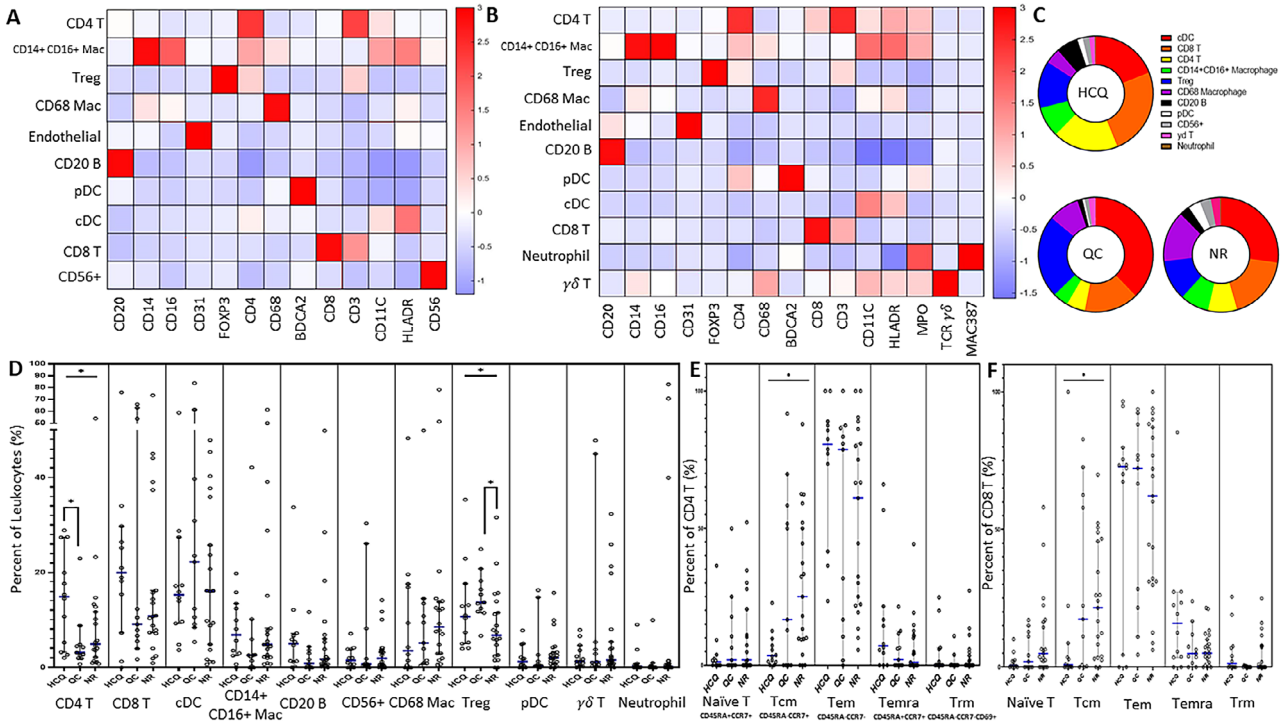

Figure 1. Immune cell composition of cutaneous lupus erythematosus (CLE) patients stratified by response to treatment with antimalarials. **A** and **B**, Heatmap showing cellular markers (horizontal axis) and immune cell clusters (vertical axis) in CLE patients, identified using Phenograph of cell cytometry panel 1 (**A**) and cell cytometry panel 2 (**B**). **C**, Relative composition of immune cell clusters in CLE patients responsive to treatment with hydroxychloroquine (HCQ), patients responsive to treatment with quinacrine (QC), and patients who did not respond to treatment with antimalarials (NR). **D**, Differences in the distribution of each immune cell cluster as a percentage of leukocytes in HCQ responders, QC responders, and nonresponders. CD4+ T cells were significantly increased in HCQ responders relative to QC responders ($P < 0.05$). Treg cells were significantly decreased in nonresponders relative to QC responders ($P < 0.05$). **E**, Distribution of CD4+ T cell subsets as a percentage of total CD4+ T cells, revealing differences in the proportion of central memory T (Tcm) cells ($P < 0.05$), with a trend toward increased Tcm cells in nonresponders. **F**, Distribution of CD8+ T cell subsets as a percentage of total CD8+ T cells. Proportions of Tcm cells were significantly different between treatment groups ($P < 0.05$) and there was a trend toward increased Tcm cells in nonresponders. Symbols represent individual patients; bars show the median and interquartile range. * $P < 0.05$. Mac = macrophage; pDC = plasmacytoid dendritic cell; cDC = conventional dendritic cell; Tem = effector memory T cell; Temra = terminally differentiated effector memory T cell; Trm = tissue-resident memory T cell.

their antimalarial response group (HCQ responder, QC responder, or nonresponder) (Figures 2C and D). There was a significant difference in the relative expression levels of phosphorylated stimulator of interferon genes (pSTING) and IFNκ between the 3 groups, with a significant increase in markers of both pathways in the QC responder group compared to the HCQ responder group ($P < 0.05$) (Figure 2C). Proinflammatory phosphorylation of JAK3 was increased in HCQ responders compared to QC responders ($P < 0.05$) (Figure 2D).

Using network analysis, we found that the numbers of Treg cells in QC responders (and to a lesser extent Treg cells in HCQ responders) were negatively correlated with the majority of proinflammatory pathways (Figures 2F and E and Supplementary Figure 2, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). In contrast, Treg cells from nonresponders were found to have little to no association with such pathways (Figure 2G and Supplementary Figure 2). The CLASI-A score was found to be positively correlated with the expression levels of proinflammatory pSTAT5 in HCQ responders ($r = 0.816$, $P < 0.05$), but no significant correlation was seen in QC responders or nonresponders (Supplementary Figure 3, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Analysis of intracellular markers identified differential inflammation marker expression between treatment response groups at the cell-type level (Supplementary Table 1, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235).

Low expression of type I IFNs in pDCs of CLE patients. Upon examination of the intracellular staining heatmaps (as shown in Figures 2A and B), we noticed that pDCs demonstrated relatively low expression of many inflammation markers, including all type I IFNs. Moreover, the numbers of pDCs were not found to be correlated with any of the global pathways (Supplementary Figure 4A, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235) or other cell counts. Only 8.5% of pDCs (interquartile range [IQR] 0.0–22.22) were IFNα+ (Supplementary Figure 4B). Across all biopsy samples from CLE

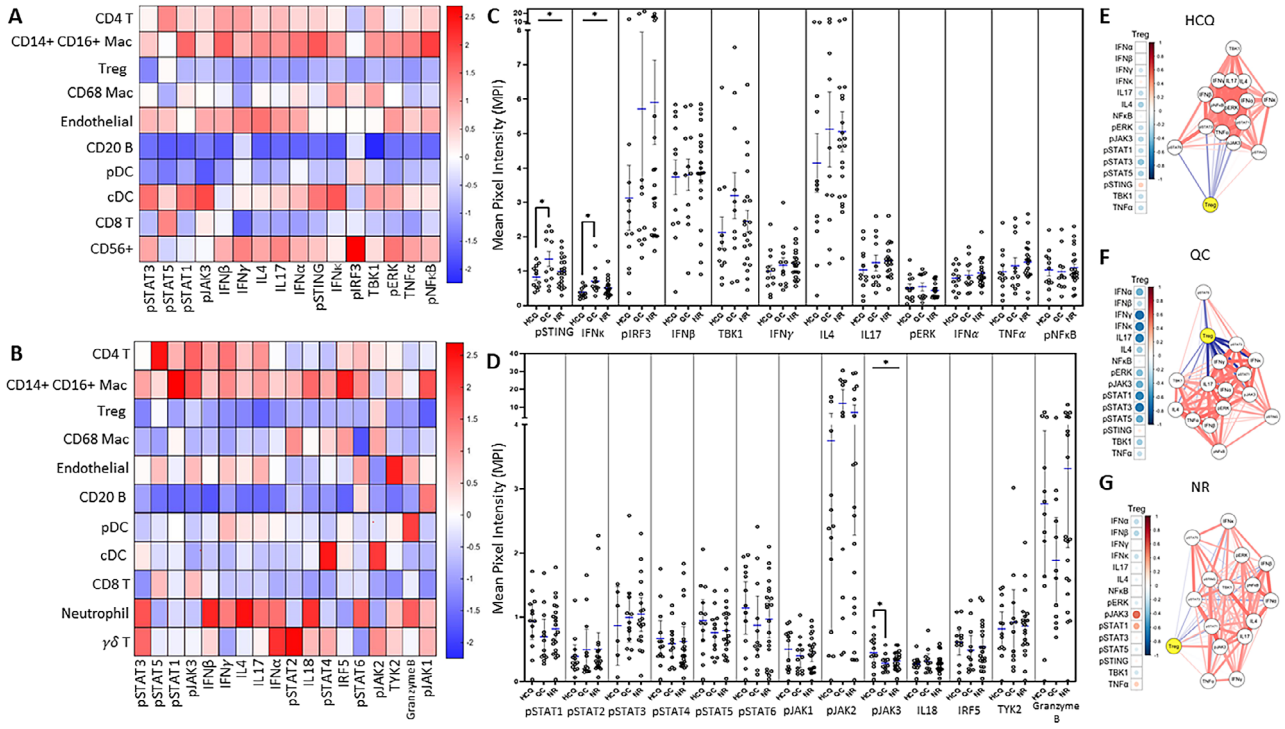

Figure 2. Global and cell-type specific intracellular inflammatory pathway expression in CLE patients stratified by antimalarial response. **A** and **B**, Heatmaps of activated intracellular pathways (horizontal axis) among immune cell clusters (vertical axis) in panel 1 (**A**) and in panel 2 (**B**). **C** and **D**, Differential pathway expression among CLE patients in each treatment group, assessed in IMC panel 1 (**C**) and panel 2 (**D**), with results expressed as the mean pixel intensity (MPI). Data in **C** show increased expression of phosphorylated stimulator of interferon genes (pSTING) and interferon-κ (IFNκ) in QC responders relative to HCQ responders ($P < 0.05$). Data in **D** show increased expression of pJAK3 in HCQ responders relative to QC responders ($P < 0.05$). **E–G**, Correlation networks showing correlations between activated pathway markers and Treg cell counts in HCQ responders (**E**) compared to QC responders (**F**) and nonresponders (**G**). The Treg cell counts of QC responders (and to a lesser extent HCQ responders) were negatively associated with the majority of pathways in panel 1. Treg cell counts of nonresponders displayed little to no association with panel 1 pathways. In **C** and **D**, symbols represent individual patients; bars show the median and interquartile range. * = $P < 0.05$. IL-4 = interleukin-4; pIRF3 = phosphorylated interferon regulatory factor 3; TBK1 = TANK-binding kinase 1; TNF = tumor necrosis factor (see Figure 1 for other definitions).

patients, pDCs were the second lowest contributor of absolute IFNα+ cells (median IFNα+ cell count 1 [IQR 0–4.5]) (B lymphocytes being the lowest contributor) (Supplementary Figure 4C). Conventional DCs and macrophages were the largest contributors to both relative and absolute cell counts of IFNα+ cells in CLE patients. Only 16.7% of pDCs were positive for IFNβ, compared to 60.7% of CD14+CD16+ macrophages. Plasmacytoid DCs were also low contributors of IFNβ+ cells (median 1 [IQR 0–7]) (B lymphocytes again being the lowest). These results suggest that pDCs are not in fact major producers of type I IFNs in CLE patients. These results also do not reveal a clear regulatory role for pDCs, as they were not found to be correlated with any other cell types or markers in the skin.

Using a weighted score accounting for cell count and MPI contributions of type I IFNs, we observed keratinocytes as collective producers of IFNα and IFNβ (Supplementary Figures 4D and E, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Myeloid lineage cells such as CD68+ macrophages, CD14+CD16+ macrophages, and cDCs made the greatest contributions to the production of IFNκ, with keratinocytes being the second highest contributor (Supplementary Figure 4F). Overall, the immune infiltrate collectively contributes the most type I IFNs, followed by epidermal sources.

Epidermal expression of cytokines and activation of inflammation pathways. The average MPI of key pathways in the epidermis for each patient is shown in Supplementary Figure 1, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235. Phosphorylation of NF-κB differed significantly between treatment response groups ($P < 0.05$), with a trend toward decreased phosphorylation of NF-κB in QC responders in the post hoc test. Unlike in the dermis, we did not observe a difference in levels of epidermal IFNκ and pSTING among the HCQ responder, QC responder, and nonresponder groups.

Strong correlations in marker expression between all epidermal and dermal pathways were identified (Supplementary Figure 1B). This is further represented by a heatmap of intracellular marker expression in the epidermis and the dermis of each CLE patient (Supplementary Figures 1C and D). There was

marked overlap in staining patterns between the dermis and epidermis, suggesting that the inflammation patterns were highly concordant between these layers.

Increased pSTING and IFNκ in QC responders. Given the up-regulation of pSTING and IFNκ in the QC responder group, we sought to identify colocalization. When both markers were visualized simultaneously, there was demonstrable overlap, suggesting that IFNκ is likely produced in the same cells with increased activation of pSTING (Figures 3A–C). Moreover, we found that pSTING staining intensity and IFNκ staining intensity were positively correlated (r = 0.676) (Figure 3D). The highest degree of staining of both pSTING and IFNκ was seen in cDCs (Figure 3A). In patients with CLE, the number of cDCs in skin biopsy samples was positively correlated with the degree of IFNκ (r = 0.571) and pSTING (r = 0.429) staining (Figure 3E).

To confirm that there are not 2 distinct populations of cDCs, we used FlowJo to visualize IFNκ and pSTING produced by cDCs and identified few single-positive events (results not shown). Levels of CD11c in cDCs and IFNκ in cDCs were also shown to be correlated, although this was expected given the relative expression profile of cDCs in our PhenoGraph-generated heatmap.

We then wanted to confirm whether cDCs positive for pSTING and IFNκ were the cells driving increased expression of these markers in QC responders. Expression of pSTING ($P < 0.01$) and IFNκ ($P < 0.05$) in cDCs was significantly increased in QC responders compared to HCQ responders (Figure 3F). To confirm that cDCs were indeed producing IFNκ, we performed mRNA in situ hybridization. We confirmed colocalization of CD11c and IFNκ using IMC (Supplementary Figure 5, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235), with colocalization of the CD11c mRNA (ITGAX) and IFNκ mRNA observed in QC responder skin (Figure 3G). Furthermore, network analysis showed a high correlation between numbers of cDCs and expression of pSTING/IFNκ in both QC responder and nonresponder patients, with the formation of a correlation triangle indicating stronger associations with each other than with any other pathway (Figures 3I and J). Single-cell staining networks revealed

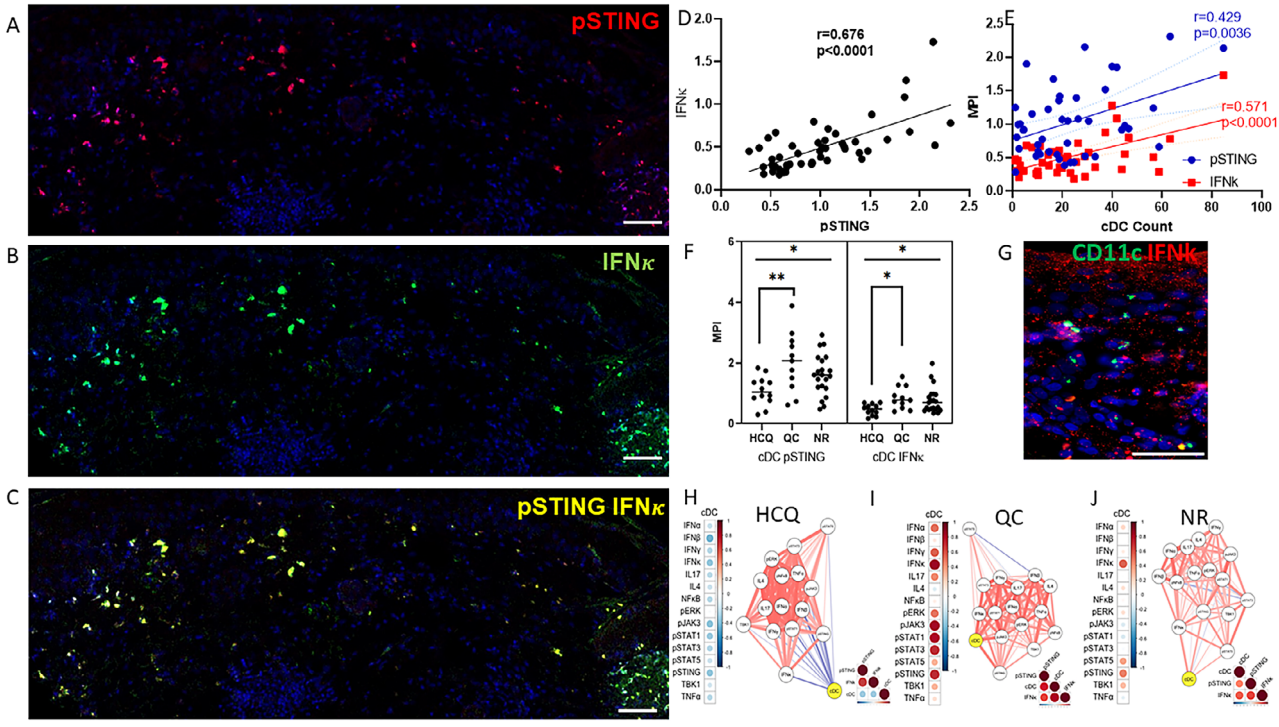

Figure 3. Increased colocalized expression of pSTING and IFNκ increased in cutaneous lupus erythematosus (CLE) patients who responded to treatment with QC. **A–C**, Images generated by imaging mass cytometry displaying staining for phosphorylated stimulator of interferon genes (pSTING) (red) (**A**), interferon-κ (IFNκ) (green) (**B**), and colocalization of pSTING and IFNκ (yellow) (**C**). **D–F**, The staining intensity of pSTING and IFNκ (mean pixel intensity) in conventional dendritic cells (cDCs) from CLE patients was assessed for correlation with each other (**D**) and with the cDC count (**E**), and levels of pSTING and IFNκ expression in cDCs were compared between the hydroxychloroquine (HCQ) responder, quinacrine (QC) responder, and nonresponder (NR) groups (**F**). Symbols represent individual patients; bars show the median and interquartile range. * = $P < 0.05$; ** = $P < 0.01$. **G**, In situ hybridization analyses of the skin of CLE patients, showing colocalization of expression of ITGAX (CD11c) mRNA (green) and IFNκ mRNA (red). Epidermal IFNκ mRNA is also visible. **H–J**, Correlation network analysis showing negative associations between cDC level and all panel 1 pathways in HCQ responders (**H**) and strong positive correlations between cDC counts and expression levels of pSTING and IFNκ in QC responders (**I**) and nonresponders (**J**). The triangle cluster formed by the cDC, pSTING, and IFNκ circles indicate that they are more strongly associated with each other than with any other pathways in panel 1. Bars in **A–C** = 100 μm; bar in **G** = 50 μm. IL-4 = interleukin-4; TBK1 = TANK-binding kinase 1; TNF = tumor necrosis factor.

cDCs to be the cell type most closely correlated to overall pSTING and IFNκ levels in QC responder patients (Supplementary Figure 2, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Previous studies have highlighted the dominant role of keratinocytes in the production of IFNκ in CLE, and in the present study we found no difference between levels of IFNκ produced by cDCs and levels of IFNκ produced in the epidermal layer ($P = 0.12$).

Neighborhood analysis and antimalarial nonresponder patient clustering. To determine spatial differences and cellular interactions, we performed neighborhood analysis of patients stratified by antimalarial response, with interactions classified as either significant interactions, significant avoidances, or nonsignificant interactions (Figures 4A–C). CD8+ T cells and γδ T cells interacted in HCQ responders and QC responders but not in nonresponder patients, with a significant negative correlation between the CLASI-A score and numbers of γδ T cells observed only in nonresponder patients ($P < 0.05$) (Supplementary Figure 3, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Similarly, Treg cells interacted with cDCs in HCQ responders and QC responders but not in nonresponder patients. CD4+ T cells interacted significantly with cDCs in HCQ responders and QC responders, but avoided cDCs in nonresponders. CD14+CD16+ macrophages interacted with endothelial cells in nonresponders, but not in HCQ responders and QC responders. CD8+ T cells interacted with pDCs in nonresponders but not in HCQ responders and QC responders.

Hierarchical patient level clustering within treatment response groups. Given the heterogeneity in immune cell composition among CLE patients, and in particular antimalarial nonresponding patients, we clustered patients according to patterns of expression in each pathway using ClustVis (Figure 4D) (12). We found that nonresponder patients could be differentiated into 3 distinct groups by pathway expression profile. Group 1 expressed IFNα, IFNγ, pSTAT1, pJAK1, pJAK3, IL-4, and IL-17. Group 2 expressed pSTAT3, pSTAT4, pSTAT5, and TANK-binding kinase 1. Group 3 expressed phosphorylated interferon regulatory factor 3 (pIRF3), granzyme B, IRF5, pSTAT2, pERK, and IL-18. Clustering based on cellular composition, global pathway expression, and cellular pathway expression

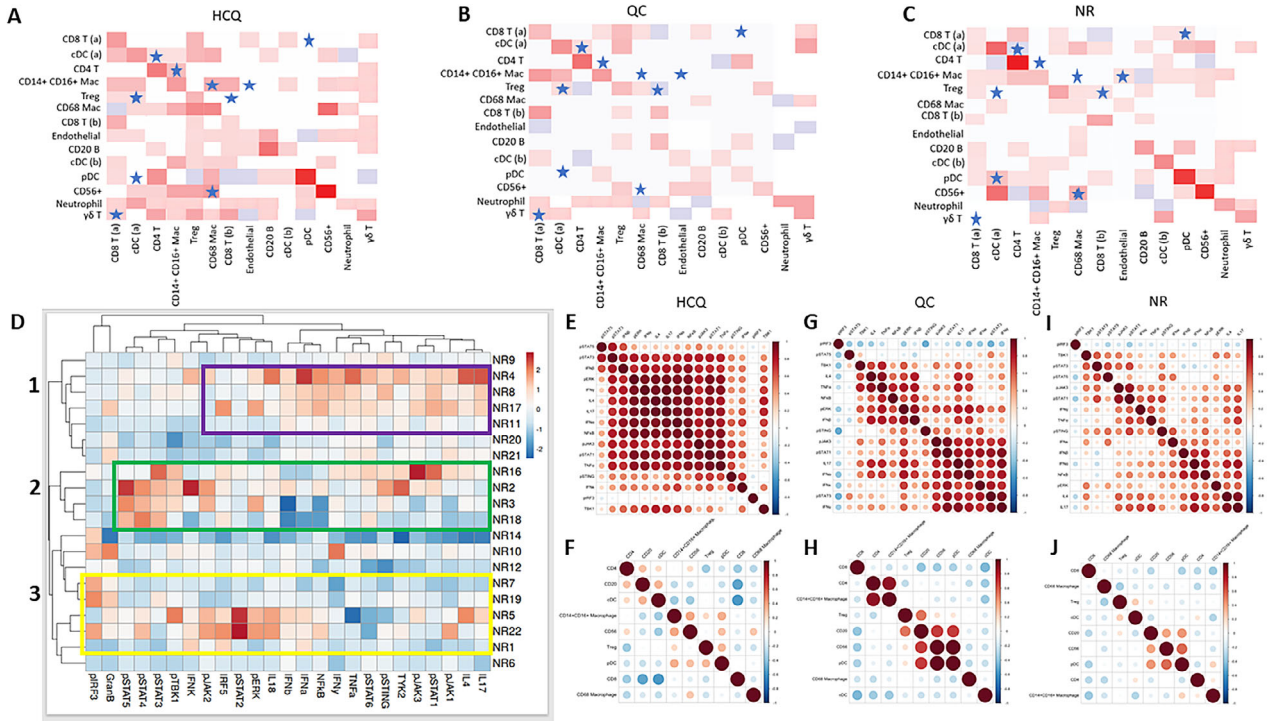

Figure 4. Neighborhood analysis of CLE patients stratified by antimalarial response and unsupervised nonresponder patient clustering. **A–C**, Neighborhood analysis heatmaps representing significant cellular interactions (red squares), significant cellular avoidances (blue squares), and insignificant cellular interactions (white squares) in CLE patients responsive to treatment with HCQ (**A**), CLE patients responsive to treatment with QC (**B**), and CLE patients who did not respond to treatment with antimalarials (**C**). Blue stars highlight differences in key spatial interactions between treatment groups. **D**, Unbiased hierarchical clustering analyses (constructed with ClustVis) of pathway expression profiles in 21 individual CLE patients in the nonresponder (NR) group, showing 3 distinct profiles (purple, green, and yellow boxes). **E–J**, Correlation matrices of intracellular markers and inflammation pathways by treatment response group. pIRF3 = phosphorylated interferon regulatory factor 3; GranB = granzyme B; TBK1 = TANK-binding kinase 1; IFN = interferon; IL-4 = interleukin-4; TNF = tumor necrosis factor; pSTING = phosphorylated stimulator of interferon genes (see Figure 1 for other definitions).

similarly revealed that nonresponder patient groups could be stratified by global and cellular expression of pSTAT3, pSTAT4, and pSTAT5 versus pSTAT2, pIRF3, and granzyme B, with differential pJAK2 expression compared to the remainder of the cohort (Supplementary Figure 6, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235).

In the QC responder group, we identified a CD8+ T cell–dominant subgroup and a separate cDC-dominant subgroup, both of which included only 3 patients (Supplementary Figure 7A, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42235). Nonresponders demonstrated a CD14+CD16+ macrophage–dominant subgroup (n = 4), a cDC-dominant subgroup (n = 6), and a CD8+ T cell–dominant subgroup (n = 5) (Supplementary Figure 7B). In the HCQ responder group, we identified a large subgroup of CD8+ T cell–dominant patients (Supplementary Figure 7C). This subgroup also demonstrated the highest degree of overall pathway activity, suggesting an important role for cytotoxic T cells in those patients.

Correlation of inflammation pathways within treatment groups but not in antimalarial nonresponders. A correlation matrix of intracellular markers demonstrated positive correlation of pathway expression of many markers among HCQ responders (Figure 4E). This may suggest coregulation or common activators of these markers in patients who respond to treatment with HCQ. Responders to treatment with QC demonstrated 2 distinct groups of inflammation markers: one group of markers regulated by TNFα, pNF-κB, and IFNβ, and the other group of markers regulated by pJAK3, pSTAT1, pSTAT3, IFNα, and IFNκ, each with strong positive correlations (Figure 4G). When evaluating antimalarial nonresponders, we found no correlation between notable groups of pathways (Figure 4I). This may be driven by pathway independence within each patient or marked heterogeneity among nonresponders overall.

For each treatment group, we performed a correlation matrix of cell counts. For HCQ responders and nonresponders (Figures 4F and J), we found minimal correlation with the leukocyte count in CLE patients. In QC responders, cell counts for 2 groups of cells showed positive correlation (Figure 4H). The data showed a positive correlation of CD4+ T cells with CD14+CD16+ macrophages. We also found a second grouping of correlated cells including pDCs, CD56+ NK cells, and CD20+ B cells.

DISCUSSION

Although antimalarials are used as first-line systemic therapy for CLE, treatment response is often unpredictable, with many patients requiring coadministration of a second antimalarial or immunosuppressive drugs (13). Accuracy in predicting treatment response can vary due to the multifactorial nature of CLE pathogenesis, and prediction is currently limited by clinical applications utilizing low-dimensional histology and cytometry. In this study, we used markers of inflammation known to be active in CLE to characterize the immunologic profiles of CLE patients who responded to treatment with HCQ (HCQ responders), patients who responded to treatment with HCQ and QC (QC responders), and patients who did not respond to treatment with antimalarials (nonresponders).

Of the 12 immune cell populations identified, numbers of CD4+ T cells and numbers of Treg cells differed significantly between the 3 treatment response groups. It was found that the treatment-naive skin of patients who later responded to treatment with HCQ contained an expanded CD4+ T cell compartment relative to QC responders. CD4+ T cell pathogenesis may play a greater role in the inflammatory process in HCQ responders as CD4+ T cells may be more susceptible to the therapeutic effects of HCQ. The nonresponder group demonstrated decreased numbers of Treg cells and increased numbers of Tcm cells, suggesting a dysregulation of immune tolerance leading to refractoriness. Treg cells in nonresponders were also weakly associated with other inflammation markers compared to Treg cells in the HCQ responders and the QC responders, suggesting defective regulation of the Treg cell pathway.

Previous studies have identified defects in the Treg cells of SLE patients and decreased Treg cell counts in CLE patients; however, conflicting data exists given the limited markers and differing definitions of Treg cell phenotypes (14). In the present study we identified Treg cells using a multitude of markers, including CD3, CD4, FoxP3, and CD25, which were clustered without supervision. These decreased Treg cell counts in nonresponders may represent defects in trafficking or peripheral induction. Alternatively, this difference may be driven by an increase in the proportions of Treg cells in QC responders. Treg cells may be recruited to the skin of QC responders in response to certain inflammatory signals up-regulated in QC responders, such as IFNκ.

IL-2 is a cytokine critical for Treg cell survival and maintenance in the peripheral immune system, and production of IL-2 is often found to be decreased in SLE patients (15). Low-dose IL-2 therapy has been shown to increase the numbers of Treg cells and ameliorate skin lesions in small SLE clinical trials (16,17). Nonresponder patients with low Treg cell counts may respond to Treg cell–mediated treatment, such as IL-2 therapy or adoptive Treg cell therapy, as an effective strategy to restore autologous immune tolerance, rather than profound immunosuppression. An increase in Tcm cells in antimalarial nonresponders may have detrimental consequences as Tcm cells are known to circulate throughout the immune system and initiate a robust and rapid inflammatory response through T helper cell and B cell support (18). Antimalarials may not be sufficient to suppress this extensive Tcm cell response in nonresponders, because HCQ is known to have differential effects on effector T cell subtypes compared to naive T cells (19).

Of the JAK/STAT pathways, pJAK3 expression was increased in HCQ responders compared to QC responders.

JAK3 is activated by cytokines of the common γ-chain family (including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), which may be inhibited by HCQ through transcriptional regulation and p38 MAPK inhibition (20,21). Patients who respond to treatment with HCQ may also respond to novel selective JAK3 inhibitors currently undergoing clinical trials, or to the existing nonselective JAK1/JAK3 inhibitor tofacitinib with the advantage of selective targeting and possibly reduced side effects of HCQ multipathway suppression (22,23).

Patients who responded to treatment with a combination of HCQ and QC had increased levels of IFNκ and pSTING, with trends in increased numbers of cDCs. The STING pathway is activated through the detection of cytosolic DNA, leading to the production of type I IFNs. This pathway is implicated in various autoimmune diseases, particularly in SLE when anti–double-stranded DNA antibodies are present (24). Ultraviolet irradiation induces the production of epidermal IFNκ in CLE patients and is thought to be a major contributor to the initial pathogenesis of the disease (25). STING gene silencing in human keratinocytes leads to a significant reduction in IFNκ production (26). The overlap and correlation of pSTING and IFNκ found through the use of IMC and fluorescence in situ hybridization, suggest a common, targetable pathway within the cDCs of QC responders. Furthermore, network analysis revealed pSTING and IFNκ to be among the pathways most strongly correlated with the number of cDCs, suggesting that the 2 markers are predominantly expressed or regulated by cDCs. In patients receiving treatment with HCQ, the addition of QC has several advantages, as QC has been shown to alternatively suppress type I IFNs when compared to other antimalarials, and QC may inhibit activation of the STING pathway as well as the ultimate production of IFNκ in select patients (27,28). In silico drug library screening identified QC as an inhibitor of STING expression and as a stronger inhibitor of IFNβ expression compared to HCQ (29). This inhibition was cooperative, further reinforcing the use of QC as an adjuvant in select patients with increased STING expression. Given the scarcity of QC in the US, these patients may benefit from treatment with alternative STING inhibitors or from the reintroduction of QC (30,31). This study also highlights the significant differences between HCQ responders and QC responders, reinforcing the need for QC availability.

Our correlation matrices demonstrated many positively correlated intracellular markers in HCQ responders and QC responders. Our data did not reveal correlation among cell types. Patients mainly converge on a pathway level, despite having noticeable differences in cell infiltrates, highlighting greater utility of pathway targeting for precision medicine. The current, evidence-based approach to treatment of CLE is to add QC to the treatment regimen of those patients who do not adequately respond to treatment with HCQ (31). These patients benefit from combination therapy, rather than QC alone. It is possible that each group of correlated inflammation markers in QC responders may be amenable to treatment with either HCQ or QC. Different subsets of nonresponder pathway correlations demonstrate heterogeneity of inflammation in these patients and support the need for personalized therapy.

IMC analysis also allows for single-cell pathway comparison. Multiplexed images may be segmented in order to create cellular outlines based on surface markers or nuclear expansion, allowing for software-assisted extrapolation of intracellular antigen expression within cellular borders (32). Using this technology, we identified multiple single-cell differences between the treatment response groups. Increased expression of IFNγ may be affected by treatment with HCQ, as multiple studies have shown inhibition of IFNγ expression by HCQ in vitro, and our data show increased expression of IFNγ in the neutrophils and cDCs of HCQ responders (33–35). The STAT1 pathway is activated by IFNγ, and gain-of-function mutations have identified a relative deficiency in IL-17 expression. In patients who respond to treatment with HCQ, increased IFNγ production may stimulate expression of STAT1, leading to compounding effects such as an observed increase in pSTAT1 in the endothelium, resulting in activation and up-regulation of adhesive molecules (36). Similarly in HCQ responders, CD68+ macrophage–expressed pSTAT1 may be increased in response to IFNγ signaling, promoting an M1 phenotype (37). Nonresponders demonstrated increased CD4+ T cell–expressed IRF5, which may contribute to HCQ refractoriness as HCQ is known to inhibit Toll-like receptor 7, Toll-like receptor 9, and downstream IRF7-mediated IFN-1, although little is known about the effects of HCQ on IRF5-mediated cytokine and autoantibody production (38–40). The effects of CD4+ T cell–expressed IRF5 may be overcome with novel cell-penetrating peptides in order to prevent homodimerization in patients who do not respond to treatment with antimalarials (41).

B cell–expressed JAK2 is known to enhance cell adhesion and survival, possibly contributing to the production of autoantibodies in QC responders (42). Novel JAK2 inhibitors such as ruxolitinib, fedratinib, and baricitinib may serve as substitutes for QC in patients who do not respond to treatment with HCQ (43). In nonresponder patients, endothelial cells and CD8+ T cells showed increased expression of pSTAT3, which may be responsive to mycophenolate mofetil, as that medication has been shown to reduce the effects of STAT3 and Th17 in SLE patients (43). Of the type I IFNs compared in this study, expression of IFNα was increased in the CD8+ T cells of nonresponders, suggesting a difference in antimalarial responsiveness based on the cell type in which type I IFNs are expressed. Little is known about the production of type I IFNs by cytotoxic T cells; further research may elucidate novel targetable pathways for CLE patients whose disease is refractory to antimalarial treatment.

Differences among treatment response groups also extended to interactions between immune cells. Treg cells in nonresponder patients did not interact with cDCs, suggesting a deficiency in tolerance as they were also decreased in number.

Moreover, γδ T cells routinely monitor skin integrity and possess suppressive functions through the production of insulin-like growth factor 1 and transforming growth factor β. In nonresponders, γδ T cells did not interact with cytotoxic CD8+ T cells; however, this interaction was present in HCQ responders and QC responders, suggesting another deficiency in the immune response mechanisms of patients who do not respond to treatment with antimalarials (44,45). The negative correlation of γδ T cells with CLASI scores that we observed exclusively in nonresponders further supports this finding. Nonresponder patients also demonstrated fewer interactions between CD4+ T cells and cDCs, but an increased number of interactions between CD14+CD16+ macrophages and endothelium, indicating that infiltration had occurred, and increased number of interactions between pDCs and CD8+ T cells, suggesting that there was cytotoxic augmentation through local type I IFNs.

Our data also demonstrated that differences in the activation of inflammatory pathways and cytokines in the epidermis may have been different between the 3 treatment response groups, with a trend toward decreased phosphorylation of NF-κB in QC responders. The overall inflammation profile of the epidermis and inflammation profile of the dermis overlapped across all treatment groups, encouraging noninvasive techniques for the diagnosis of CLE, such as tape stripping (46). Sampling of keratinocytes may in fact yield information regarding the inflammation profile of the dermis, making the performance of skin biopsy unnecessary (47).

Treatment response prediction historically involves clinical data from randomized controlled trials that identify differences between treatment groups; however, such groups are often heterogeneous, and a single patient may not conform to either group on an individual level. Given the heterogeneity of the immune infiltrate among our groups of CLE patients, we implemented an unbiased patient clustering system using ClustVis (12). Nonresponder patients stratified into 3 groups according to pathway expression, highlighting diverse pathophysiologic patterns driven by either IFNγ, IL-4, IL-17, pJAK1, and pJAK3, or by pSTAT3, pSTAT4, and pSTAT5, or by pSTAT2, pIRF3, and granzyme B. Such individual differences may guide the ultimate selection of treatment, given the wide array of immunosuppressives and the known differences in pathway suppression. CLE patients may benefit greatly from personalized medicine approaches such as those that determine patient-specific targetable disease features.

We also identified a surprising paucity of type I IFN+ pDCs in our CLE patients, with the major producers being myeloid lineage cells. Our data demonstrated a relatively low MPI of type I IFN+ pDCs. The absolute counts and percentages of IFNα+ and IFNβ+ pDCs were also minimal. These results cut across a decade-long paradigm that places type I IFNs produced by pDCs at the center of lupus pathogenesis (48). The prominent role of type I IFNs in LE is well established (49,50). Several in vivo studies have shown that IFNα production, in response to CpG challenge, is exclusively dependent on pDCs (51–53). It is also well known that pDCs are capable of producing type I IFNs abundantly and rapidly, producing over 1,000 times more type I IFNs than other cell types under appropriate stimulation (54,55). Naturally, it seems logical to assume that pDCs play a crucial role in autoimmune connective tissue diseases such as LE.

However, these results do not entirely contradict the findings in the current literature on the pathophysiologic processes of lupus. Recently, Psarras et al implicated a largely exhausted and unimportant role for pDCs in the pathogenesis of LE (56). These findings represent a crucial junction in the understanding of pDC biology and their role in autoimmunity. Indeed, previous studies have demonstrated decreased circulating pDCs in SLE patients (48,57). Some have hypothesized that type I IFN–producing pDCs were instead present in the end organs such as the skin and kidneys (49). Our results suggest that pDCs are not, in fact, major drivers of type I IFN production in the setting of CLE, and that myeloid cell lineages are the major contributors of type I IFNs. A subset of NK cells also express type I IFNs; however, like pDCs, they are relatively scarce in tissue. Given the success of anti–blood dendritic cell antigen 2 (58) therapy in patients with LE and the recent and promising initial success of VIB7734 (59), a monoclonal antibody directed against immunoglobulin-like transcript 7 (another pDC-specific marker), pDCs likely play an important role for some CLE patients. Ultimately, we believe these results highlight the complex and unclear role pDCs play in the pathogenesis of LE. Studies to confirm these findings and elucidate the role of pDCs in CLE are currently under way by our group.

This study was limited by sample size and the use of markers determined to be critical to CLE pathogenesis and is not all-inclusive. Cell clustering is limited by the resolution of IMC, and thus may cluster overlapping cells that were ultimately refined manually. A lack of multiple hypothesis testing limits the conclusions that can be drawn from this discovery pilot study; however, key findings were confirmed by manual identification of IMC images and fluorescence in situ hybridization exploration. By using IMC analysis for CLE patients stratified by antimalarial response, we were able to identify significant differences in their immune profiles. These differences will guide future therapies and encourage a more personalized approach to treatment, one that combines clinical, histopathologic, and immunologic sources of data.

ACKNOWLEDGMENTS

We thank Dr. Dan Traum, PhD and Dr. Kyong-Mi Chang, PhD for their assistance with conducting imaging mass cytometry.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Vazquez had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Patel, Vazquez, Werth.

Acquisition of data. Patel, Vazquez, Werth.
Analysis and interpretation of data. Patel, Vazquez, Chin, Keyes, Yan, Diaz, Grinnell, Sharma, Li, Feng, Sprow, Dan, Werth.

REFERENCES

1. Fabbri P, Cardinali C, Giomi B, Caproni M. Cutaneous lupus erythematosus: diagnosis and management. Am J Clin Dermatol 2003;4: 449–65.
2. Klein R, Moghadam-Kia S, Taylor L, Coley C, Okawa J, LoMonico J, et al. Quality of life in cutaneous lupus erythematosus. J Am Acad Dermatol 2011;64:849–58.
3. Chang AY, Ghazi E, Okawa J, Werth VP. Quality of life differences between responders and nonresponders in the treatment of cutaneous lupus erythematosus. JAMA Dermatol 2013;149:104–6.
4. Patel J, Borucki R, Werth VP. An update on the pathogenesis of cutaneous lupus erythematosus and its role in clinical practice. Curr Rheumatol Rep 2020;22:69.
5. Walling HW, Sontheimer RD. Cutaneous lupus erythematosus: issues in diagnosis and treatment. Am J Clin Dermatol 2009;10:365–81.
6. Kalia S, Dutz JP. New concepts in antimalarial use and mode of action in dermatology. Dermatol Ther 2007;20:160–74.
7. Wozniacka A, Carter A, McCauliffe DP. Antimalarials in cutaneous lupus erythematosus: mechanisms of therapeutic benefit. Lupus 2002;11:71–81.
8. Zeidi M, Kim HJ, Werth VP. Increased myeloid dendritic cells and TNF-α expression predicts poor response to hydroxychloroquine in cutaneous lupus erythematosus. J Invest Dermatol 2019;139: 324–32.
9. Sontheimer RD, Thomas JR, Gilliam JN. Subacute cutaneous lupus erythematosus: a cutaneous marker for a distinct lupus erythematosus subset. Arch Dermatol 1979;115:1409–15.
10. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
11. Albrecht J, Taylor L, Berlin JA, Dulay S, Ang G, Fakharzadeh S, et al. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an outcome instrument for cutaneous lupus erythematosus. J Invest Dermatol 2005;125:889–94.
12. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res 2015;43:W566–70.
13. Borucki R, Werth VP. An evidence-based approach to refractory cutaneous lupus erythematosus. Arthritis Rheumatol 2020;72: 1777–85.
14. Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, et al. Global natural regulatory T cell depletion in active systemic lupus erythematosus. J Immunol 2005;175:8392–400.
15. Furtado GC, de Lafaille MA, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. J Exp Med 2002;196:851–7.
16. Humrich JY, von Spee-Mayer C, Siegert E, Alexander T, Hiepe F, Radbruch A, et al. Rapid induction of clinical remission by low-dose interleukin-2 in a patient with refractory SLE. Ann Rheum Dis 2015; 74:791–2.
17. Von Spee-Mayer C, Siegert E, Abdirama D, Rose A, Klaus A, Alexander T, et al. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. Ann Rheum Dis 2016;75:1407–15.
18. Raphael I, Joern RR, Forsthuber TG. Memory CD4(+) T cells in immunity and autoimmune diseases [review]. Cells 2020;9:531.
19. Van Loosdregt J, Spreafico R, Rossetti M, Prakken BJ, Lotz M, Albani S. Hydroxychloroquine preferentially induces apoptosis of CD45RO+ effector T cells by inhibiting autophagy: a possible mechanism for therapeutic modulation of T cells. J Allergy Clin Immunol 2013;131:1443–6.
20. Chen M, Cheng A, Chen YQ, Hymel A, Hanson EP, Kimmel L, et al. The amino terminus of JAK3 is necessary and sufficient for binding to the common gamma chain and confers the ability to transmit interleukin 2-mediated signals. Proc Natl Acad Sci U S A 1997;94:6910–5.
21. Roldan EQ, Biasiotto G, Magro P, Zanella I. The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): a role for iron homeostasis? Pharmacol Res 2020;158:104904.
22. Robinson MF, Damjanov N, Stamenkovic B, Radunovic G, Kivitz A, Cox L, et al. Efficacy and safety of PF-06651600 (Ritlecitinib), a novel JAK3/TEC inhibitor, in patients with moderate-to-severe rheumatoid arthritis and an inadequate response to methotrexate. Arthritis Rheumatol 2020;72:1621–31.
23. Farmer LJ, Ledeboer MW, Hoock T, Arnost MJ, Bethiel RS, Bennani YL, et al. Discovery of VX-509 (decernotinib): a potent and selective janus kinase 3 inhibitor for the treatment of autoimmune diseases. J Med Chem 2015;58:7195–216.
24. Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. Nat Rev Immunol 2021:1–22.
25. Sarkar MK, Hile GA, Tsoi LC, Xing X, Liu J, Liang Y, et al. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. Ann Rheum Dis 2018;77: 1653–64.
26. Sunthamala N, Thierry F, Teissier S, Pientong C, Kongyingyoes B, Tangsiriwatthana T, et al. E2 proteins of high risk human papillomaviruses down-modulate STING and IFN-κ transcription in keratinocytes. PLoS ONE 2014;9:e91473.
27. Cardoso EC, Pereira NZ, Mitsunari GE, Oliveira LM, Ruocco RM, Francisco RP, et al. TLR7/TLR8 activation restores defective cytokine secretion by myeloid dendritic cells but not by plasmacytoid dendritic cells in HIV-infected pregnant women and newborns. PLoS ONE 2013;8:e67036.
28. Alves P, Bashir MM, Wysocka M, Zeidi M, Feng R, Werth VP. Quinacrine suppresses tumor necrosis factor-α and IFN-α in dermatomyositis and cutaneous lupus erythematosus. J Investig Dermatol Symp Proc 2017;18:S57–63.
29. An J, Woodward JJ, Sasaki T, Minie M, Elkon KB. Cutting edge: antimalarial drugs inhibit IFN-β production through blockade of cyclic GMP-AMP synthase-DNA interaction. J Immunol 2015;194: 4089–93.
30. Thim-Uam A, Prabakaran T, Tansakul M, Makjaroen J, Wongkongkathep P, Chantaravisoot N, et al. STING mediates lupus via the activation of conventional dendritic cell maturation and plasmacytoid dendritic cell differentiation. iScience 2020;23:101530.
31. Chang AY, Piette EW, Foering KP, Tenhave TR, Okawa J, Werth VP. Response to antimalarial agents in cutaneous lupus erythematosus: a prospective analysis. Arch Dermatol 2011;147:1261–7.
32. Schapiro D, Jackson HW, Raghuraman S, Fischer JR, Zanotelli VR, Schulz D, et al. histoCAT: analysis of cell phenotypes and interactions in multiplex image cytometry data. Nat Methods 2017;14:873–6.
33. Da Silva JC, Mariz HA, da Rocha LF Jr, de Oliveira PS, Dantas AT, Duarte AL, et al. Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients. Clinics (Sao Paulo) 2013;68:766–71.
34. Van den Borne B, Dijkmans B, De Rooij H, Le Cessie S, Verweij C. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. J Rheumatol 1997;24:55–60.

35. Jeong JY, Choi JW, Jeon KI, Jue DM. Chloroquine decreases cell-surface expression of tumour necrosis factor receptors in human histiocytic U-937 cells. Immunology 2002;105:83–91.
36. Sikorski K, Chmielewski S, Przybyl L, Heemann U, Wesoly J, Baumann M, et al. STAT1-mediated signal integration between IFNγ and LPS leads to increased EC and SMC activation and monocyte adhesion. Am J Physiol Cell Physiol 2011;300:C1337–44.
37. Kim HS, Kim DC, Kim HM, Kwon HJ, Kwon SJ, Kang SJ, et al. STAT1 deficiency redirects IFN signalling toward suppression of TLR response through a feedback activation of STAT3. Sci Rep 2015;5: 1–15.
38. Manni M, Gupta S, Ricker E, Chinenov Y, Park SH, Shi M, et al. Regulation of age-associated B cells by IRF5 in systemic autoimmunity. Nat Immunol 2018;19:407–19.
39. Savitsky DA, Yanai H, Tamura T, Taniguchi T, Honda K. Contribution of IRF5 in B cells to the development of murine SLE-like disease through its transcriptional control of the IgG2a locus. Proc Natl Acad Sci U S A 2010;107:10154–9.
40. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, et al. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 2005;434:243–9.
41. Banga J, Srinivasan D, Sun CC, Thompson CD, Milletti F, Huang KS, et al. Inhibition of IRF5 cellular activity with cell-penetrating peptides that target homodimerization. Sci Adv 2020;6:eaay1057.
42. Montresor A, Toffali L, Mirenda M, Rigo A, Vinante F, Laudanna C. JAK2 tyrosine kinase mediates integrin activation induced by CXCL12 in B-cell chronic lymphocytic leukemia. Oncotarget 2015;6: 34245–57.
43. Davis RR, Li B, Yun SY, Chan A, Nareddy P, Gunawan S, et al. Structural insights into JAK2 inhibition by ruxolitinib, fedratinib, and derivatives thereof. J Med Chem 2021;64:2228–41.
44. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, et al. A role for human skin-resident T cells in wound healing. J Exp Med 2009;206:743–50.
45. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 2008; 14:818–29.
46. Berekméri A, Tiganescu A, Alase AA, Vital E, Stacey M, Wittmann M. Non-invasive approaches for the diagnosis of autoimmune/autoinflammatory skin diseases-a focus on psoriasis and lupus erythematosus [review]. Front Immunol 2019;10:1931.
47. Merola JF, Wang W, Wager CG, Hamann S, Zhang X, Thai A, et al. RNA tape sampling in cutaneous lupus erythematosus discriminates affected from unaffected and healthy volunteer skin. Lupus Sci Med 2021;8:e000428.
48. Blomberg S, Eloranta ML, Cederblad B, Nordlin K, Alm GV, Rönnblom L. Presence of cutaneous interferon-α producing cells in patients with systemic lupus erythematosus. Lupus 2001;10:484–90.
49. Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. Lupus Sci Med 2019;6: e000270.
50. Crow MK, Ronnblom L. Type I interferons in host defence and inflammatory diseases. Lupus Sci Med 2019;6:e000336.
51. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. Cell 2008;135:37–48.
52. Asselin-Paturel C, Brizard G, Pin JJ, Brière F, Trinchieri G. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. J Immunol 2003;171: 6466–77.
53. Kumagai Y, Takeuchi O, Kato H, Kumar H, Matsui K, Morii E, et al. Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. Immunity 2007;27:240–52.
54. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med 1999;5:919–23.
55. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23: 275–306.
56. Psarras A, Alase A, Antanaviciute A, Carr IM, Yusof MY, Wittmann M, et al. Functionally impaired plasmacytoid dendritic cells and non-haematopoietic sources of type I interferon characterize human autoimmunity. Nat Commun 2020;11:6149.
57. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon- α/β-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237–43.
58. Furie R, Werth VP, Merola JF, Stevenson L, Reynolds TL, Naik H, et al. Monoclonal antibody targeting BDCA2 ameliorates skin lesions in systemic lupus erythematosus. J Clin Invest 2019;129:1359–71.
59. Karnell JL, Wu Y, Mittereder N, Smith MA, Gunsior M, Yan L, et al. Depleting plasmacytoid dendritic cells reduces local type I interferon responses and disease activity in patients with cutaneous lupus. Sci Transl Med 2021;13:eabf8442.