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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 NF□B are higher in HCQ+QC-responders, and we have performed sub-analyses demonstrating a correlation between pSTING and IFN□, as well as increased cDC-labeled pSTING and IFN□□□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 interventions.

15. SUBJECT TERMS

Cutaneous lupus erythematosus, immune cells, pathways

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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.

Preliminary 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.

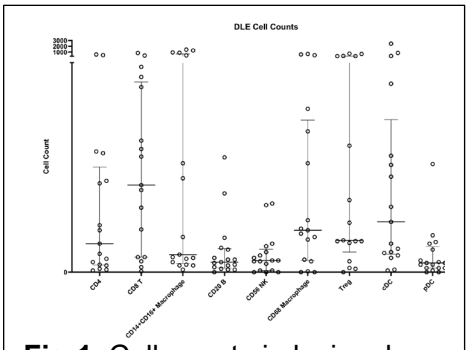

Fig 1. Cell counts in lesional DLE skin.

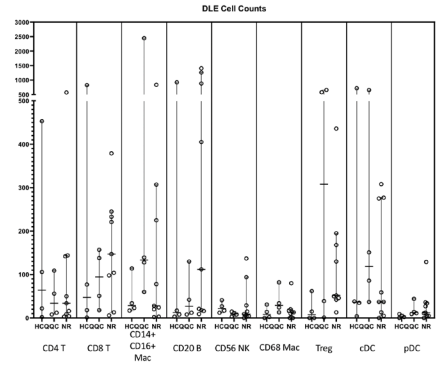

Fig 2. DLE cell counts according to antimalarial response.

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).

In situ hybridization using RNAscope techniques co-localized pSTING and IFNκ to cDCs (Figure 4). This is a novel observation that will be pursued further.

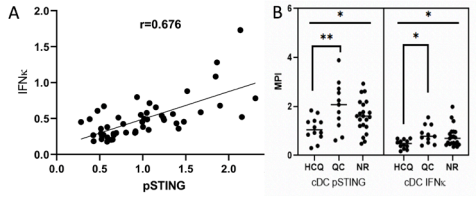

Fig. 3. Strong correlation between pSTNG and IFNκ.

How were the results disseminated to communities of interest?

Two abstracts were submitted to the Society of Investigative Dermatology and 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). Additional data in an abstract has been submitted to the American College of Rheumatology 2021 meetings.

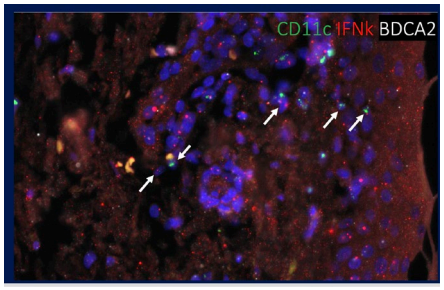

Fig. 4. Co-localization of IFNk mRNA to cDCs and not pDCs.

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.

What do you plan to do in the next reporting period to accomplish the goals?

We have also isolated mRNA from the skin samples and will do real time PCR analysis to further substantiate and expand our tissue mass cytometry results.

We are starting to utilize K-means clustering on the mass cytometry data we've been collecting throughout this past year to divide patients into immunophenotypically-distinct subgroups. We then plan on using heatmaps to visualize the differences between these subgroups in terms of cell population, pathway, and single cell protein expression levels. Special attention will be paid towards differences in treatment response and clinical subtype within each subgroup. We are also running machine learning algorithms (Random Forest predictive models) on individual subgroups to explore the possibility of predicting treatment response using mass cytometry data. Such analysis will help in determining the viability of an algorithmic-based immunophenotype approach to predicting CLE treatment response. We will also be using the proximity values from the Random Forest predictive runs to refine and generate additional subgroups which we are hopeful will help reveal immunophenotype distinctions that could potentially explain the mechanistic differences driving differing CLE clinical subtypes and treatment responses.

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.

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Funding Support	

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Funding Support	Rheumatology Research Foundation

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Funding Support	

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Contribution to project	Identifying lupus patients and response from database
Funding Support	

8. Special Reporting requirements: None

9. Appendix

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 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

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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.

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

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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.

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

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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.

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Single-cell composition and architecture of cutaneous lupus

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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.

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

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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.