Arthritis & Rheumatology
Vol. 74, No. 12, December 2022, pp 2024–2031
DOI 10.1002/art.42283

AMERICAN COLLEGE of RHEUMATOLOGY
Empowering Rheumatology Professionals

BRIEF REPORT

Imaging Mass Cytometry Reveals Predominant Innate Immune Signature and Endothelial–Immune Cell Interaction in Juvenile Myositis Compared to Lupus Skin

Jessica L. Turnier,[1] Christine M. Yee,[2] Jacqueline A. Madison,[3] Syed M. Rizvi,[2] Celine C. Berthier,[4] Fei Wen,[2] and J. Michelle Kahlenberg[5]

Objective. Cutaneous inflammation can signal disease in juvenile dermatomyositis (DM) and childhood-onset systemic lupus erythematosus (cSLE), but we do not fully understand cellular mechanisms of cutaneous inflammation. In this study, we used imaging mass cytometry to characterize cutaneous inflammatory cell populations and cell–cell interactions in juvenile DM as compared to cSLE.

Methods. We performed imaging mass cytometry analysis on skin biopsy samples from juvenile DM patients (n = 6) and cSLE patients (n = 4). Tissue slides were processed and incubated with metal-tagged antibodies for CD14, CD15, CD16, CD56, CD68, CD11c, HLA–DR, blood dendritic cell antigen 2, CD20, CD27, CD138, CD4, CD8, E-cadherin, CD31, pan-keratin, and type I collagen. Stained tissue was ablated, and raw data were acquired using the Hyperion imaging system. We utilized the Phenograph unsupervised clustering algorithm to determine cell marker expression and permutation test by histoCAT to perform neighborhood analysis.

Results. We identified 14 cell populations in juvenile DM and cSLE skin, including CD14+ and CD68+ macrophages, myeloid and plasmacytoid dendritic cells (pDCs), CD4+ and CD8+ T cells, and B cells. Overall, cSLE skin had a higher inflammatory cell infiltrate, with increased CD14+ macrophages, pDCs, and CD8+ T cells and immune cell–immune cell interactions. Juvenile DM skin displayed a stronger innate immune signature, with a higher overall percentage of CD14+ macrophages and prominent endothelial cell–immune cell interaction.

Conclusion. Our findings identify immune cell population differences, including CD14+ macrophages, pDCs, and CD8+ T cells, in juvenile DM skin compared to cSLE skin, and highlight a predominant innate immune signature and endothelial cell–immune cell interaction in juvenile DM, providing insight into candidate cell populations and interactions to better understand disease-specific pathophysiology.

INTRODUCTION

Juvenile dermatomyositis (DM) and childhood-onset systemic lupus erythematosus (cSLE) are multisystem inflammatory diseases with overlapping yet distinct clinical phenotypes and unique tropism for major organ involvement. Cutaneous inflammation is often the first recognized symptom at disease onset, and substantial clinical and histopathologic overlap exists between skin lesions (1). Both juvenile DM and cSLE skin lesions demonstrate interface dermatitis, characterized by lymphocytic infiltrate and apoptotic keratinocytes at the dermoepidermal junction and also share an association with type I interferon (IFN)

Dr. Turnier's work was supported by a Cure JM Foundation Research Grant, Rheumatology Research Foundation Investigator Award, an NIH LRP Pediatric Research Renewal Award (award 2L40-AR-070531-04), and a Michigan Institute for Clinical & Health Research (MICHR) Pilot Grant (UL1-TR-002240). Dr. Wen's work was supported by the NIH (awards S10-OD-020053 and P30-CA-046592), the Taubman Institute Innovative Program, and the National Science Foundation (award 1653611). Dr. Kahlenberg's work was supported by the NIH (awards R01-AR-071384 and K24-AR-076975), and the Taubman Institute Innovative Program.

[1]Jessica L. Turnier, MD: Division of Pediatric Rheumatology, Department of Pediatrics, University of Michigan, Ann Arbor; [2]Christine M. Yee Auy, BS, Syed M. Rizvi, PhD, Fei Wen, PhD: Department of Chemical Engineering, University of Michigan, Ann Arbor; [3]Jacqueline A. Madison, MD: Division of Pediatric Rheumatology, Department of Pediatrics, and Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor; [4]Celine C. Berthier, PhD: Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor; [5]J. Michelle Kahlenberg, MD, PhD: Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor.

Author disclosures are available at https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42283&file=art42283-sup-0001-Disclosureform.pdf.

Address correspondence via email to Jessica Turnier, MD, at turnierj@med.umich.edu.

Submitted for publication March 11, 2022; accepted in revised form June 23, 2022.

activation (2). Cutaneous inflammation has further been demonstrated to associate with systemic disease activity and chronicity in juvenile DM and cSLE (3); however, we are limited in our understanding of pathogenic mechanisms and immune cells that drive cutaneous inflammation and disease-specific phenotypes.

Imaging mass cytometry (IMC) is a powerful tool to study disease phenotypes through simultaneous analysis of multiple protein targets while preserving tissue architecture and lending insights into cellular microenvironment and interactions (4). A recent publication harnessing IMC for adult DM skin immunophenotyping identified 13 unique immune cell populations and described a predominant myeloid signature, with abundant CD14+ macrophages and CD11c+ myeloid dendritic cells (mDCs), in addition to lymphoid cells (5). Prior use of mass cytometry to characterize cSLE blood defined a $CD14^{high}$ monocyte cytokine signature that was inducible in peripheral blood from healthy controls after treatment with cSLE plasma (6). Improving our understanding of immune cell populations and cell–cell interactions central to tissue inflammation is key to informing the development of targeted treatment for juvenile DM and cSLE patients.

In the present study, we use IMC to characterize similarities and differences in inflammatory cells and cell–cell interactions at a single-cell level within juvenile DM lesional skin compared to cSLE lesional skin. Our findings identify differences in cell populations, including CD14+ macrophages, plasmacytoid DCs (pDCs), and CD8+ T cells, in juvenile DM versus cSLE, and highlight a predominance of innate immune cells and endothelial cell–immune cell interactions in juvenile DM skin, providing insight into immune cell populations and cellular interactions as candidates for further study.

PATIENTS AND METHODS

Human subjects, skin biopsy samples, and clinical data acquisition. Formalin-fixed paraffin-embedded (FFPE) skin biopsy samples previously obtained for clinical care at the University of Michigan were obtained after institutional review board approval. Diagnoses at the time of biopsy for juvenile DM or cSLE were made by a pediatric rheumatologist and were verified by chart review of clinical findings, laboratory data, imaging, and histopathology. All juvenile DM patients (n = 6) met the 2017 EULAR/American College of Rheumatology (ACR) classification criteria (7), with 1 patient having skin-predominant disease. All cSLE patients (n = 4) met the 1997 ACR classification criteria for SLE (8) at time of biopsy, with the exception of 1 patient with isolated cutaneous lupus at diagnosis who later developed features of systemic disease 3 years after biopsy. Lesional skin was from varied locations, including the elbow (n = 3), finger (n = 2), arm (n = 2), cheek, scalp, and thigh (all n = 1). Clinical data were collected retrospectively by chart review (Supplementary Table 1, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42283).

IMC sample preparation and image processing. We performed IMC on all skin biopsy samples to identify and quantify immune cell populations that were present. FFPE tissue slides were heated for 2 hours at 60°C, deparaffinized, and rehydrated. Slides were placed in Tris–EDTA (pH 9) antigen retrieval buffer and heated at 96°C for 30 minutes. After cooling, slides were blocked in 3% bovine serum albumin and incubated with metal-tagged antibodies. Our antibody panel included the following markers: CD14, CD15, CD16, CD56, CD68, CD11c, HLA–DR, blood dendritic cell antigen 2, CD20, CD27, CD138, CD4, CD8, E-cadherin, CD31, pan-keratin, and type I collagen. Stained tissue was ablated, and raw data were acquired using the Hyperion imaging system (Fluidigm). Multiplexed cytometry by time-of-flight mass spectrometry imaging data were preprocessed using commercial acquisition software (Fluidigm), converted to TIFF images, and then segmented into individual cells using CellProfiler version 3.1.8.

IMC data analysis. For dimensionality reduction, we used visualization of t-distributed stochastic neighbor embedding (t-SNE) to determine phenotypic diversity of cell populations. The Phenograph unsupervised clustering algorithm was used to determine cell marker expression (9). A heatmap was generated to demonstrate median Z score marker expression of cells in each cluster. Neighborhood analysis was performed by permutation test using histoCAT (10) with a permutation number of 999 and a P value threshold of 0.01.

Microarray data analysis to evaluate innate and adaptive signatures. We previously performed microarray gene expression analysis on all lesional skin samples (11). Using the xCell webtool (http://xcell.ucsf.edu) (12), we generated innate and adaptive transcriptional immune signatures from samples. Each patient signature was generated for innate immune signatures by adding xCell enrichment scores from DCs, pDCs, macrophages, and monocytes (94, 38, 259, and 303 genes, respectively) and for adaptive immune signatures by adding scores from B cells, CD4+ T cells, and CD8+ T cells (135, 158, and 116 genes, respectively).

Statistical analysis. Cell populations were quantified by number of cells per mm^2 of tissue and translated into percentage of total immune cells identified in each patient sample. Differences in cell populations between juvenile DM and cSLE were assessed in GraphPad Prism 8 software using Student's 2-tailed t-test, with P values less than 0.05 considered significant.

RESULTS

Key differences in absolute number of immune cell populations within skin lesions in juvenile DM and cSLE. Childhood-onset SLE skin lesions had an overall higher inflammatory cell infiltrate compared to juvenile DM (Figure 1A).

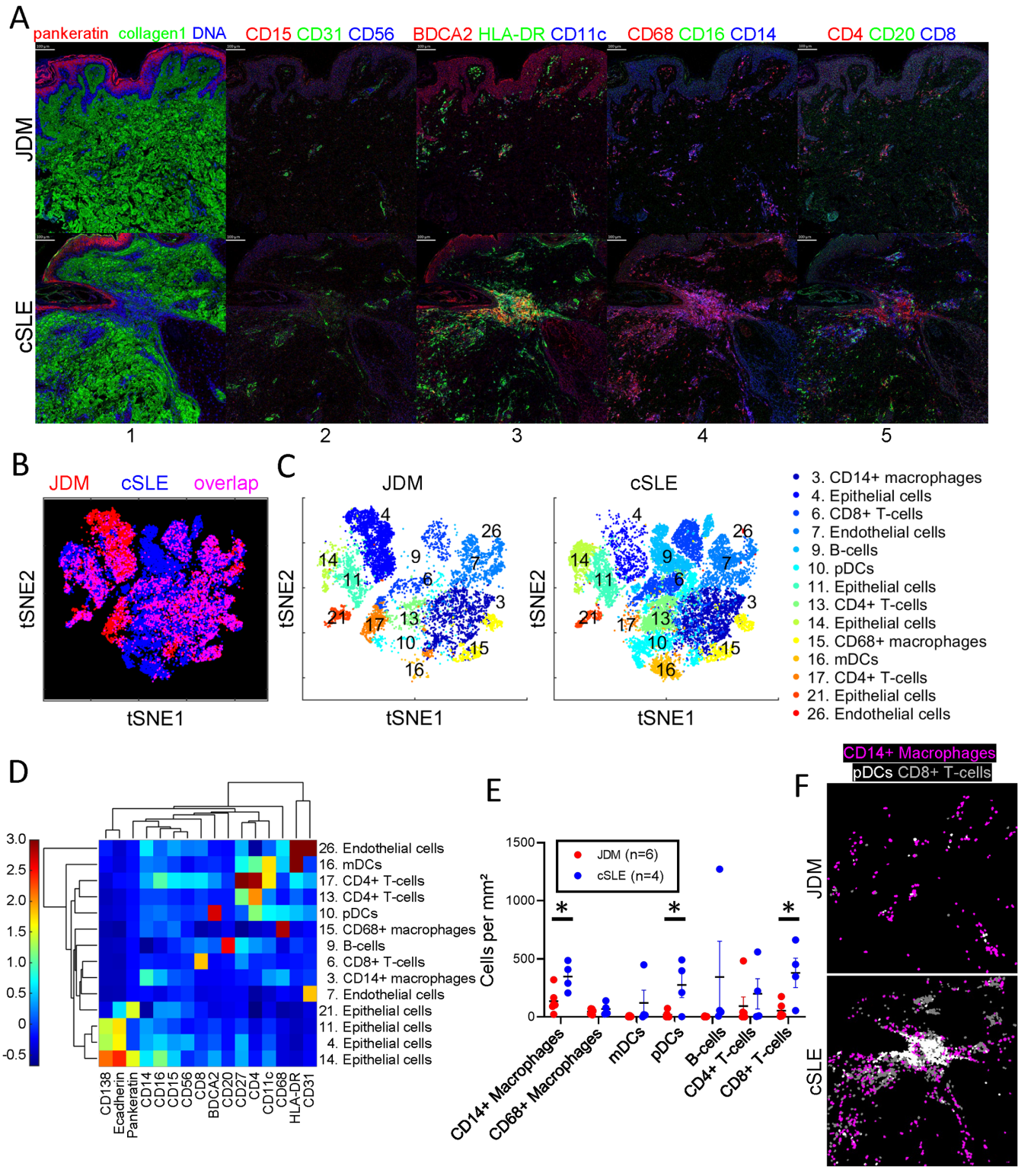

Figure 1. CD14+ macrophages, plasmacytoid dendritic cells (pDCs), and CD8+ T cells were increased in childhood-onset systemic lupus erythematosus (cSLE) compared to juvenile dermatomyositis (JDM) lesional skin. **A**, Multiplexed images demonstrating staining for cellular markers in JDM and cSLE skin samples, represented by different colors in panels 1–5. Bars = 100 μm. **B** and **C**, Analysis by t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction demonstrating overlay of identified JDM and cSLE cell clusters (**B**) and individual t-SNE plots by disease (**C**). **D**, Phenograph clustergram and heatmap showing marker expression by cell cluster. **E**, Quantification of immune cell types per disease based on marker expression. Bars show the mean ± SEM. * = $P < 0.05$. **F**, Representative images demonstrating higher quantities of CD14+ macrophages, pDCs, and CD8+ T cells in cSLE compared to JDM. Magnification is the same as in **A**. BDCA2 = blood dendritic cell antigen 2; mDCs = myeloid DCs.

Using the t-SNE dimensionality reduction tool, we visualized cell clusters that overlapped between diseases and those more predominant in either juvenile DM or cSLE (Figures 1B and 1C and Supplementary Figure 1, https://onlinelibrary.wiley.com/doi/10.1002/art.42283). Overall, we identified 26 unique cell clusters in juvenile DM and cSLE skin (Supplementary Figure 1), of which we were able to definitively identify 14 cell populations using marker expression patterns (Figure 1D), including 8 immune cell populations: CD14+ macrophages (cluster 3), CD68+ macrophages (cluster 15), mDCs (cluster 16), pDCs (cluster 10), B cells (cluster 9), CD4+ T cells (clusters 13 and 17), and CD8+ T cells (cluster 6).

While all immune cell populations were present in both diseases, there were differences in cell numbers per cluster according to disease. Notably, cSLE skin showed increased CD14+ macrophages, pDCs, and CD8+ T cells (Figure 1E). This is demonstrated visually by the spatial distribution of labeled cells in juvenile DM compared to cSLE skin (Figure 1F). Interestingly, we noted 2 CD4+ T cell populations, with cluster 17 additionally displaying CD11c and CD27 coexpression (Figure 1D). CD4+ T cells from cluster 17 were more concentrated in juvenile DM skin (Figures 1B and C) and could potentially represent a more highly activated, migratory T cell population (13).

Overall immune cell composition differs in juvenile DM skin lesions compared to cSLE skin lesions. While CD14+ macrophages were the predominant immune cell population in both juvenile DM and cSLE, juvenile DM had an overall higher percentage of CD14+ macrophages relative to total immune cell composition (46.1% versus 30%) (Figure 2A and Supplementary Table 2A, https://onlinelibrary.wiley.com/doi/10.1002/art.42283). In contrast, cSLE exhibited a higher overall percentage of pDCs and CD8+ T cells than juvenile DM (13.5% versus 3.9%, and 21% versus 13%, respectively). In juvenile DM, the composition of identified immune cells from highest to lowest percentage included CD14+ macrophages (46.1%) followed by CD68+ macrophages (24%), CD8+ T cells (13%), CD4+ T cells (11.7%), pDCs (3.9%), mDCs (0.9%), and B cells (0.5%) (Figure 2A and Supplementary Table 2A). Of note, B cells were scarce in all juvenile DM samples. In cSLE, the most populous immune cells were also CD14+ macrophages (30%),

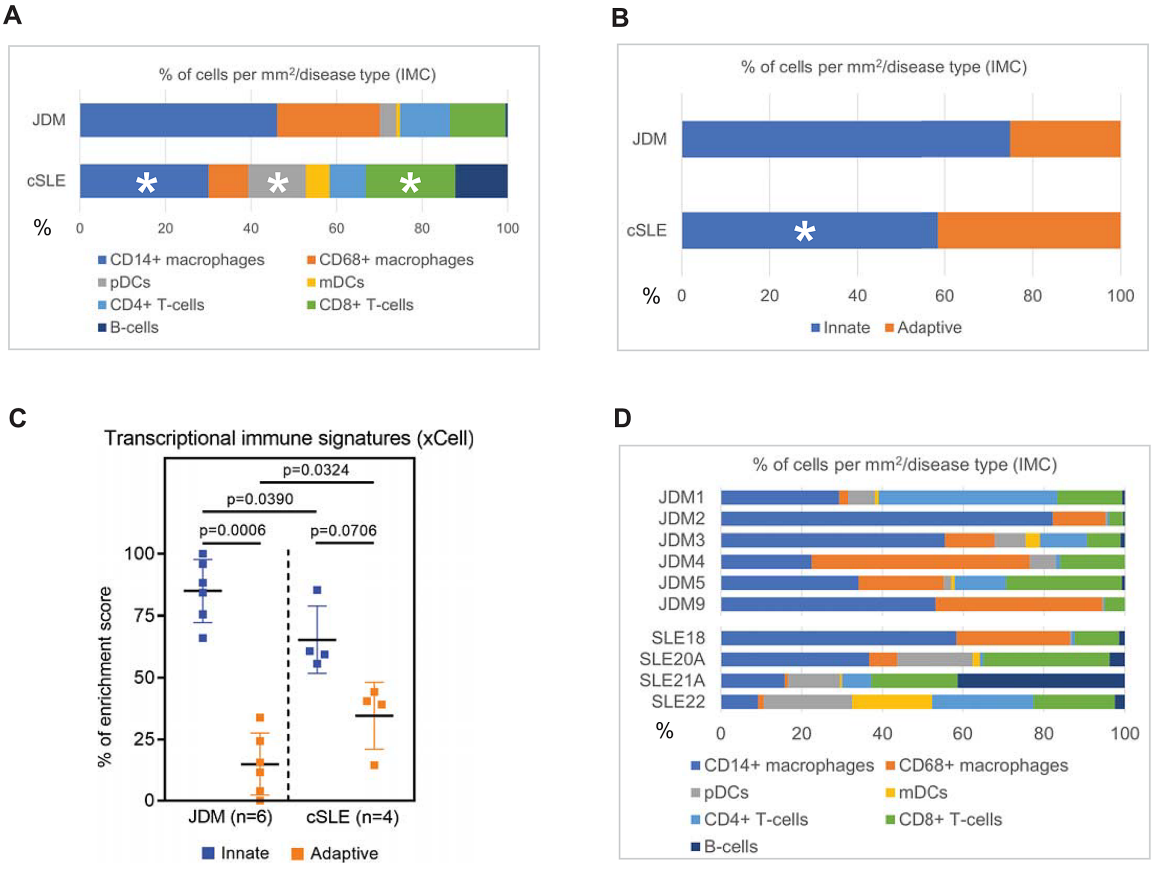

Figure 2. Overall immune cell composition in JDM compared to cSLE skin was predominantly innate immune cells. **A** and **B,** Bar graphs showing percent composition of immune cell types by disease (**A**) and innate versus adaptive immune cell categorization, with CD14+ and CD68+ macrophages, pDCs, and mDCs categorized as innate, and CD4+ and CD8+ T cells and B cells as adaptive (**B**). * = $P < 0.05$ versus JDM. **C,** Innate versus adaptive immune cell enrichment from skin microarrays of the same patients. Bars show the mean ± SD. **D**, Bar graph showing individual patient sample immune cell composition. IMC = imaging mass cytometry (see Figure 1 for other definitions).

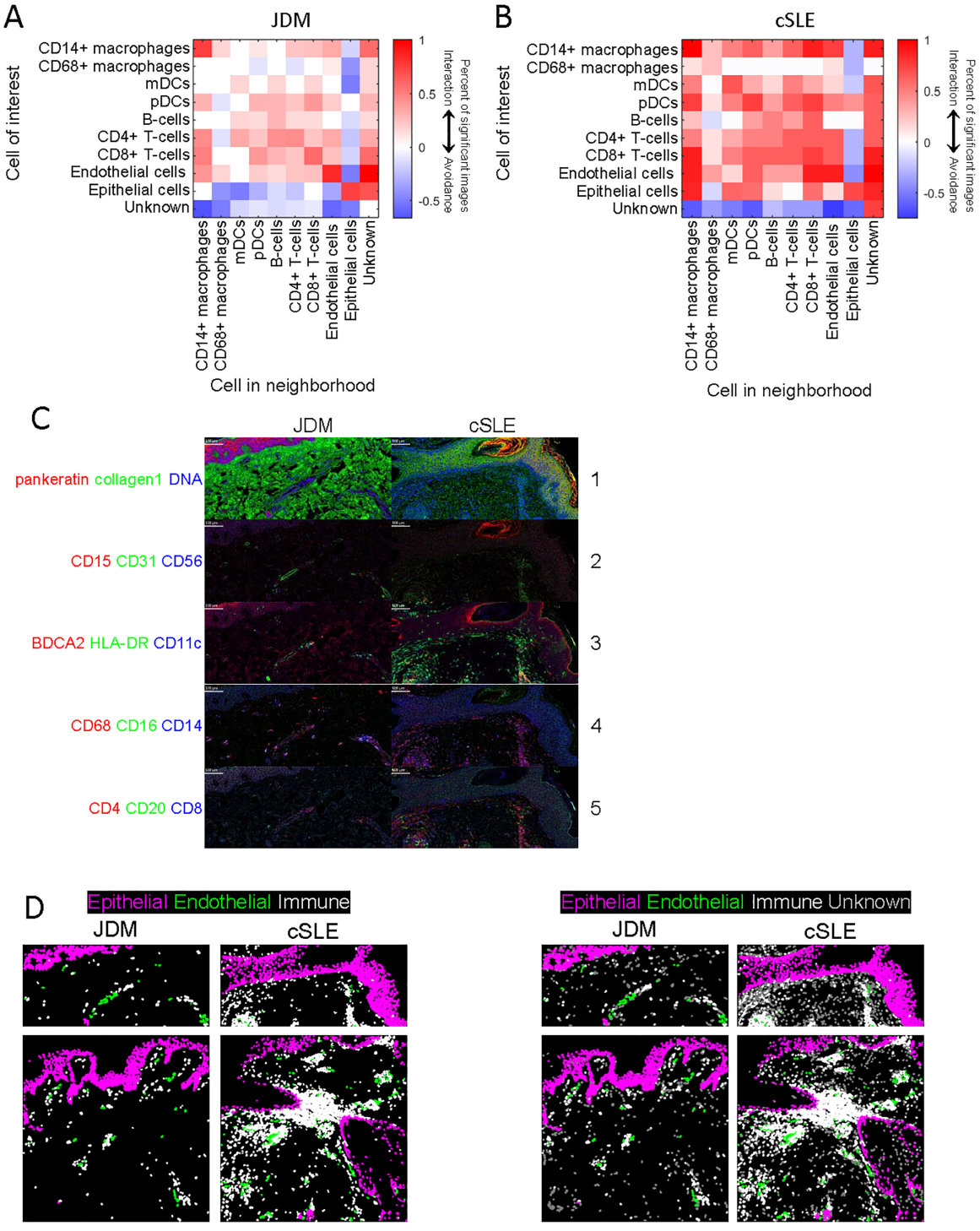

Figure 3. Cell–cell interactions in JDM and cSLE lesional skin using neighborhood analysis. **A** and **B**, Heatmaps highlighting differences in cell–cell interactions in JDM (**A**) and cSLE (**B**) lesional skin using permutation tests for neighborhood analysis. Red represents a positive association ($P < 0.01$), white represents an insignificant association, and blue represents a negative association ($P < 0.01$). **C**, Multiplexed images demonstrating staining for cellular markers in JDM and cSLE skin samples, represented by different colors in panels 1–5. Bars = 100 μm. **D**, Demonstration of increased epithelial cell–immune cell interaction in cSLE compared to JDM lesional skin, and an overall more prominent endothelial cell–immune cell interaction in JDM. Magnification is the same as in **C**. See Figure 1 for definitions.

followed by CD8+ T cells (21%), pDCs (13.5%), B cells (12.2%), CD68+ macrophages (9.3%), CD4+ T cells (8.4%), and mDCs (5.6%).

Higher innate immune signature, relative to adaptive immune signature, demonstrated by juvenile DM skin lesions compared to cSLE skin lesions. Upon grouping

cells into an innate immune (macrophages and DCs) and adaptive immune (T cells and B cells) categorization, juvenile DM demonstrated a stronger innate immune signature compared to cSLE (74.9% versus 58.4%) (Figure 2B and Supplementary Table 2B, https://onlinelibrary.wiley.com/doi/10.1002/art.42283). The increased innate immune signature in juvenile DM skin lesions observed using IMC was also seen at the transcriptional level using xCell cell types enrichment analysis (see Patients and Methods) (11) (Figure 2C).

Clinical cohort characteristics and inflammatory heterogeneity within individual skin lesions. A high degree of variability existed in immune cell composition within individual patient skin lesions (Figure 2D), and these cellular data are accompanied by clinical and histopathologic data in Supplementary Table 1 (https://onlinelibrary.wiley.com/doi/10.1002/art.42283). While all juvenile DM skin lesions consistently had macrophages composing ≥30% of inflammatory infiltrate, the degree of T cell infiltrate varied (Figure 2D). The 2 juvenile DM patients (referred to in Figure 2 as JDM1 and JDM5) with skin-predominant disease at diagnosis had more T cell infiltrate, although these patients were also treatment-naive. The 2 juvenile DM patients (JDM2 and JDM9) with prolonged disease duration at biopsy (5–6 versus 0 years for the rest of the juvenile DM cohort) demonstrated predominant innate immune signatures, although both were also receiving immunosuppressive therapy with at least methotrexate (Figure 2D). In the cSLE patient with isolated cutaneous lupus at biopsy and discoid lupus phenotype (referred to in Figure 2 as SLE21A), B cells predominated in the skin lesion (14) (Figure 2D and Supplementary Table 1).

Endothelial cell–immune cell interactions characterize juvenile DM skin lesions. Using neighborhood analysis to examine immune cell–immune cell interactions, juvenile DM demonstrated fewer overall interactions between immune cells (Figures 3A–C). In cSLE, pDCs and mDCs exhibited more interaction with other immune cells compared to juvenile DM. In both juvenile DM and cSLE, CD68+ macrophages had the least interaction with other cells. Both CD4+ and CD8+ T cells demonstrated interaction with most immune cells in both juvenile DM and cSLE (Figures 3A–C).

We then examined predicted cell–cell interactions with 2 important skin populations within both diseases: endothelial and epithelial cells. Intriguingly, cSLE skin displayed a higher number of positive cell–cell interactions for our identified immune cell populations with both epithelial and endothelial cells (Figures 3A and B). In contrast, juvenile DM skin demonstrated a striking contrast between endothelial and epithelial cell–immune cell interactions, with positive endothelial cell–immune cell interaction and epithelial cell–immune cell avoidance (Figure 3A). Of note, in juvenile DM, CD14+ macrophages displayed the strongest interaction with endothelial cells. This finding of endothelial cell–immune cell interaction and epithelial cell–immune cell avoidance in juvenile DM was confirmed by visualizing spatial distribution of labeled cells, with a lack of noted proximity between immune and epithelial cells near the dermoepidermal junction but the presence of immune cells surrounding vasculature (Figure 3D). These data suggest that pathologic immune education in skin may involve not only immune cell–immune cell interactions, but that the epidermis may play a stronger role in pathogenic responses in cSLE compared to juvenile DM.

DISCUSSION

In this study, we provide the first characterization of immune cell populations and cell–cell interactions within pediatric dermatomyositis and lupus lesional skin using IMC. We identified a more prominent innate immune signature in juvenile DM as compared to cSLE skin. While CD14+ and CD68+ macrophages were the most numerous immune cells composing juvenile DM skin lesions, cSLE had a more even distribution of innate and adaptive immune cells. Skin lesions in cSLE demonstrated denser inflammatory cell infiltrate, notably with higher absolute numbers of CD14+ macrophages, pDCs, and CD8+ T cells and an overall higher number of cell–cell interactions compared to juvenile DM. Unlike cSLE, juvenile DM patients had few B cells in skin lesions. When considering cell–cell interactions in juvenile DM, compared to cSLE, juvenile DM patients displayed a prominent endothelial cell–immune cell interaction and no significant epithelial cell–immune cell interactions with identified cell populations.

The use of IMC in this study allowed us to define immune cell populations in juvenile DM and cSLE with more granularity than previously possible. Our finding that CD14+ macrophages comprise the top immune cell population in juvenile DM skin is consistent with IMC data recently reported by Patel et al on adult DM lesional skin (5). In that study, CD14+ macrophages were also found to positively associate with skin disease activity (5). In contrast to that study, mDCs were not as prominent in juvenile DM skin within our cohort. A direct comparison of all cell populations identified between our cohort and the published adult DM cohort is challenging, given the use of different marker panels and the presence of unidentified clusters in both studies. There is likely more macrophage diversity in both juvenile DM and cSLE skin than we were able to identify using our marker panel. While we identified 2 macrophage populations, 4 populations were identified in adult DM skin, including CD14+, CD14+CD16+, phosphorylated stimulator of IFN genes–positive (p-STING+), and MAC387+ macrophages. The p-STING+ macrophage population in adult DM also displayed CD68 coexpression and may be included within our identified CD68+ macrophage population.

The finding of a stronger innate immune signature versus adaptive immune signature in juvenile DM compared to cSLE at both transcriptional and protein levels suggests differences in

pathophysiology. Consistent with this, our previously published gene expression data identified a stronger type II IFN signature in cSLE skin lesions compared to juvenile DM skin lesions (11), supporting a larger role for adaptive immunity in cSLE. While innate immunity likely plays an important role in both juvenile DM and cSLE pathogenesis, the influence of innate immune mechanisms in regulation of cutaneous inflammation in juvenile DM as compared to cSLE has not been well studied to date. In juvenile DM, skin disease as compared to muscle disease is often more resistant to treatment, and we may need to consider different treatment targets, potentially targeting the innate immune system, to improve skin disease and underlying vasculopathy.

The CD4:CD8 T cell ratio in juvenile DM skin within our study (0.9) was more equivalent than that identified in adult DM to date (5). In contrast, we identified a much lower CD4:CD8 T cell ratio in cSLE (0.4). The finding of an overall higher number of CD4+ T cells coexpressing CD11c in juvenile DM skin (cluster 17; Figures 1B and C) suggests that these cells could potentially represent invariant natural killer T (iNKT) cells or another activated T cell population (13). Invariant NKT cells represent less studied immune cells that bridge innate and adaptive immune response and serve as regulators of the immune response through secretion of cytokines, including IFNγ, and play a role in cytotoxicity (13).

Our data suggest a striking contrast of positive endothelial cell–immune cell and avoidant epithelial cell–immune cell interactions in juvenile DM skin, supporting the notion that an underlying vasculopathic process occurs in skin, reflected clinically by pronounced nailfold capillary abnormalities that we often see in children. Previous reports that DM is characterized by marked expression of *MXA*, an IFN-inducible gene, in endothelial cells, whereas in SLE, *MXA* expression is often more prominent near areas of interface dermatitis would also align with our data (15). We do not fully understand the mechanisms connecting IFN to disease pathogenesis. Through further study of the relation of IFN to endothelial cell–immune cell interactions in juvenile DM, we may uncover disease-specific mechanisms.

It is important to emphasize that our findings should be interpreted in the context of markers present on our IMC panel. Other immune cells that potentially play important roles in juvenile DM can be included in future IMC antibody panels to further characterize immune cell subtypes and their variations in inflammatory cytokine and chemokine expression. Our study was also limited by small sample size and clinical heterogeneity within patient phenotypes and treatment status. Given retrospective data collection, we lacked the ability to collect detailed skin or systemic disease activity measures or paired fresh tissue or blood. Future analysis will include fresh tissue with paired blood to allow for in-depth clinical/mechanistic characterization.

Overall, the results of this study pave the way to better understand immunophenotypes in pediatric myositis and lupus and lend insight into the use of molecular and single-cell signatures to target treatment based on predominant cell types in lesional tissue.

ACKNOWLEDGMENTS

We express our many thanks to the myositis and lupus patients for generously sharing their samples for our work. We thank the Cure Juvenile Myositis and Rheumatology Research Foundation for supporting our work. We also thank the support of the University of Michigan CyTOF Core and the George M. O'Brien Michigan Kidney Translational Research Core Center (P30DK081943).

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. Turnier 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. Turnier, Wen, Kahlenberg.
Acquisition of data. Turnier, Yee, Madison, Rizvi, Wen, Kahlenberg.
Analysis and interpretation of data. Turnier, Yee, Berthier, Wen, Kahlenberg.

REFERENCES

1. Smith ES, Hallman JR, DeLuca AM, et al. Dermatomyositis: a clinicopathological study of 40 patients. Am J Dermatopathol 2009; 31:61–7.
2. Wenzel J, Tuting T. An IFN-associated cytotoxic cellular immune response against viral, self-, or tumor antigens is a common pathogenetic feature in "interface dermatitis." J Invest Dermatol 2008;128: 2392–402.
3. Christen-Zaech S, Seshadri R, Sundberg J, et al. Persistent association of nailfold capillaroscopy changes and skin involvement over thirty-six months with duration of untreated disease in patients with juvenile dermatomyositis. Arthritis Rheum 2008;58:571–6.
4. Giesen C, Wang HA, Schapiro D, et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat Methods 2014;11:417–22.
5. Patel J, Maddukuri S, Li Y, et al. Highly multiplexed mass cytometry identifies the immunophenotype in the skin of dermatomyositis. J Invest Dermatol 2021;141:2151–60.
6. O'Gorman WE, Kong DS, Balboni IM, et al. Mass cytometry identifies a distinct monocyte cytokine signature shared by clinically heterogeneous pediatric SLE patients. J Autoimmun 2017. E-pub ahead of print.
7. Lundberg IE, Tjärnlund A, Bottai M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Arthritis Rheumatol 2017;69:2271–82.
8. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
9. Levine JH, Simonds EF, Bendall SC, et al. Data-driven phenotypic dissection of AML reveals progenitor-like cells that correlate with prognosis. Cell 2015;162:184–97.
10. Schapiro D, Jackson HW, Raghuraman S, et al. histoCAT: analysis of cell phenotypes and interactions in multiplex image cytometry data. Nat Methods 2017;14:873–6.
11. Turnier JL, Pachman LM, Lowe L, et al. Comparison of lesional juvenile myositis and lupus skin reveals overlapping yet

unique disease pathophysiology. Arthritis Rheumatol 2021;73: 1062–72.

12. Aran D, Hu Z, Butte, AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 2017;18, 220.
13. Qualai J, Li LX, Cantero J, et al. Expression of CD11c is associated with unconventional activated T cell subsets with high migratory potential. PLoS One 2016;11:e0154253.
14. Abernathy-Close L, Lazar S, Stannard J, et al. B cell signatures distinguish cutaneous lupus erythematosus subtypes and the presence of systemic disease activity. Front Immunol 2021;12: 775353.
15. Magro CM, Segal JP, Crowson AN, et al. The phenotypic profile of dermatomyositis and lupus erythematosus: a comparative analysis. J Cutan Pathol 2010;37:659–71.