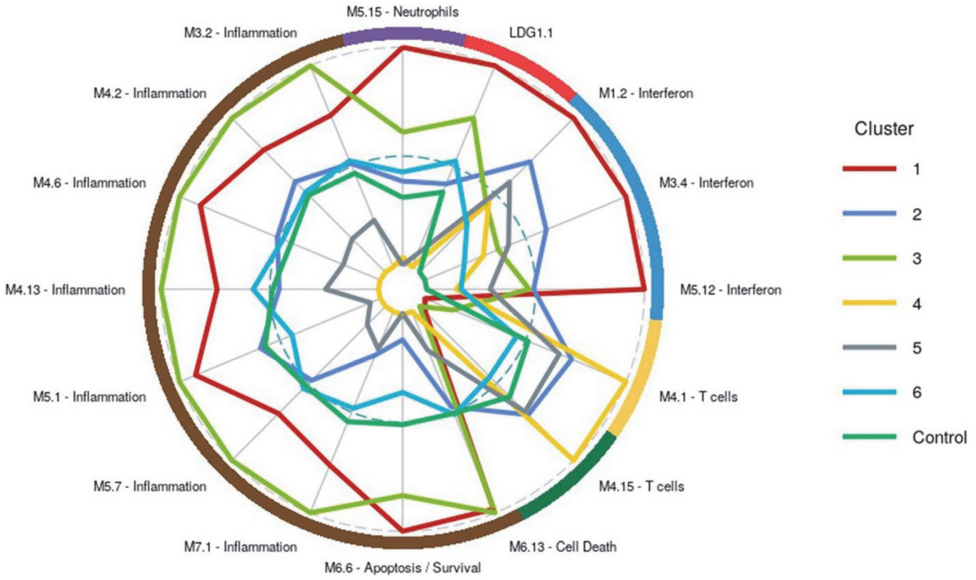

Abstract 801 Figure 2 Molecular profiles of six CLE patient clusters. Radar plots showed modified z- scores of relative gene expression module scores in each of the molecularly-defined patient cluster indicated by legend. Cluster 1 had increased interferon (M1.2, M3.4, M5.12) module scores. Clusters 1 and 3 had increased inflammation (M2.2, M4.2, M4.6, M4.13, M5.1, M5.7, and M7.1), neutrophil (M5.15), low density granulocyte (LDG1.1), cell death (M6.13), and apoptosis (M6.6) module scores. Clusters 2, 4, and 5 had elevated T cell (M4.1 and M4.15) module scores.

feron-related proteins, neutrophils, and cell death processes could be driving the inflammatory response in these subgroups. Three different clusters had a predominant T cell signature, which were supported by lymphocyte counts (figure 2).

Conclusion Our data support a diverse molecular profile in CLE that further adds to the clinical variations of this skin disease, and may affect disease course and treatment selection. Future studies with a larger and diverse CLE patient cohort are warranted to confirm these findings.

Cutaneous lupus

802 LYMPHATIC DYSFUNCTION IN LUPUS CONTRIBUTES TO CUTANEOUS PHOTOSENSITIVITY AND LYMPH NODE B CELL RESPONSES

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Patients with systemic lupus erythematosus (SLE) are photosensitive, developing skin inflammation with even ambient ultraviolet radiation (UVR), and this cutaneous photosensitivity can be associated with UVR-induced flares of systemic disease, with increased autoantibodies and further end organ injury.

Mechanistic insight into the link between skin disease and autoimmunity is limited. Signals from skin are transmitted directly to the immune system via lymphatic vessels, and here we show evidence for potentiation of UVR-induced lymphatic flow dysfunction in SLE patients and murine models. Improving lymphatic flow by manual lymphatic drainage (MLD) or with a transgenic model reduces both cutaneous photosensitivity and lymph node B cell responses. Mechanistically, improved flow restrains B cell responses by activating a fibroblastic reticular cell-monocyte axis. Our results point to a lymphatic flow- lymph node stromal axis as a link between photosensitivity and autoimmune responses and as a therapeutic target in lupus, have implications for understanding skin-immune interactions in other diseases such as skin cancer, and suggest the possibility of MLD as an immediately available, cost- effective adjunctive treatment in lupus and related diseases.

803 PLASMACYTOID DENDRITIC CELLS ARE NOT MAJOR PRODUCERS OF TYPE 1 INTERFERONS IN CUTANEOUS LUPUS

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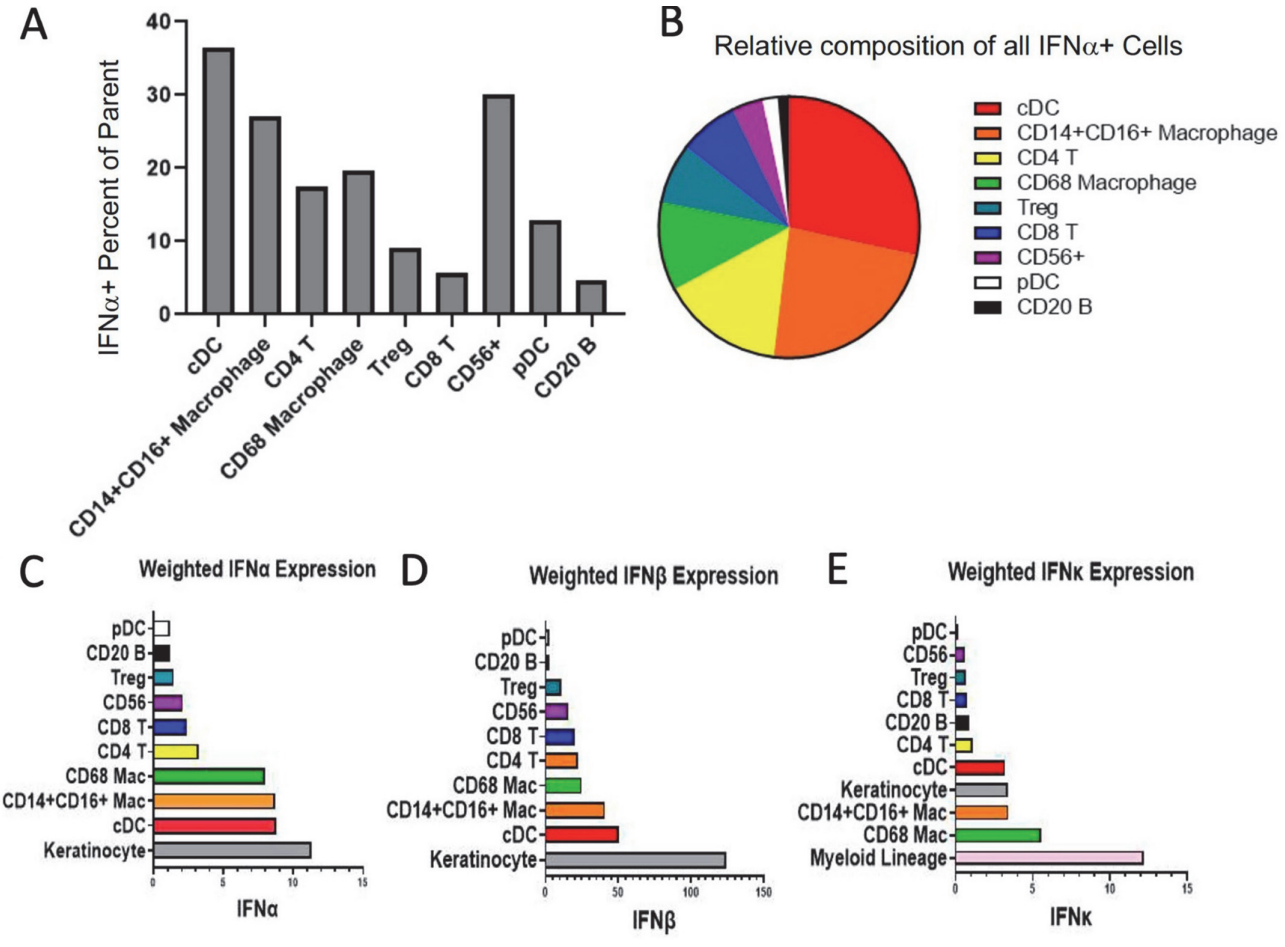
10.1136/lupus-2022-lupus21century.51

Background Type 1 interferons (IFN-1) are major drivers of disease activity in systemic (SLE) and cutaneous lupus erythematosus (CLE). Plasmacytoid dendritic cells (pDCs) are the major producers of IFN-1 during viral infection. Therefore, pDCs have been hypothesized to be the primary IFN-1

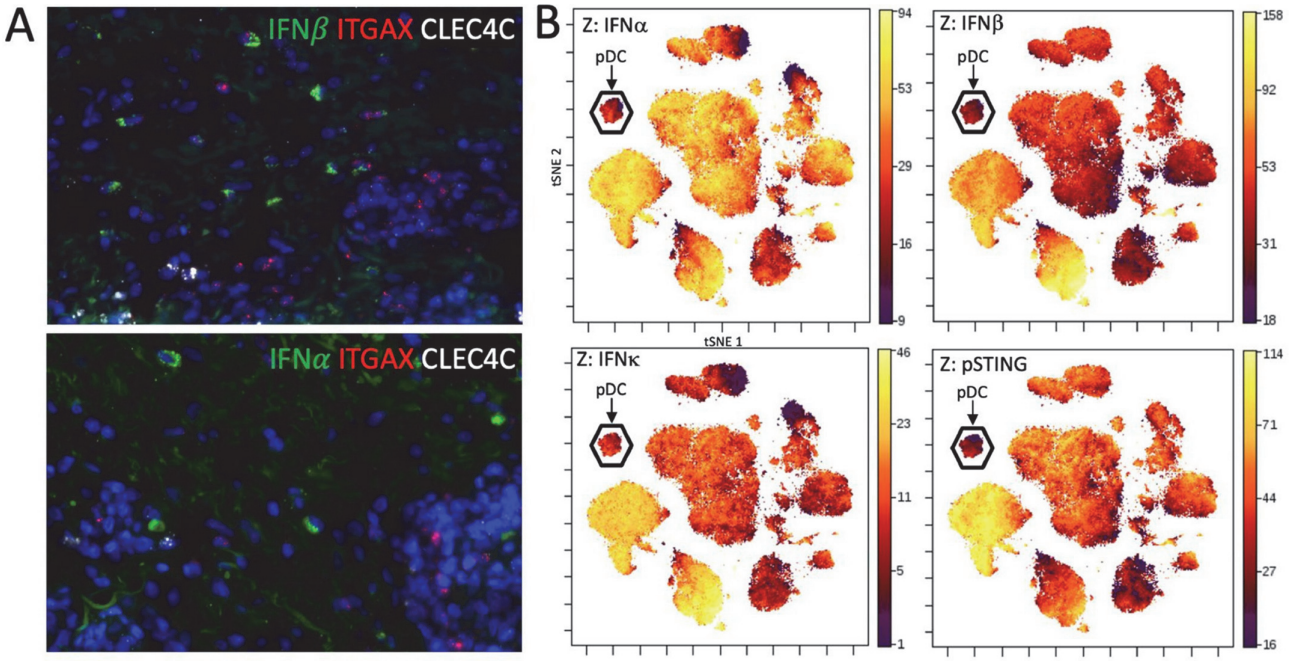
producing cell in lupus. IFN-1 production by pDCs has not been extensively studied due to reliance on interferon gene signatures as a proxy for IFN-1 levels as well as a reliance on pDCs from healthy controls for *in vitro* studies. However, it has been known that pDCs are reduced in the circulation in SLE and have also been more recently shown to have an exhausted phenotype, suggesting that pathogenic pDCs may be located in the end organs.

Methods Well characterized CLE patients were recruited from a prospective database. We performed imaging mass cytometry on archived treatment naïve CLE skin (n=48) and a subset were also used for mRNA *in situ* hybridization. Fresh 4mm punch biopsies (n=3) and blood (n=4) were obtained from CLE patients and healthy controls (n=3) for multiplexed flow cytometry or cytometry by time of flight (CyTOF).

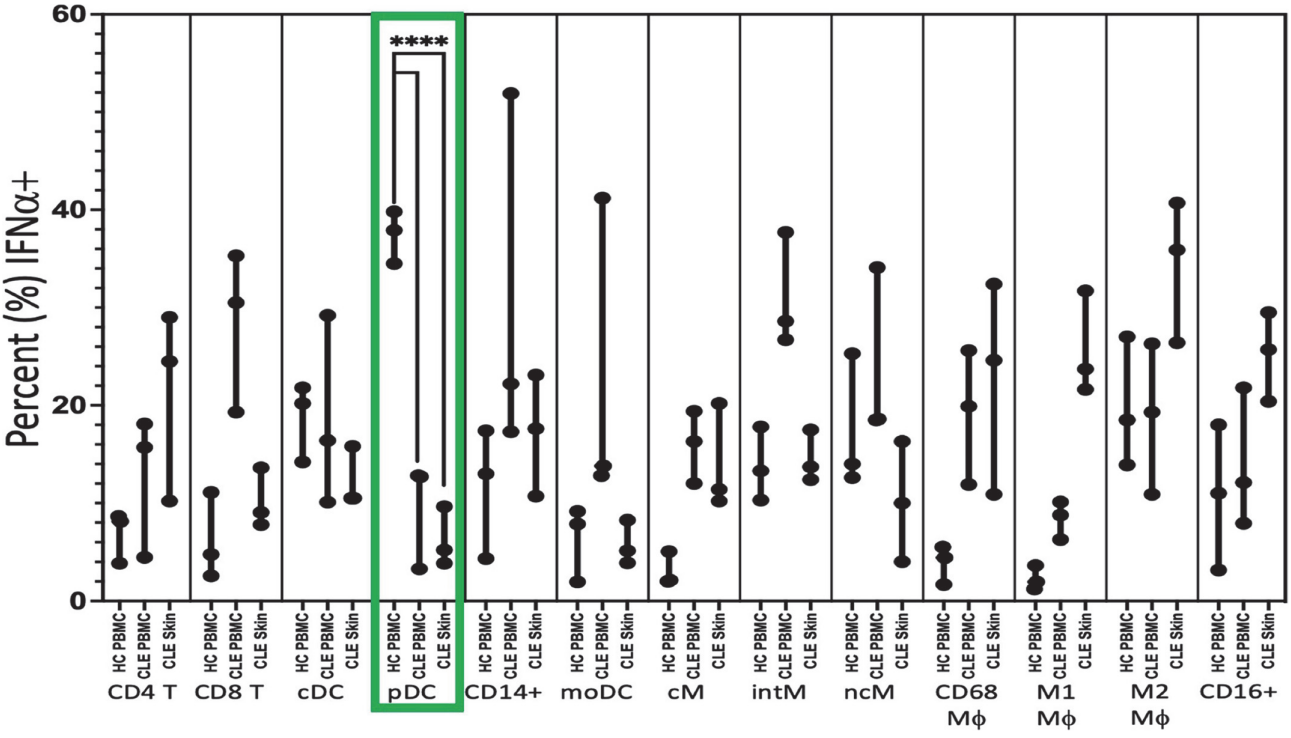
Results In lesional CLE skin, only 8.5% (IQR 0.0-22.22) of pDCs were IFNα+ (figure 1A,B). Across all CLE biopsies, pDCs were the second lowest contributor of absolute IFNα + cells (Median 1; IQR 0-4.5), with B lymphocytes being the smallest contributor. Classic dendritic cells (cDCs) and macrophages (Mϕ) were the largest relative and absolute contributors of IFNα in CLE (figure 1C). For IFNβ, only 16.7% of pDCs were positive, compared to 60.7% of CD14+CD16+ Mϕ. pDCs were also the second lowest contributors of IFNβ+ cells (Median 1; IQR 0-7), with B lymphocytes again being the lowest (figure 1D). For IFNκ, the largest contributor was CD68 Mac, CD14+CD16+ Mac, and cDCs (figure 1E). IFN-1 mRNA was detected in ITGAX (CD11c) positive cDCs but we did not identify colocalization of pDC- specific gene, CLEC4C (BDCA2), and IFN-1 mRNA (figure 2). Flow cytometry on leukocytes eluted from fresh lesional biopsies showed a median of 5.2% of pDCs were positive for IFNα (IQR 3.85 – 9.64) which was significantly lower than the median of 37.9% of HC PBMC pDCs (34.5 – 39.8) ($p<0.0001$). In comparison, other leukocytes in CLE skin displayed greater positivity for IFNα such as CD4 T cells (Median, IQR) (24.5%, 10.2 – 29), CD14+ cells (17.6%, 10.7 – 23.1), Classical Monocytes (CD14++CD16-) (11.4%, 10.2 – 20.2), Intermediate Monocytes (CD14++CD16+) (13.7%, 12.4 – 17.5), CD68 + Macrophages (24.6%, 10.9 – 32.4), M1 Macrophages



Abstract 803 Figure 1 A) IFNα+ Percent of Parent for cell types identified in CLE skin shown in order of descending absolute contribution of IFNα + cell counts in CLE. 12.83% of pDCs are positive for IFNα. B) Composition of IFNα+ cells (absolute counts) across all aggregated CLE biopsies. C) Weighted (cell count x MPI) IFNα expression. D) Weighted IFNβ expression. E) Weighted IFNκ expression with myeloid lineage consisting of CD68 Mac, CD14+CD16+ Mac, and cDCs revealing dominant IFN-1 contribution from myeloid cells followed by keratinocytes.



Abstract 803 Figure 2 A) Representative images of mRNA in situ hybridization of IFNα and IFNβ mRNA with markers for conventional dendritic cells (ITGAX) and plasmacytoid dendritic cells (CLEC4C). No observable overlap of type 1 interferon mRNA and CLEC4C was seen. B) tSNE plot of PBMCs from a CLE patient stained and acquired by CyTOF. Plasmacytoid dendritic cells, identified principally on CD123 expression, demonstrate little type 1 interferon staining as well as activation of the STING pathway (a signaling molecule upstream of type 1 interferon transcription) on the Z axis.



Abstract 803 Figure 3 Flow cytometry of leukocytes eluted from CLE skin demonstrating significantly fewer IFNα+ plasmacytoid dendritic cells (pDCs) in cutaneous lupus erythematosus (CLE) skin (Median 5.2%, p<0.0001) and peripheral blood mononuclear cells (PBMCs) (12.7%, p<0.0001) than healthy control PBMCs (37.9%). Abbreviations: cDC: classical dendritic cell; pDC: plasmacytoid dendritic cell; moDC: monocyte derived dendritic cell; cM: classical monocyte; intM: intermediate monocyte; ncM: nonclassical monocyte; Mf: macrophage.

(CD68+CD163-) (23.7%, 21.6 – 31.7), M2 Macrophages (CD68+CD163+) (35.9%, 26.4 – 40.7), and CD16+ cells (25.7%, 20.4 – 29.5) (figure 3). Further verification using a Z-axis overlay of intracellular markers on tSNE plots of immune cell clusters identified by CyTOF confirmed low expression of IFN-1and the interferogenic pathway, phosphorylated stimulator of interferon genes (pSTING), in the pDCs (figure 2B).

Conclusions Taken together, these findings suggest pDCs may not play the central role in CLE as major IFN-1 producers and myeloid cells are larger contributors of IFN-1 in numbers and as a percent. pDCs may have a pathogenic role in CLE through IFN-1-independent mechanisms.

901 SYMPOSIUM: MOLECULAR BIOLOGY AND IMMUNOLOGY OF PAIN

Stephen G Waxman. *Depts. of Neurology, Neuroscience and Pharmacology, Yale Medical School and VAMC West Haven CT Chasing Men on Fire: Genes regulating pain sensibility in humans*

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Given the need for more effective treatments, there is a pressing need for a better understanding of chronic pain, including pain in SLE. Discovery of peripheral sodium channels (Nav1.7, Nav1.8, Nav1.9) and of pain resilience genes (KCNQ2, KCNQ3) opens up the possibility of targeting peripheral generators of pain (primary sensory neurons) without affecting the heart or CNS, thus enabling new and more effective pain therapies devoid of CNS side effects or addictive potential.

In this lecture I will review several lines of recent progress. Molecular genetics has validated peripheral sodium channels Nav1.7, 1.8 and 1.9 as strong drivers of firing of peripheral pain-signaling neurons and thus of human pain. Building upon this, recent studies have begun to provide proof of concept that Nav1.7-specific blockers can reduce pain. In parallel, genomically-guided pharmacogenomic approaches indicate that the goal of patient-specific, personalized pain therapy is an achievable objective.

Molecular genetics has also begun to identify pain resilience genes, pointing toward another set of molecular targets for pain therapy.

While there is still a lot of work to do, the goal of more effective, non-addictive treatments for chronic pain appears to be in sight.

Lay summary We are beginning to understand, in exciting detail, the molecular drivers of human pain. This new knowledge is bringing us closer to the goal of more effective, non-addictive treatments for chronic pain.

Molecular Biology of Lupus

902 LOSS-OF-FUNCTION VARIANTS IN *SAT1* CAUSE X-LINKED CHILDHOOD-ONSET SYSTEMIC LUPUS ERYTHEMATOSUS

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Objectives Families that contain multiple siblings affected with childhood-onset of systemic lupus erythematosus (SLE) likely have strong genetic predispositions. We performed whole-exome sequencing (WES) to identify familial rare risk variants and to assess their effects in lupus.

Methods Sanger sequencing validated the two ultra-rare, predicted pathogenic risk variants discovered by WES and identified additional variants in 562 additional SLE patients. Effects of a splice site variant and a frameshift variant were assessed using a Minigene assay and CRISPR/Cas9-mediated knock-in (KI) mice, respectively.

Results The two familial ultra-rare, predicted loss-of-function (LOF) *SAT1* variants exhibited X-linked recessive Mendelian inheritance in two unrelated African-American families. Each LOF variant was transmitted from the heterozygous unaffected mother to her two sons with childhood-onset SLE. The p. Asp40Tyr variant affected a splice donor site causing deleterious transcripts. The young hemizygous male and homozygous female *Sat1*p.Glu92Leufs*6 KI mice spontaneously developed splenomegaly, enlarged glomeruli with leukocyte infiltration, proteinuria and elevated expression of type I interferon inducible genes. *SAT1* is highly expressed in neutrophils and