Insights into the cellular basis of skin inflammation in systemic autoimmune rheumatic diseases from single-cell technologies

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ABSTRACT
Cutaneous inflammation is a common feature of several systemic autoimmune rheumatic diseases (SARDs) including systemic lupus erythematosus (SLE), undifferentiated connective tissue disease (UCTD), mixed connective tissue disease (MCTD) and dermatomyositis (DM) but is less common in other SARDs such as primary Sjogren's syndrome (pSS). It is important to understand whether the pathophysiological processes underlying skin inflammation are different or shared between SARDs to develop targeted therapies. This review will discuss commonalities and differences between inflammatory skin disease in SARDs focusing on histopathology and describe newer insights obtained from single-cell technologies.

Introduction

Skin inflammation is prominent in systemic autoimmune rheumatic diseases (SARDs) including systemic lupus erythematosus (SLE) and dermatomyositis (DM). It is currently unclear to what extent the pathogenesis of skin inflammation is shared between SARDs. Single-cell technologies offer the opportunity to resolve the mechanism of skin inflammation at the single cell level and identify both unique and shared pathways. This narrative review will discuss the histological appearance of skin lesions in SARDs and describe recent insights gained from single-cell analyses.

Histopathology of skin in SARDs

Clinical management of skin involvement in patients with SARDs is often supported by routine clinical histopathology and immunohistochemistry. These methods assist diagnosis and disease classification through identification of characteristic structural changes, leukocyte infiltration, and immunoglobulin and/or complement deposits (1, 2). Whilst these modalities are relatively blunt, they have highlighted several shared and distinct features between SARDs.

Cutaneous lupus erythematosus (CLE) is an autoimmune, inflammatory skin disorder encompassing distinct clinical and histological subtypes. Whilst some histological changes are relatively specific to acute (ACLE), subacute (SCLE) and chronic CLE (CCLE), other features such as epidermal acanthosis, basal vacuolisation and keratinocyte (KC) necrosis are shared (Table I). ACLE can be localised or generalised and occurs in 50–60% of SLE patients (3, 4). ACLE is typically photosensitive and non-scarring, often occurring in sun-exposed areas. Localised ACLE occurs in up to half of patients at diagnosis (4-6), and includes the malar ("butterfly") rash. Generalised ACLE usually presents as a widespread photodermatosis. SCLE typically has mono- or poly-cyclic, non-scarring lesions which can be annular, or papulosquamous in nature although these can occur concurrently (7, 8). SCLE is often photosensitive occurring on sun-exposed areas. Discoid lupus erythematosus (DLE) is a scarring form of CCLE and occurs in up to 8.4% of SLE patients (9). DLE has hyper- or hypo-pigmented, scaly, plaque-like lesions frequently on the scalp (leading to alopecia), face, ears, neck and back (7, 8, 10). DLE can also affect mucous membranes, commonly the oral cavity although other sites can be involved. Despite differences in clinical appearance, distribution and the degree of scarring, there are similar histological features in SCLE and DLE, including follicular plugging, mucin deposition and immunoglobulin G (IgG) deposition at the dermo-epidermal junction (DEJ), which has led to the suggestion that SCLE and DLE exist on a spectrum, related to severity and duration, rather being discrete entities (see Table I and Fig. 1) (7). Although some of these features occur in ACLE, dermal oedema may be more common in ACLE, and DEJ Ig is more likely to be IgM than IgG (7). It is likely, therefore, that ACLE is pathologically distinct from SCLE or DLE.

Some histological findings may be shared between different SARDs. Gottron's papules (hyperkeratotic erythematous lesions typically over the extensor surfaces of the metacarpophalangeal and interphalangeal joints), heliotrope rash (red/purple eyelid discolouration) and the shawl sign (erythematous lesions over the neck, upper back, chest, and shoulders) are cutaneous features of DM. Histologically, Gottron's papules have dermal mucin deposits and basement membrane thickening similar to SCLE, but differ in other aspects; *e.g.* infiltrating lymphocytes are typically peri-vascular in DM but peri-eccrine in SCLE (11, 12). Annular erythema (AE) is an uncommon rash, described clinically similar to SCLE, typically characterised as a polycyclic annular and indurated erythematous rash, seen only in 9% of primary Sjögren's syndrome (pSS) patients but can also occur due to infection, medications, or chronic conditions such as liver or thyroid disease. In pSS, AE appears predominantly in Asian patients and like SCLE, is associated with anti-Ro/SSA antibodies (13). However, histologically, AE lesions have absent BM thickening, follicular plugging and epidermal atrophy (14).

Systemic sclerosis (SSc) is an autoimmune disease typified by progressive thickening of the skin, classified as limited when only affecting the areas distal to the forearms, lower limbs or face, with diffuse disease defined by the presence of proximal skin thickening (15). It is clinically distinct from other SARDs with prominent vascular damage, collagen deposition and fibrosis in addition to histological changes also observed in other SARDs, such as lymphocytic infiltration, dermal mucin deposition and pigment incontinence (15, 16), although in early disease, histological changes may be non-specific (17).

Approach to single cell analysis of human skin

Compared to bulk RNA sequencing techniques, single cell RNA sequencing (scRNA-seq) can identify gene expression at an individual cell level rather than as an 'average' across a whole tissue sample. Using unbiased clustering, the transcriptional profile of individual cells can be used to identify individual cell types within a population and novel subsets that occur in disease. Cell subpopulations, or transcriptional differences within populations can be compared between anatomical sites, disease subtypes and even cellular response to therapy.

Briefly, in scRNA-seq, tissue is digested to produce a single cell suspension, from which RNA is reverse transcribed and amplified to generate a library (18). These libraries are sequenced, and fragments aligned to the human genome to identify transcripts or genes. For data visualisation, poly-dimensional data need be reduced into 2 or 3-dimensions using matrix factorisation methods such as a Principal Component Analysis (PCA) or t-distributed stochastic neighbour embedding (t-SNE) (19, 20)

In healthy human skin, all principal constituent cell types can be identified using scRNA-seq including keratinocytes (KC), fibroblasts (FB), vascular and lymphatic endothelial cells (EC), melanocytes and immune cells (both myeloid and lymphocytic) (21).

Single cell analysis of healthy skin

Keratinocyte and fibroblast heterogeneity

In healthy skin, across 3 anatomical sites (foreskin, trunk, and scalp), Cheng *et al.* (2018*)* (22) identified up to 8 distinct keratinocyte (KC) clusters defined principally by stage of differentiation. Basal KC predominantly expressed *KRT5* and *KRT14* but during differentiation and maturation, keratin production switched to *KRT1*/*KRT10* (correlating to suprabasal and spinous cells). In granular cells, expression of late differentiation markers such as *LOR* (loricin) and *FLG* (filaggrin) was increased.

Sub-clustering of basal KC identified 3 groups characterised by expression of *CXCL14*/*DMKN*, *CCL2*/*IL1R2* and *AREG*/*EGRF* respectively. The *AREG*/*EGFR* group were only present in foreskin, demonstrating that unique KC populations may exist at specific anatomical sites, which may have direct relevance for skin inflammation. For example, a subpopulation of scalp KCs had increased expression of inflammatory *S100* genes which the authors propose may explain the predilection of psoriasis for the scalp.

Classically, it was considered that there are two subtypes of dermal fibroblast (FB) (papillary and reticular) with distinct functions. However, Philipeos *et al.* (2018) identified 5 FB subclusters in a single donor (23). Whilst some cell populations, such as those defined by *COL6A5* expression, were only present in the superficial dermis, other populations, (*e.g.* those expressing *MFAP5*) were spread throughout the dermis (23). A further study of 3 donors identified 6 transcriptionally distinct dermal FB subsets and confirmed that conventional FB markers were present in all 6 of these FB subtypes; demonstrating a disconnect between histological techniques and cell function (24). Novel FB subsets may occur in inflammation, He *et al.* (2020) identified a *COL6A5+*/*COL18A1+* FB subset expressing CCL2 and CCL19 in lesional AD skin (24).

Myeloid cells

Within the myeloid cluster of cells (defined as LIN-/HLA-DR+), Xue *et al.* (2020) identified 3 macrophage and 6 dendritic cell (DC) subclusters (25). Some traditional markers for macrophages or DC (*e.g. LGAL3* and *CD123*) were not specific to these cells and occurred in non-myeloid populations. The 3 macrophage subsets were defined by expression of *CCR1*, *TREM2* and *MARCO*. Within the DC cluster, classical DCs (cDCs), and Langerhan's cells were identified along with a small subset strongly expressing *LAMP3*, proposed to be a mature DC subset.

Vascular cell heterogeneity

Significant heterogeneity in the endothelium exists between different tissues. In scRNA-seq datasets, dermal EC form a discrete cell cluster, tran-

Table I. Histological findings of autoimmune inflammatory skin disease.

Acute cutaneous lupus (7, 10, 72)	Sub-acute cutaneous lupus (7, 72, 73)	Chronic cutaneous lupus/discoid lupus (7, 72, 73)
Epidermal atrophy/acanthosis Mild basal degeneration Dermal oedema Positive direct IF for IgM at the DEJ Lymphocytic/neutrophilic infiltrates Pigmentary incontinence Basal vacuolisation Keratinocyte necrosis Interface dermatitis	Epidermal acanthosis Interface dermatitis Follicular plugging Superficial and deep lymphocytic infiltrate Thickened BM Dermal mucin deposition IgG deposition at the DEJ Peri-eccrine lymphocytic infiltrate Pigmentary incontinence Basal cell vacuolisation Keratinocyte necrosis	Epidermal acanthosis Interface dermatitis Follicular plugging Superficial and deep lymphocytic infiltrate Thickened BM Dermal mucin deposition IgG deposition at the DEJ Peri-eccrine lymphocytic infiltrate Pigmentary incontinence Basal cell vacuolisation Keratinocyte necrosis
Dermatomyositis (12, 74)	**Sjögren's syndrome (Annular erythema) (14, 75)**	**Systemic sclerosis (15)**
Hyperkeratosis Dyskeratotic keratinocytes Epidermal acanthosis Dermal mucin deposition Perivascular inflammatory cell infiltrates Basal cell vacuole degeneration Vascular dilatation/thickening Thickened BM Pigmentary incontinence	Peri-vascular and peri-eccrine lymphocytic infiltration BM Ig deposition BM vacuolisation and degeneration Skin spongiosis	Perivascular and diffuse cellular infiltrates Increased collagen deposition and fat replacement Epidermal acanthosis Mucin deposition Parakeratosis Adnexal loss Epidermal papillae loss Pigmentary incontinence Papillary telangiectasia

IF: immunofluorescence; BM: basement membrane; IgG: immunoglobulin G; IgM: immunoglobulin M; DEJ: dermo-epidermal junction.

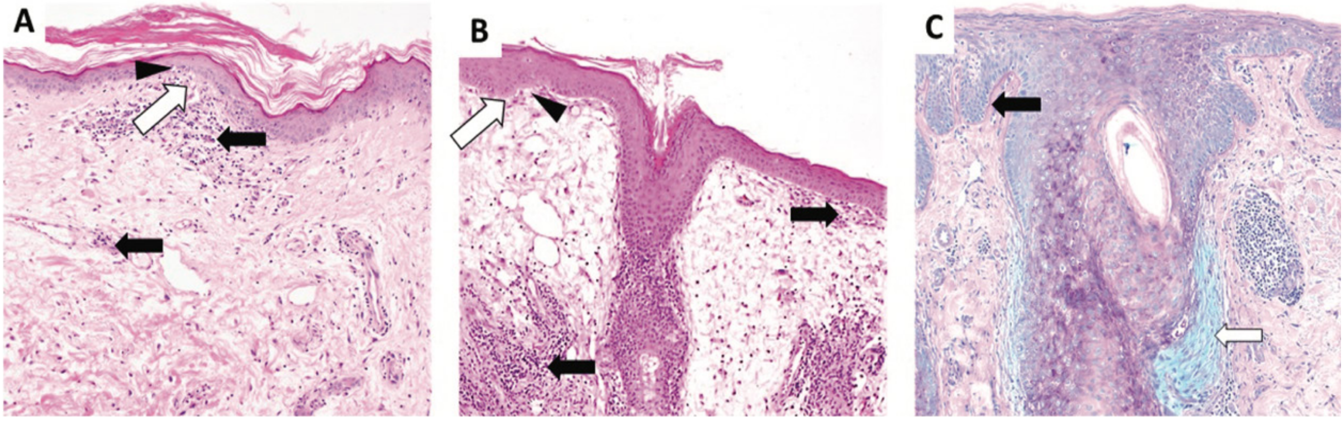

Fig. 1. Typical histological features of CLE.
Similar histological features observed in (**A**) ACLE and (**B**) DLE including interface dermatitis (white arrow), superficial and deep lymphocyte infiltrates (black arrow) and basal keratinocyte vacuolisation (arrow head). (**C**) mucin deposition (white arrow) and thickened basement membrane (black arrow) can be present in both ACLE and DLE (and dermatomyositis – not shown).

scriptionally distinct from EC found in other organs (although share several transcripts with EC from adipose tissue) (26). Gene expression in dermal ECs varies between arteriolar, capillary, post-capillary, and venules. For example, arteriole cells have increased expression of genes related to cell-cell junctions, whilst adhesion molecules are increased in post-capillary EC. Similarly, genes related to oxidative phosphorylation are increased in arteriolar endothelium, but glycolysis-related genes increased in capillary, post-capillary, and venule cells.

Cell populations in inflammatory skin disease

Histological, immunohistochemical and flow cytometry-based experimental studies have identified several cell populations in the skin of SARD patients suggesting that there may be shared pathological mechanisms, but also unique processes occurring in some conditions. The study of these differences at a cellular level has been made possible by scRNA-seq (27) which has demonstrated increased interferon-stimulated gene (ISG) expression in T and B cells, natural killer (NK), antigen presenting cells (macrophages and DC) and KCs (Table II and III).

Systemic lupus erythematosus

Keratinocytes (KC) play an important role in the CLE disease and as mentioned, histology have shown KC necrosis in lesional CLE skin. Furthermore, KC in the presence of UV exposure induces apoptosis, cytokine and chemokine (such as IL-6, CXCL10 and IFN-α) production, and auto-antibody (such as SSA/Ro52) production (28).
Billi *et al.* (2022) identified subpopulations across various cell types including

Table II. Studies of single cell RNA sequencing in autoimmune inflammatory skin disease.

Author (year)	Condition (n)	Skin samples (n)	Main findings	scRNA-seq method (sequencing)
Der *et al*. (2017)(34)	CLE (12) *vs*. HC (5)	Non-lesional, non-sun-exposed skin (12) *vs*. HC (5)	28 upregulated genes and 2 downregulated genes in KCs from non-sun-exposed skin in patients with lupus nephritis compared to HC: notably ISGs (IFITM1, IFI6, STAT1, IFI27)	Fluidigm C1 (Illumina Nextera XT)
Apostolidis *et al*. (2018)(63)	DSSc (1) *vs*. age and sex matched HC (1)	Dorsal forearm skin from Diffuse SSc (1) and HC (1)	88 cells from HC skin and 96 cells from SSC skin. 4 upregulated genes in SSc EC (VWF, THBS1, APLNR and HSPG2) when compared to HC	SmartSeq2 (Illumina Nextera XT)
Der *et al*. (2019)(35)	SLE/LN (21) *vs*. HC (3)	Non-lesional, non-sun exposed (17) *vs*. HC (3)	Enriched IFN pathway in both kidney (tubular cells) and skin (KCs). In KCs increased IFN pathway activation associated with proliferative LN. Pathway enrichment analysis in KC show upregulation of ECMs.	Fluidigm C1 HT. (Illumina Nextera XT)
Tsoi *et al*. (2020)(55)	HC (2), DM (2), CLE (2) (plus DM (36), HC (5), CLE (90) for bulk)	Upper thigh HC (2), DM (2), CLE (2) For bulk: DM (43), HC (5), CLE (43 SCLE and 47 DLE)	Increased IL18 in DM KCs (lesional and non-lesional) compared to HC or CLE. In bulk sequencing, 5 gene score (IFN, LCE2D, LCE1B, KRT80, and TPM4) distinguishes DM from CLE expression.	10x Genomics Chromium (Illumina NovaSeq 6000)
Mirizio *et al*. (2020)(71)	LSSc (3)	1 pair lesional skin samples (fresh and frozen)	18 cell clusters from 14,901 cells, 9 main cell grouping including KC, pericytes, APCs and immune cells (T cells, NK cells, mast cell), ECs and melanocytes. CryoStor® CS10 preservation compared to fresh specimen demonstrated similar transcriptomic expression of genes.	10x Genomics Chromium (Illumina NextSeq)
Gaydosik *et al*. (2021)(67)	DSSc (32) *vs*. HC (10)	DSSc (27) and HC (10)	9 T cell clusters from 3729 CD3+ T cells. Each cluster differing in DEGs; convention CD4+ T cells in 3 clusters, CD8 T cells in 1, Treg in 1, IL26 in 1 cluster, CXCL13 in 1, TRDC in 1 and proliferation genes in 1. Unique recirculating CXCL13+ (T follicular helper) T-cell subset in dSSc skin lesions.	10x Genomics Chromium (Illumina KAPA Library)
Tabib *et al*. (2021)(64)	DSSC (12) *vs*. HC (12)	Mid-forearm sample 12 DSSc (12) *vs*. HC (10)	10 fibroblasts cell types; similar between DSSc and HC skin although SFRP2^{hi}/PRSS23+ fibroblasts mostly within DSSc population. SFRP2^{hi}/SFRP4 myofibroblasts mostly in DSSc skin and expressed WNT pathway genes.	10× Genomics Chromium (Illumina NextSeq-500)
Xue *et al*. (2022)(68)	DSSC (12) *vs*. age and sex matched HC (10)	Dorsal forearm skin from DSSc (12) and HC (10)	12 myeloid cell clusters of which 4 DSSc-specific (other clusters comparable between HC and DSSc skin). Increased IFNγ and innate immune pathways genes in DDSc CCR-1+ macrophages. Expansion of perivascular FCN-1+ myeloid cells in some patients with DDSc Increased numbers of pDCs in DDSc.	10× Genomics Chromium (Roche KAPA Universal Library)
Billi *et al*. (2022)(29)	DLE (3), SCLE (4) *vs*. 14 HC.	Paired lesional and sun-protected non-lesional skin (7). Various anatomical sites from HC (14)	10 major cell types with 26 distinct cell clusters. Increased ISG expression in CLE KCs compared to HC (especially basal and spinous KCs). Of 10 FB subclusters, 1 was mostly comprised of cells from CLE patients (SFRP2+ FBs with high ISG score). Across 9 T cell clusters there was increased ISG expression (13-15% in CLE compared to <1% in HC), including non-lesional Treg Less variability in the 9 myeloid subclusters between HC and CLE but fewer Langerhans's cells in CLE	10x Genomics Chromium (Illumina NovaSeq 6000)
Zheng *et al*. (2022)(31)	DLE (8) SLE (10) *vs*. HC (5)	23 skin biopsies total, sites including face, cheek, after the ear, neck and forehead	Higher proportion of immune cells (B cell and NK cells) in DLE than SLE or HC. Within 12 KC subtypes, differentiating KC subtype associated with high expression of ISGs (FIH1, IFIT2, IFITM3), chemokines (CXCL10, CXCL11) and antigen processing and presentation related molecules (HLA-DRA and HLA-DRB1). Amplification 5 of KC subsets in DLE and SLE including CCL20+ subset. Of 9 clusters FBs, 4 clusters of dermal FNs increased in SLE including CXCL1+ and HLA+. Subcluster of epidermal and dermal T cells defined by high ISG expression in DLE/SLE Trajectory analysis suggests increased differentiation of T cells in DLE and SLE. 5 B cell subclusters with expansion of dermal B cells and ISG+ epidermal and dermal plasma cells in SLE/DLE Increased ISG in epidermal macrophages/DCs in SLE and increased pDC genes in both an epidermal and dermal subset of macrophages/DCs, enriched in DLE/SLE. Increased ISG and HSP-70 gene expression within NK cell subclusters.	10x Genomic Chromium (Illumina NovaSeq 6000)

Author (year)	Condition (n)	Skin samples (n)	Main findings	scRNA-seq method (sequencing)
Zheng *et al*. (2022)(32)	SCLE (3) *vs*. HC (3)	1 from each SCLE patient. HC scRNA data obtained from the GEO database (GSE162183)	Expansion of differentiating, basal, terminally differentiated and inner root sheath keratinocytes in SCLE. 4 FB subsets (pericytes, secretory reticular, secretory papillary and SFRP4+) expanded in SCLE vs HC All lymphocyte subsets expanded in SCLE except Treg which were reduced. Amongst 8 myeloid cells sub-clusters, increased M1 macrophages, pDC, LAMP3+ DC, Langerhans cells, and Cycling cells in SCLE than those in HCs. Increased ISG expression in KC, FB, T cells, B cell and myeloid cells in SCLE.	10x Genomic Chromium (Illumina NovaSeq 6000)
Dunlap *et al*. (2022)(30)	DLE (3), SCLE (4) *vs*. HC (14)	Paired lesional and non-lesional skin	Mixed population of T cells (CD4+, CD8+, CD4-CD8-) T cells expressing high ISG signature. ISG-high T cells in lesional and non-lesional CLE skin. Increased total CD4+ and CD8+ memory T cells in CLE, but relative reduction in one CD8+ Trm subset. Increased ISG signature in T cells from CLE compared to SSC and increased activation of peripheral and follicular helper T cells in CLE compared to HC or SSc skin.	10x Genomic Chromium (Illumina NovaSeq 6000)

KC: keratinocyte; FB: fibroblast; EC: endothelial cell; ISG: Interferon-stimulated gene; LN: lupus nephritis; SSc: systemic sclerosis; DSSc: diffuse systemic sclerosis; DM: dermatomyositis; pDC: plasmacytoid dendritic cell; HC: healthy control; SCLE: subacute cutaneous lupus; ACLE: acute cutaneous lupus.

T, B cells, FB, myeloid cells and KC in lesional and non-lesional skin of 7 CLE patients (6 with SLE) (29). In KC subpopulations, three ISG^{high} subclusters in CLE compared to healthy control (HC) was identified with increased IFN pathway activation in non-lesional noted in basal KCs. Although current scRNA-seq methodologies lack the sequencing depth to measure IFN transcripts directly, other studies have demonstrated that lupus KCs are key producers of type 1 IFNs (notably IFNκ) (30).

Across 2 scRNA-seq studies, Zheng *et al*. identified 6 (in SCLE) and 12 (in DLE) KC subclusters, 4 were common to DLE and SCLE expressing *KRT1, KRTDAP, COL17A1, GJB2, UBE2c* and *TOP2A*) (31, 32). The cytokine CCL20 was increased in DLE and SCLE KCs compared to HC (33), suggesting that CLE KCs have role in chemotaxis.

Der *et al*. (2017) demonstrated the potential for skin biopsies in lupus nephritis (LN) to be a biomarker by observing KC production of Type 1 IFN (34). Examining non-lesional, non-sun exposed skin in 12 LN patients and 5 HC with scRNA-seq, thirty differentially expressed genes (DEGs) were identified in KC from LN patients compared to HC. There was significant up-regulation of IFN pathway genes in non-inflamed/non-sun exposed skin. In 2019, the same authors confirmed increased type 1 IFN pathway activation in KC from patients with proliferative LN but not membranous glomerulonephritis (35).

In both SCLE and DLE, there are clonal expansion of T cells, with increased CD4^{+}, and CD8^{+} cells expressing the chemokine receptors CCR4, CCR6 and CCR10 through immunostaining of CLE skin tissue (36, 37). Recruitment of effector Th1 lymphocytes may be driven by increased expression of CXCL9, 10 and 11, in response to type I IFN (28, 38, 39). There are increased Th1 T cells in lesional and non-lesional CLE; more prominent in DLE than SCLE (37). In one study, there were more CXCR5+ cells in the skin of SLE and CLE patients compared to HC skin. Furthermore, in DLE, IL-8/CXCL8 expression correlated positively with the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) damage score, but not disease activity, suggesting that IL-8-driven neutrophil or granulocyte chemotaxis may have a role in later stages of inflammation and/or wound healing (37).

Immunohistochemistry performed by Schmidt *et al*. (2017) showed that the total number of cutaneous CD4^{+} T cells are similar in CLE patients (SCLE, DLE and lupus tumidus) compared to other inflammatory dermatoses including psoriasis, lichen planus and atopic dermatitis (AD). There are fewer CD4^{+} Foxp3^{+} regulatory T cells (Tregs) in CLE than SLE and other inflammatory skin conditions (40). This reduction in Foxp3^{+} Tregs is more marked in lesional *versus* non-lesional SLE skin but doesn't differ between the CLE subtypes (41). Interestingly, whilst the numbers of skin Tregs was lower in all CLE subtypes, the number of circulating Tregs was only lower in SLE, suggesting differences between CLE with and without systemic involvement. However, Billi *et al*. identified 9 T cell subsets and noted similar proportions of Treg in HC, non-lesional and lesional skin samples, although a higher ISG signature in the Treg of non-lesional skin compared to HC was found (29).

Zhao *et al*. identified differences in a subpopulation of non-circulatory T cells, the CD4^{+} tissue-resident memory (Trm) cells, between ACLE, SCLE, and DLE with HC skin with scRNA-seq (42). They noted more Trm cells in SCLE and localised DLE compared to ACLE and HC. Absent in Melanoma 2 (AIM2), which has been implicated in disease due to its effects on IFN-dependent B cell stimulation (43), had higher expression in CLE compared to HC, with highest expression in SCLE and localised DLE. Furthermore, as AIM2 expression was not observed on immunofluorescence in HC CD4^{+} T cells, AIM2+ Trm cell may be useful in CLE diagnosis.

Further identification of differences in T cell populations in CLE by Dunlap *et al*. (2022), identified 13 cellular subsets categorised as CD4^{+} and CD8^{+} T cells and NK cells; one subset, containing

Table III. Summary table of discussed findings.

Condition	Cell type	Histopathology, immunohistochemistry and fluorescence	scRNA-seq
Cutaneous Lupus Erythematosus	Keratinocytes	KC necrosis in histology Apoptosis, cytokine and chemokine production	KC subtypes with ISG^{high} KC subtypes common to DLE and SCLE IFN gene upregulation in non-lesional CLE
	T cells	Increased CD4+, CD8+ T cells expressing chemokine receptors CCR4, CCR6 and CCR10. Effector Th1 recruitment through expression of CXCL9, 10 and 11. Th17 increased in skin of SCLE and DLE Reduced Foxp3+ Treg in CLE than SLE	Subpopulation of CD4+/CD8+ and CD4/8- T cells expressing ISGs found in CLE Subpopulation of non-circulatory T cells (CD4+ Trm) between different types of CLE. AIM2+ Trm cells observed in CLE but not in HC Subpopulations identified expressing ISG increased in CLE
	B cells	Ig deposition at the DEJ (positive lupus band test) Increased B cell deposition compared to other inflammatory cutaneous dermatoses	5 epidermal and 7 dermal B cell clusters identified; 1 epidermal and 2 dermal expressed ISG and 3 expressing Hsp70 coding genes.
	Plasmacytoid dendritic cell	Periadnexal and perivascular deposition in the epidermis and DEJ	Increased deposition in CLE with subclusters identified. CD16+ pDC overrepresented in CLE in lesional and non-lesional skin Mixture of expressed markers in epidermis, $ISG^{high,}$ CD16+ pDC in dermis
Dermatomyositis	KC	Dyskeratotic keratinocytes are observed in mostly in lesional skin	Increased IL-18 expression in KC in pair skin compared to SCLE. 5 gene signature allowing for identification of DM *vs*. CLE.
	T cells	Perivascular CD4+, CD8+, T cells, CD40+ Th cell in the dermal basal epidermal layers	No applicable scRNA-seq study
Sjögren's Syndrome	T cells	Increased number of Th17+ T cell in the middle to deep dermis in Annular Erythema. Increased Foxp3+ Treg in Annular Erythema compared to healthy skin.	No applicable scRNA-seq study
Systemic Sclerosis	T cells	Increased CD3+ T cells. Increased Th2 in later stages	T cell cluster identified; 1 expressing proliferating genes in SSc CXCL13-expression T cell cluster found in early disease ISG expressing T cells are greater in CLE than SSc
	B cells and plasma cells	Increased CD20+ B cells and CD138+ plasma cells	No applicable scRNA-seq study
	FB	Production of ECM by dermal fibroblasts	PRSS23 expressing $SFRB2^{hi}$ FB from SSc patients expressing genes correlating with disease severity.
	Macrophages, myeloid cells	Increased in established disease, producing pro-fibrotic cytokines including TGFβ	FCGR3A expressing myeloid population with increased CCL2 expression. Ficolin-1 (FCN-1) positive cells predominantly located in perivascular tissue
	NK cells	Increased in established disease, producing pro-fibrotic cytokines including TGFβ	No applicable scRNA-seq study

scRNA-seq: single cell RNA sequencing; CLE: cutaneous lupus erythematosus; KC: keratinocytes; ISG: interferon-stimulated gene; DLE: discoid lupus erythematosus; SCLE: subacute cutaneous lupus erythematosus; IFN: interferon; Treg: regulatory T cell; Trm: tissue resident memory T cell; DEJ: dermo-epidermal junction; DC: dendritic cell; pDC: plasmacytoid dendritic cell; SSc: systemic sclerosis; FB: fibroblasts; NK cells: natural killer cells.

a mixture of $CD4^+$, $CD8^+$ and CD4/8- (double negative) T cells, expressing ISGs (30). Zheng *et al*. (2022) also identified increased T, B and NK cells in DLE and SCLE compared to HC(32). An ISGs gene set (*IFI6, IFI44, IFITM3, ISG20, IFI27, ISG15* and *IFI44L*) was increased in dermal T cells from DLE compared to HC, although other ISGs (*IFI6, IFI44, IFIH1,* and *DDX58*) were present in both epidermal and dermal T cells. Although Tregs were present in both the epidermis and the dermis, GO analysis showed enrichment of cell adhesion and T cell activation pathways in dermal Tregs cells suggesting different functions (and possibly origin) between spatial locations. 5 NK cell clusters with differential gene expression between epidermal and dermal cells were identified. Th17 cells are also implicated in CLE, with increased tissue levels of IL-17A in the skin of SCLE and DLE patients and in non-lesional skin of SLE patients. In SCLE and SLE, there is an association between the percentage of IL-17A+ lymphocytes in the tissue and the presence of anti-SSA/Ro antibodies (44). However, these findings may not be specific to SLE as the Th17 axis is implicated in several other inflammatory skin conditions including allergic contact dermatitis, AD, cutaneous T cell lymphoma, Behçet's disease and psoriasis (45). Although others have reported Th17 in SCLE but not DLE skin (but dominant Th1 cells and type II IFN) (46), interestingly, this is not noted in the studies by Dunlap and Zheng *et al.* (30, 32).

Although Ig deposition at the DEJ occurs in SLE (lupus band test), relatively little is known about the role of B cells in CLE. Early studies identified increased CD20+ B cells in DLE compared to lichen planus and HC (47, 48), but a subsequent study reported increased total (CD20+) and mature (CD27+) B cells in DLE and ACLE, but not SCLE (49). Interestingly, the inflammatory infiltrate may change over time in DLE as $CD4^+$ T cells, CD163+ macrophages, $CD8^+$ T cells and CD20+ B cells are dominant in early lesions, with fewer $CD8^+$ T cells in later/scarred lesions (36). Utilising scRNA-seq, Zheng *et al.* (31) identified 5 epidermal and 7 dermal B cell clusters in the skin of DLE patients, 3 of which (1 epidermal, 2 dermal) expressed ISGs (*CD38*, *IGHG4* and *IGHG1*) and 3 (1 of which was ISG^{hi}), expressed Hsp70 coding genes, which have a role in antigen presentation.

Histologically, pDC have been identified through the expression of CD123 in SCLE, DLE and lupus tumidus located in the periadnexal and perivascular areas of the epidermal and DEJ(50) and thought to cause disease through IFN production (51). Three studies, Billi *et al.* (29), Zheng *et al.* (2021) (31) and Zheng *et al.* (2022) (32), demonstrated increased pDC (characterised by *JCHAIN, GZMB* and *MZB1*) and epidermal Langerhans cells (characterised by CD207) in CLE lesions. 5 clusters each of macrophage/DC were identified, with ISG expression in both epidermal and dermal cell populations. In the epidermis, the ISG^{hi} population expressed a mixture of macrophage and DC markers (suggested a mixed population) whilst the dermal ISG^{hi} population was comprised of CD16+ DCs.

Billi et al. identified 9 myeloid cell subsets in CLE and HC, some of which were only observed in CLE (29). Whilst, as in other cell types, a high ISG signature defined a unique T cell subset only seen in SLE, in GO analysis the IFN pathway was also upregulated in both Treg and peripheral/follicular helper T cells also seen in the study by Dunlap *et al.* Within the myeloid population, CLE was characterised by fewer Langerhans's cells and increased CD16+ DC (expressing *FCGR3A*/CD16a). It is proposed that these CD16+ DCs arise from non-classical monocytes although confirmatory studies are required.

Dermatomyositis

Histologically, Gottron's lesions have increased superficial dermal $CD4^+$ and basal epidermal $CD8^+$ T cells and a $CD40^+$ Th cell infiltrate in the dermal/basal epidermal layer, although overall fewer T cells compared to DLE. Additionally, in DM, the lymphocyte infiltrate is predominantly peri-vascular rather than peri-eccrine as observed in CLE (52, 53). In lesional DM skin, there are fewer Foxp3+ Tregs compared to psoriasis vulgaris, suggesting pathogenesis in DM more closely resembles CLE than psoriasis (41, 54). Consistent with this observation, the numbers of IL-10- and TGFβ-producing cells in the skin, and serum levels of IL-10 and TGFβ are also reduced in DM patients compared to psoriasis (52, 54).

Single-cell analysis of HC, SCLE and DM (2 in each group) confirmed increased expression of IL-18 in KC, but not myeloid cells, in lesional and non-lesional DM compared to SCLE. In the same study, bulk RNA-seq identified that a 5 gene signature (*IL-18*, *LCE2D*, *LCE1B, KRT80* and *TPM4)* could differentiate DM from SCLE with an AUROC of 0.98 (55).

Sjögren's syndrome

pSS shares some autoantibodies (notably anti-SSA/Ro) with SLE and reflecting this, a small study of AE lesions in pSS demonstrated increased numbers of Th17+ T cells in the middle-to-deep dermis in AE (as seen in SCLE) compared to either psoriasis or AD (in psoriasis, there are superficial dermal Th17+ cells) (14). However, the total number and proportion of Foxp3+ Treg was higher in both AE and SLE skin compared to healthy skin (14). The increase in Th17+ cells may therefore reflect changes in IL-2 and IL-6 expression, rather than the number of Foxp3+ Tregs (56). Although it has been proposed that activated (CD45Ro+) memory T cells could differentiate AE from other inflammatory dermatoses, increased CD45Ro + cells are not specific to AE, and can be found, for example, in keloid scars (57). There are currently no single-cell studies of cutaneous inflammation in pSS.

Systemic sclerosis

In established SSc, there are increased cutaneous eosinophils, plasma cells, Th1/Th2 cells, macrophages, NK and perivascular B cells (16, 58). The number of T cells (CD3+), myeloid cells (CD68+), B cells (CD20+) and plasma cells (CD138+) are higher in involved skin compared to uninvolved skin(58). In the inflammatory stage of the disease, cytokines including TNF-α, IL-1/-12/-17 and -22 are increased in the skin (59). IL-17 may be important in the inflammatory stage, increasing inflammation whilst inhibiting fibrosis (60). In the later (fibrotic) stages, Th2 cells, NK cells, and macrophages produce IL-4, -6, -10 and -13, and platelet derived growth factor (PDGF), while B cells drive collagen production by dermal FBs (61, 62). These FBs produce TGFβ which perpetuates fibroblast activation and collagen and extracellular matrix production in a feed-forward process which may also be relevant to scarring in DLE (16, 60).

Four studies have identified unique cell subsets in SSc patients. In 2018, Apostolidis *et al.* identified 10 cell clusters including 1 cluster of EC based on increased expression of *VWF, PECAM1* and *CDH5* expression in one dSSC patient (63). With the DEGs, there was increased expression of *APLNR* (a marker of endothelial damage) *VWF and THBS1* (markers of vascular dysfunction) and *HSPG2* (a component of ECM).

Tabib *et al.* (2021) identified that SSc FB formed a distinct cluster with significant transcriptomic differences (64). Sub-clustering of *SFRP2^{hi}* FB identified a population expressing *PRSS23* predominantly comprised of cells from SSc patients (64). These cells expressed genes which have been shown to correlate with SSc severity including *THBS1*, *CDH11* and *CCL2* (65, 66). Interestingly, there may be a further transcriptional sub-cluster within the *SFRP2^hi^/PRSS23+* FB population, the relevance of which warrants further investigation.

Gaydosik *et al*. (2021) identified 9 T cell clusters in SSc and HC skin; 3 conventional $CD4^+$ T cell clusters, one likely comprising actively proliferating T cells, and 1 each of $CD8^+$ T cells and Treg cells (67). A cluster expressing proliferating genes, was increased in SSc compared to HC suggesting active expansion of T cells in SSc skin. A *CXCL13*-expressing cluster was unique to SSc, predominantly in the earlier stages of disease (67). The origin of the *CXCL13+* T cells remains uncertain, but trajectory analyses suggested a close relation to Tregs. Notably, Zheng *et al*. (2022), utilised the scRNA-seq dataset from Gaydosik *et al*. and compared between CLE and SSc skin, finding that although the major cellular subtypes were found to co-cluster, ISG expression by T cells was greater in CLE than in SSc.

Xue *et al*. (2021) sequenced myeloid cells from 12 diffuse cutaneous SSc patients(68). Comparable to CLE, a *FCGR3A* expressing myeloid cell population was identified. It is likely that this population of cells are present in small numbers in HC and most SSc patients, but significantly expanded in a subgroup of SSc. These cells had increased chemokine expression including the SSc severity biomarker *CCL2*. Similarly, ficolin-1 (FCN-1)-positive cells were identified, predominantly from 2 patients. These were distinct from the *FCGR3A+* macrophages and expressed increased levels of some classical monocyte markers (*e.g. S100A9, SOD2*) but not others (*ITGAM, TLR2, CLEC7A*). FCN-1+ cells were associated with disease severity and predominantly located in perivascular tissue suggesting a role in cell migration.

Although both SLE and SSc skin demonstrate an ISG signature, there may be important differences in the cells producing or responding to IFN in the skin between SARDs. For example, infiltrating T cells in SLE express an ISG signature was not observed in T cells from SSc skin (30). Bioinformatic analysis also identified increased activation and exhaustion genes in CLE T cells compared to SSc.

Limitations of scRNA-seq

Depending on disease stage, several histological changes in the skin such as epidermal acanthosis, mucin deposition or peri-vascular cellular infiltrate are shared between SARDs. For disease classification, treatment or prognostication, routine clinical histology is therefore imprecise. More detailed analyses are required to interrogate the molecular and cellular basis of skin inflammation. Single-cell technologies offer the opportunity to delineate the inflammatory processes to identify targets, and common pathological processes within clinically heterogeneous conditions.

However, despite the advantages of scRNA-seq, there remain several important limitations. Firstly, there is no standardised method for sample storage, cell isolation, dead cell/debris removal, cell capture and library generation in skin tissue (20). As mentioned, PCA or tsne are utilised for reduction of data for visualisation. These approaches cannot represent the true complexity of data as clustering algorithms, including K-means and hierarchical clustering, may give different results depending on the distribution of data and/or parameters selected during analysis. Datasets are often large requiring significant computer processing capacity and dedicated bioinformatics expertise (69).

As the starting material for analysis in scRNA-seq is relatively small, low capture efficacy can affect data quality (70). Furthermore, significant noise in the data due to observed zeros, which include technical (false) zeros (or drop-outs) and true zeros, which may be difficult to differentiate from one another (19, 20). Variations in cell number and read depth, which may in some cases relate to the tissue type and/or differentiation state of individual cells, may be further complicated by technical noise, leading to challenges in downstream analysis (20, 69). It is important to note that cellular cryopreservation does not adversely affect RNA quality (71). Transcripts expressed at low levels, including cytokines, are not reliably detected and confirmatory experiments or combination of scRNA-seq with other single cell -omics are likely required.

Conclusion

The pathophysiological processes in the skin of patients with SARDs are diverse with certain clinical manifestations and histological appearances shared amongst disease groups. Newer techniques such as scRNA-seq have allowed the identification of novel cell subsets within cellular populations previously considered homogenous, with some subsets only unique to disease. Single-cell methods offer insight into the function of these cells, and trajectory mapping can predict how these cells arise *in vivo*. In addition to biological insights, these studies can also aid biomarker discovery. For example, in SLE, shared IFN signatures between the kidney and non-inflamed skin offers the possibility of skin-based biomarkers to assess or monitor major organ disease. Basket trials targeting shared pathways across different SARDs could rapidly accelerate drug development in these uncommon diseases.

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