Review

Macrophage heterogeneity governs the balance between fibrosis and tissue protection in systemic sclerosis

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ABSTRACT

Systemic sclerosis (SSc), especially its life-threatening interstitial lung disease (SSc-ILD), urgently requires targeted therapies. This review highlights how macrophage heterogeneity, transcending the obsolete M1/M2 paradigm, acts as a central orchestrator of the pathogenic "fibro-immune axis" linking vascular injury, immune dysregulation, and fibroblast-mediated fibrosis. Single-cell RNA sequencing and spatial multi-omics reveal distinct, context-dependent macrophage subsets with specialised roles across SSc tissues: secreted phosphoprotein-1 (SPP1$^+$) macrophages dominate SSc-ILD, promoting fibrosis via epithelial-mesenchymal transition and reciprocal activation of fibroblasts through IL-6/ERK signalling; FCGR3A$^+$ macrophages drive skin inflammation and fibrosis via NF-κB and TGF-β pathways; while TREM2$^+$ macrophages exhibit protective lipid clearance functions in skin. These subsets primarily originate from monocytes recruited through pathways such as CCL2-SPP1-ARG1 and CXCL4. Crucially, spatial analyses uncover "fibrotic niches" where macrophages and fibroblasts engage in pathogenic crosstalk, explaining the efficacy of IL-6 blockade (tocilizumab) in early SSc-ILD. Despite these advances, challenges remain in resolving temporal dynamics and spatial signalling resolution. This integrated perspective establishes macrophage heterogeneity as a fundamental determinant of SSc progression and a critical frontier for mechanism-based therapeutics.

Introduction

Systemic sclerosis (SSc) is a devastating autoimmune disorder characterised by progressive vasculopathy, immune dysregulation and uncontrolled fibrosis affecting the skin and internal organs, particularly the lungs. Interstitial lung disease (ILD) accounts for 35% of SSc-related mortality, with a 5-year survival around 70%, underscoring the urgent need for targeted therapies (1, 2). Despite advances in understanding its pathogenesis, therapeutic options remain limited, partly due to unresolved complexities in the contributions of immune cells. Skin biopsies have revealed elevated infiltration of monocytes/macrophages in early-stage SSc skin fibrosis (3). Among these, macrophages have emerged as pivotal orchestrators of fibro-inflammatory cascades (4-6), bridging vascular injury, immune activation and fibroblast-driven fibrosis, a triad termed the "fibro-immune axis" (7).

Historically, macrophage roles in SSc were oversimplified into the M1/M2 dichotomy, a model derived from bulk-tissue analyses that masked critical heterogeneity and context-dependent plasticity (8). This binary framework fails to explain clinical observations, such as the efficacy of anti-IL-6R (tocilizumab) observed only in early SSc-ILD (9), or the accelerated fibrosis observed in SSc compared to idiopathic pulmonary fibrosis (IPF). The advent of single-cell RNA sequencing (scRNA-seq) and spatial multi-omics has revolutionised macrophage heterogeneity profiling in SSc, revealing novel subsets with distinct roles in SSc pathogenesis. Unlike previous scRNA-seq that primarily provided descriptive characterisation of cellular subtypes (10), this study integrates multi-omics data to identify several chemokine axes as the central driver of macrophage subsets differentiation, thereby redefining the molecular hierarchy in fibrotic pathogenesis.

Overview of SSc immune process

SSc is a multifaceted autoimmune connective tissue disorder of unclear aetiology. The pathogenesis of SSc

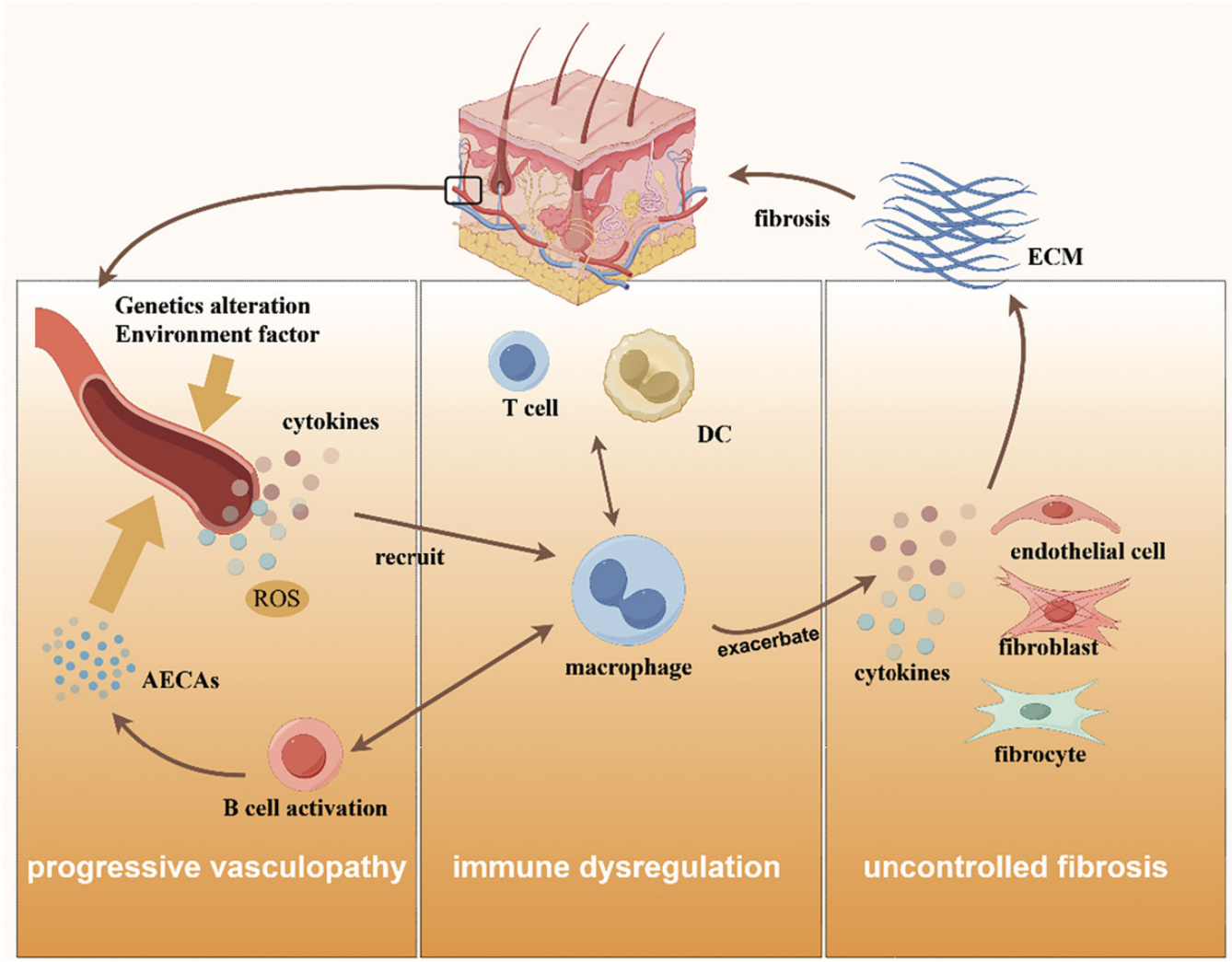

Fig. 1. The overview of the potential mechanisms of SSc fibrosis. Initial vascular injury is partly caused by environmental factors, genetic mutations and autoimmune reaction from antibodies (AECAs). Vascular structural and functional abnormalities lead to excessive release of ROS and pro-inflammatory cytokines. The altered expression of cell adhesion molecules induces the infiltration of lymphocytes and macrophages. Recruited macrophages further exacerbate inflammatory signalling by producing more chemokines. Local fibroblasts, ECs and fibrocytes are activated and differentiated, resulting in vasculopathy, inflammation, autoimmunity and an excessive production of ECM.
AECAs: anti-endothelial cell antibodies; ROS: reactive oxygen species; DC: dendritic cell; ECs: endothelial cells; ECM: extracellular matrix.

revolves around a triad of interconnected process: vasculopathy, immune dysregulation, and progressive fibrosis (11) (Fig. 1). Vascular injury, particularly targeting endothelial cells (ECs), is considered a pivotal initiating event, leading to an imbalance between vasoconstrictors and vasodilators, which results in capillary damage, vessel occlusion, and tissue hypoxia (12). This process is driven by complex interactions between genetic predisposition and environmental factors, including epigenetic modifications which amplify ECs damage and initiate aberrant immune response. Furthermore, anti-endothelial cell antibodies (AECAs) exacerbate early vascular injury by promoting the release of pro-inflammatory mediators such as cytokines, chemokines and reactive oxygen species (ROS) (13). These factors recruit immune cells, including macrophages and lymphocytes, to form perivascular infiltrates that sustain chronic inflammation (14). Persistent inflammation activates myofibroblasts, the primary drivers of excessive extracellular matrix (ECM) deposition, culminating in tissue fibrosis (15). Importantly, the vasculopathy not only drives fibrotic progression but also substantially increases the risk of major cardiovascular events (16). These findings underscore the critical need to elucidate the underlying mechanisms of fibrosis to develop more effective targeted therapies in SSc patients.

Reevaluating limitations of the M1/M2 paradigm

Macrophages are the dominant regulators and immune cell population in SSc, whose dysfunction directly or indirectly contributes to the fibrosis process and abnormal regeneration. Notably, macrophages can sense their microenvironment and change how they behave through phenotypic plasticity under different microenvironment (17), usually recognised as the M1/M2 macrophage dichotomy, a traditional classification and an over-simplified in vitro model where monocyte-de-

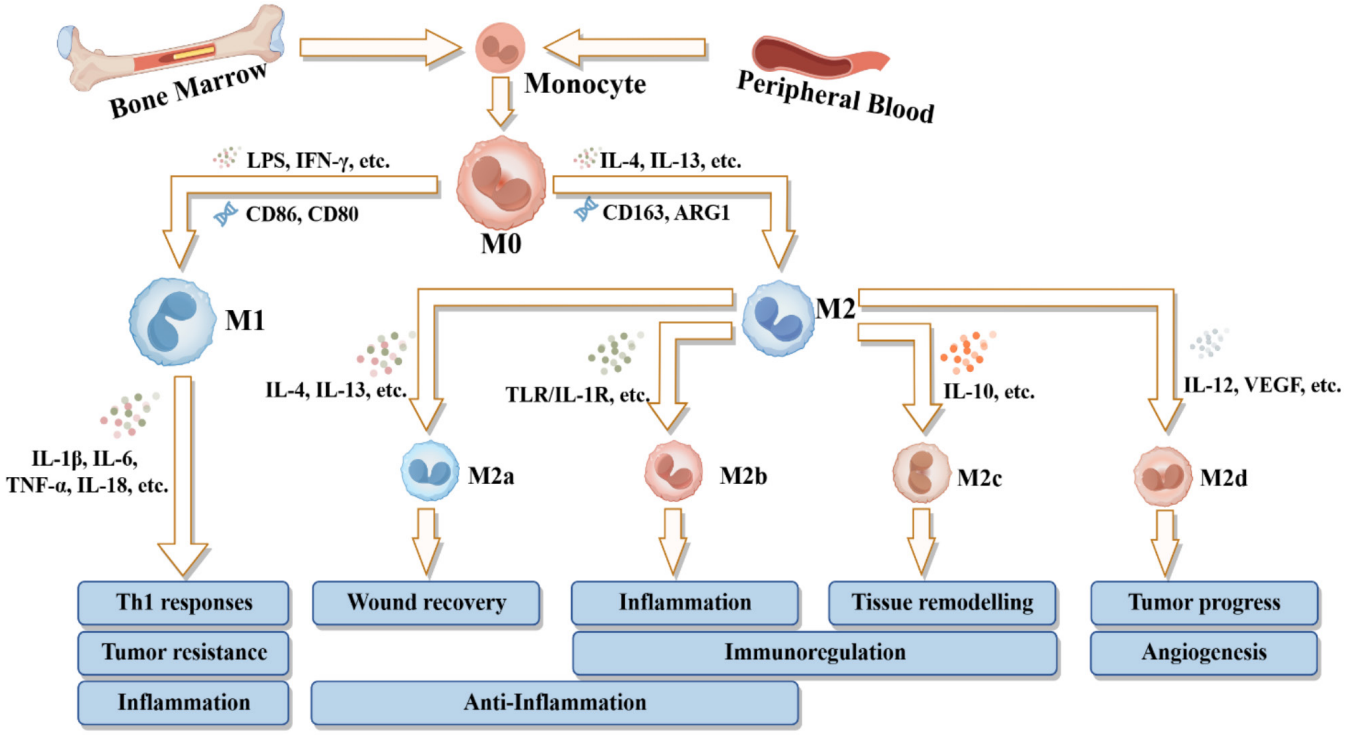

Fig. 2. M0 macrophages, which originate from monocytes, polarise into classically activated M1 macrophages under pro-inflammatory stimuli such as LPS and IFN-γ, and express markers like CD86 and CD80, and secrete cytokines including IL-1β, IL-6, TNF-α, and IL-18. M1 macrophages promote Th1 responses, tumour resistance, and inflammation. Alternatively, under anti-inflammatory or regulatory signals, such as IL-4 and IL-13, M0 macrophages polarise into M2 macrophages, which express markers such as CD163 and ARG1. M2 macrophages further subdivide into subsets: M2a (induced by IL-4/IL-13, secreting IL-4 and IL-13, involved in wound recovery and anti-inflammation); M2b (induced by TLR/IL-1R ligands, secreting IL-10, contributing to inflammation and immunoregulation); M2c (induced by IL-10, secreting IL-10, supporting tissue remodelling and anti-inflammation); and M2d (induced by IL-12 and VEGF, promoting tumour progression and angiogenesis).
LPS: lipopolysaccharide; IFN-γ: interferon gamma; TNF-α: tumour necrosis factor alpha; ARG1: arginase 1; TLR: toll-like receptor; VEGF: vascular endothelial growth factor.

rived M1 macrophages were defined by LPS/interferon-γ as "pro-inflammatory" (18). Monocyte-derived M2 macrophages were defined by IL-4/IL-13 as "reparative"/ "profibrosis" (19). Furthermore, these macrophages are divided into: M2a (induced by IL-4 or IL-13); M2b (induced by agonists of Toll-like receptors or IL-1R); M2c (induced by IL-10 and glucocorticoid hormones); M2d (induced by vascular endothelial growth factor, VEGF) (20, 21) (Fig. 2).

Nowadays, many researchers have discussed the accuracy of this dichotomy model which fails to capture the functional complexity of macrophages, as many macrophages often simultaneously highly express genes linked to inflammation and fibrosis, resisting clear categorisation. Besides, SSc macrophages frequently exhibit hybrid phenotypes co-expressing CD86, TNF-α (M1-associated markers) and CD163, TGF-β (M2-associated markers), as evidenced by elevated mixed markers in SSc-ILD patients and co-detection of IL-12B (M1-associated) with TGM2 (M2-associated) in $SPP1^+$ lung macrophages (22). For instance, in SSc-ILD, a distinct $TLR4^+$ M2 monocyte/macrophage population ($CD1c^-CD80^-TLR4^+CD163^+CD204^+CD206^+$) is significantly enriched in peripheral blood and lung tissue, displaying both pro-inflammatory (TLR4) and pro-fibrotic (CD163, CD206) features, further highlighting the inadequacy of the M1/M2 framework. These cells co-localise with $CD68^+$ fibrotic regions in the lungs of SSc-ILD patients (absent in healthy control lung samples), secreting TGF-β and CCL18, deriving fibroblast activation and collagen deposition, and their elevated presence in peripheral blood correlates with SSc-ILD severity (23). Crucially, macrophage phenotypes are not static but exist on a dynamic continuum, continuously reshaped by disease-specific microenvironmental cues such as cytokine gradients, metabolic stress, tissue damage, and even drug usage (24, 25). In early diffuse cutaneous SSc (dcSSc, <5 years), macrophages express inflammatory genes (*e.g.*, IL-6, TGF-β), deriving fibroblast activation, particularly in patients with anti-topoisomerase 1 antibodies (ATA), while in late-stage dcSSc (>5 years), they shift toward a pro-fibrotic phenotype, sustaining ECM remodelling (26). This inherent dynamism generates transitional and intermediate states that co-express hybrid M1/M2 markers, which evade binary classification. It's been proved in many other diseases that traditional polarisation models systematically overlook these fluid states, which critically orchestrate fibro-inflammatory cascades during the pathogenesis of many diseases (27).

The advent of scRNA-seq has transformed macrophage classification by allowing high-resolution analysis of these previously overlooked pheno-

types. These observations highlight the limitations of the M1/M2 paradigm in capturing macrophage plasticity and hybrid phenotypes in SSc. Ultimately, advanced technologies like scRNA-seq are essential to uncover dynamic macrophage states for better understanding fibro-inflammatory processes.

Mapping macrophage heterogeneity

scRNA-seq resolves transcriptional heterogeneity by capturing genome-wide expression profiles of individual cells, enabling the identification of rare subpopulations and their functional states within complex tissues. In SSc, this technology is pivotal for deconvoluting macrophage diversity beyond the traditional M1/M2 dichotomy, revealing disease-specific subsets that drive distinct pathogenic mechanisms. Defining these subtypes is critical for understanding their roles in fibro-inflammatory cascades and ultimately improving clinical outcomes for SSc-ILD and skin fibrosis.

scRNA-seq identifies subgroups of macrophages

Despite therapeutic advances, SSc-ILD remains a major therapeutic challenge due to its early onset and rapid progression compared to idiopathic pulmonary fibrosis (28, 29). scRNA-seq has identified disease-specific macrophage subsets that drive fibro-inflammatory cascades through spatially resolved mechanisms, transcending the limitations of M1/M2 paradigms.

FABP4$^+$ macrophages, FCN1$^+$ macrophages, and SPP1$^+$ macrophages have also been identified in SSc. SPP1$^+$ macrophages are mainly found in SSc-ILD patients, while FABP4$^+$ macrophages and FCN1$^+$ macrophages are found both in SSc and healthy controls (28, 30).

FABP4$^+$ macrophages exhibit high expression of lipid-binding and metabolic genes such as FABP4, INHBA, and ATP1B1, indicating a strong connection to alveolar lipid processing and homeostatic maintenance (31). Compared to their role in IPF, FABP4$^+$ macrophages in SSc-ILD demonstrate unique transcriptional signatures, notably upregulated type I interferon signalling relative to IPF counterparts (32). Their persistent presence suggests a potential role in shaping immune regulation and tissue metabolic balance in SSc, although their direct fibrotic interactions remain to be elucidated.

FCN1$^+$ macrophages (also classified as monocyte-derived dendritic-like cells) are also present in both healthy and diseased SSc tissues. Characterised by high expression of monocyte markers such as FCN1, CD14, EREG and S100A8, they are enriched in early monocyte-to-macrophage transition states. FCN1$^+$ myeloid cells correlate with activated monocyte-driven clusters and exhibit proximity to conventional type-2 dendritic cell populations (33). Trajectory analyses place them as intermediates in differentiation cascades, linking peripheral monocyte recruitment to phenotypically mature macrophages, suggesting FCN1$^+$ monocytes/macrophages may serve as key transitional populations in the SSc macrophage landscape, resulting in early immune infiltration and subsequent specialisation into disease-associated subsets.

Critically, scRNA-seq reveals that SSc-ILD macrophages uniquely exhibit constitutive MAPK signalling driven by oxidative stress and loss of the ANNEXIN-FPR inhibitory axis, distinguishing from IPF macrophages, which retain this feedback mechanism (32, 34). This dysregulation is sustained by BCLAF1 and NFE2L2 regulons, explaining SSc-ILD accelerated fibrosis. SPP1$^+$ macrophage is the most enriched cluster in these macrophages, has specific proliferating properties, marked by upregulation of SPP1, MERTK, and LGMN (35, 36), and has hybrid gene expression related to M1 and M2 phenotype at the same time (37). scRNA-seq had detected the transcriptional activation of the epithelial-mesenchymal transition (EMT) pathway, and the SPP1$^+$ macrophages could induce EMT of alveolar epithelial cells, leading to pulmonary fibrosis in SSc-ILD (32, 38).

It has been proven that SPP1 produced by macrophages drives monocytes(39), some of which differentiate into specific macrophages, activating the VEGF and VISFATIN signalling pathways, further driving the downstream p38-MAPK pathway (34). Ingenuity pathway analysis suggests potential upstream drivers of SPP1$^+$ macrophages as well as SSc-ILD phenotype, including LPS, TGF-β, and IL-6, as well as other cytokines (28, 40, 41). This result explains that IL-6 inhibition with tocilizumab preserves lung function over 48 weeks in patients with early SSc-ILD (42).

Currently, in SSc skin lesions, FCGR3A^{hi} macrophages exhibit a pathogenic triad: their pro-phagocytic activity via elevated FCGR1A/FCGR2A, pro-inflammatory activity through NF-κB-dependent IL-6/TNF secretion, and pro-fibrotic activity by TGF-β/JAK-STAT/WNT activation (43). Besides, these clusters of macrophages show upregulated expression of genes correlated with the severity of SSc, such as MSR1, CD163, F13A1, CCL2, and MS4A4A (44). Gene ontology (GO) functional profile suggests FCGR3A$^+$ macrophages are activated through TLR signalling (33), consistent with several recent studies that placed TLR signalling upstream of inflammatory and pro-fibrotic changes in SSc (45, 46). Patients with expanded FCGR3A$^+$ macrophages display elevated expression of genes correlated with the severity of dcSSc as assessed by the modified Rodnan Skin Score (mRSS) and accelerated fibrosis progression, confirming their role as drivers of the "vascular injury-fibrosis axis" in SSc pathogenesis (33).

Other discoveries have turned attention towards the central role of the TREM2 (a sensor recognising multiple lipid species) receptor in diverse pathologies as a major pathology-induced immune signalling hub for sensing tissue damage (47). An upregulation of TREM2$^+$ macrophage alteration in human skin lesions of dcSSc and localised scleroderma has been found during skin fibrosis progression. This elevated expression is mainly seen in macrophage subpopulations but not in other cell types from mouse injured skin (48), consistent with reported studies that TREM2 is mainly limited to macrophages (49, 50). Functional analysis

Table I. Enriched macrophage cell populations in SSc and their pathogenic function.

Macrophage subtypes	Gene signature	Disease specificity	Sample	Pathogenic function (associated pathways)
TLR4^{+}M2	TLR4, CD163, CD204	disease-specific: enriched in SSc-ILD, absent in health.	lung/peripheral blood	pro-fibrotic (TLR4 signalling)
FABP4^{+}	IRF9, FABP4	constitutively present in healthy lung; expanded in SSc-ILD	lung	lipid metabolism (type I IFN signalling)
SPP1^{+}	MERTK, LGMN, SPP1	disease-specific: enriched in SSc-ILD, minimal in healthy lung	lung	pro-fibrotic (IL-6/ERK; CCL2-SPP1-ARG1 signalling; CXCL4; p38-MAPK)
FCN1^{+}	FCN1, CD14, IL-1B	present in health and SSc (enriched in disease)	lung/skin	inflammatory
CCR1^{+}	MMP9, HMOX1	homeostatic in skin; pathologically activated in SSc	skin	pro-fibrotic (ECM remodelling, Interferon-γ Pathway)
MARCO^{+}	SEPP1, CCL13	homeostatic in skin; pathologically activated in SSc	skin	pro-fibrotic (IFN type I and γ)
TREM2^{+}	TREM2, APOE, PLTP, CCR2, LY6C2	disease-induced: minimal in healthy skin, enriched in SSc fibrosis	skin	anti-fibrotic (NLRP3 inflammasome activation)
FCGR3A^{+}	TGFB1, FCGR3A, MS4A4A	disease-specific: absent in health, defines severe SSc skin involvement	skin	pro-fibrotic/ pro-phagocytic/ pro-inflammatory (NF-κB pathway; TGF-β pathway)
CD206^{hi}MHCII^{lo}	MERTK, ARG1	Inactive in normal skin, activated in SSc skin fibrosis	skin	pro-fibrotic

of TREM2^{+} Macrophages demonstrates a reduction of several genes related to inflammation as well as fibrosis; meanwhile, an upregulation of genes related to phagocytosis, extracellular matrix remodelling and cell survival, which corresponds to the previous study (50). Critically, this subset highly expresses lipid transport genes (PLTP, APOE), which enhance lipophagy to clear toxic lipids released by damaged cells, and this result is consistent with a recent report that TREM2^{+} macrophages harbour an intrinsic proinflammatory function mediated by MS4A7-dependent NLRP3 inflammasome activation (51). Nevertheless, prolonged lipid droplets exposure and efferocytosis promote further polarization of macrophages toward an anti-inflammatory phenotype, thereby blocking lipotoxicity-induced fibroblast activation (48, 52). However, recent studies also implicate TREM2 in promoting tissue fibrosis, particularly in IPF. Notably, TREM2^{+} macrophages often co-express SPP1, driving M2 polarization via the JAK2/STAT3 signalling pathway, which accelerates lung fibrosis in IPF (53). This overlap between TREM2^{+} and SPP1^{+} macrophage subsets underscores their potential synergistic role in fibrotic niches. Ultimately, whether this synergistic role exists in SSc requires further investigation.

In the bleomycin-induced SSc skin fibrosis mouse model, scRNA-seq combined with flow cytometry identified a dominant CD206^{hi}MHCII^{lo} macrophage population, distinct from classical M2 macrophages, which typically express both CD206 and high MHC II following IL-4/IL-13 stimulation. Neutrophil infiltration is a prerequisite for the accumulation of these macrophages. This subset co-expresses high levels of MerTK, ARG1, IL-10, and TGF-β1, hallmarks of alternative activation, but its notably low MHCII suggests reduced antigen-presenting capacity and skew toward a regulatory, tissue-remodelling phenotype (54). Correspondingly, soluble CD206 levels were elevated in the serum of dcSSc patients, correlating with disease activity (55). Functional inhibition using CD206-targeted peptides significantly reduced TGF-β1 production, reinforcing the pathogenic relevance of this population (56). Collectively, these findings substantiate the central role of CCR1^{+}, MARCO^{+}, TREM2^{+}, FCGR3A^{+}, and CD206^{hi}MHCII^{lo} M2-like macrophages as proliferative, regulatory, and fibrotic actors within the SSc skin immune microenvironment (Table I). Results above illustrate the diversity of macrophage subsets identified by scRNA-seq in SSc-ILD and skin lesions, imply targeting key pathways like IL-6 and TGF-β in these subsets holds promise for mitigating fibrosis progression.

Differentiation source of macrophage subsets

Tissue-resident alveolar macrophages (TR-AMs) originate from embryonic yolk sac and foetal liver progenitors, colonising the lung shortly after birth and persisting throughout their lifespan (57). In contrast, following injury or inflammation, circulating CCR2^{+} monocytes are recruited to the alveoli via chemokines and differentiate into monocyte-derived macrophages (Mo-AMs). Unlike TR-AMs, these monocyte-derived macrophages often exhibit heightened inflammatory and pro-fibrotic gene expression, contributing significantly to fibrotic remodelling in fibrosis diseases such as SSc (58).

To date, there are few lineage-tracing studies for TREM2^{+} macrophages in SSc skin, and it remains controversial whether they derive from circulating monocytes or embryonic progenitors

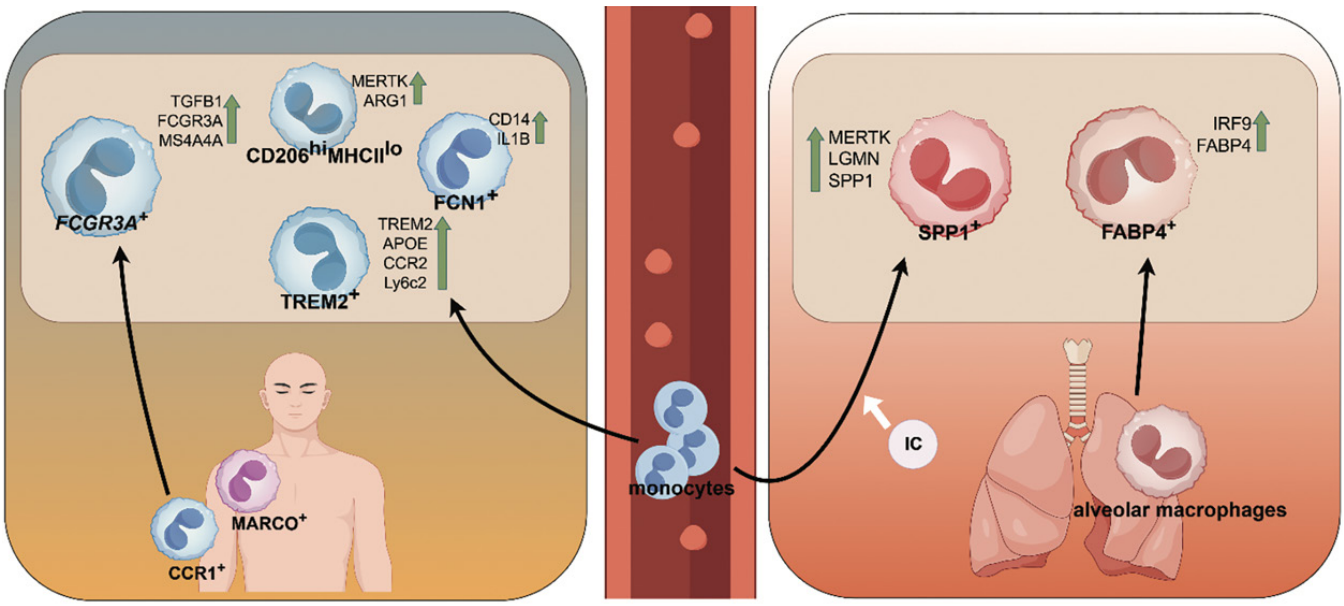

Fig. 3. In the lung, monocyte-derived macrophages adopt pro-fibrotic SPP1$^+$ phenotypes, partially driven by IC stimulation, and alveolar macrophages transition into FABP4$^+$ macrophages under disease conditions. In the skin, monocytes give rise to transcriptionally diverse subsets, including TREM2$^+$, FCN1$^+$ and CD206^{hi}MHCII^{lo} macrophages. Resident CCR1$^+$ and MARCO$^+$ macrophages are also present in healthy skin and may transition into FCGR3A$^+$ macrophages under pathological conditions.
IC: immune complex; CCR1: C-C chemokine receptor type 1; MARCO: macrophage receptor with collagenous structure; FCGR3A: Fcγ receptor IIIa; TREM2: triggering receptor expressed on myeloid cells 2; FCN: Ficolin-1; SPP1: secreted phosphoprotein 1; FABP4: fatty acid-binding protein 4.

(59, 60). scRNA-seq profile detected higher expression of CCR2, LY6C2, and PLAC8 genes in TREM2$^+$ macrophages, indicating a likely origin from circulating monocytes (48).

Trajectory pseudo-time (a scRNA-seq algorithm that tracks the relationship between transcriptomes of single cells) analysis of myeloid cells in SSc patients revealed that FCGR3A$^+$ macrophages are closely related and even admixed with CCR1$^+$ macrophages, while also exhibit spatial and transcriptional proximity to MARCO$^+$ macrophages, suggesting CCR1$^+$ macrophages and/or MARCO$^+$ macrophages may act as progenitors of FCGR3A$^+$ macrophages (33). Notably, both CCR1$^+$ and MARCO$^+$ macrophages are present in healthy skin, where they are known to play essential roles in maintaining tissue homeostasis and promoting wound healing (61). However, under pathological conditions such as SSc, these resident macrophage subsets undergo aberrant activation, proliferation, or phenotypic switching, contributing to the emergence of FCGR3A$^+$ macrophages, driving fibrosis and immune dysregulation (62). This transition underscores the plasticity of tissue-resident macrophages and highlights their context-dependent roles, which may be therapeutically targeted to modulate macrophage polarisation and fibrotic progression in SSc (63).

Emerging evidence implicates CD14$^+$CD68$^+$ monocytes and CD14$^+$ macrophages as pivotal mediators in SSc pathogenesis, with their abundance positively correlating with clinical severity indices such as mRSS and ILD-GAP index (64). Beyond numerical increases, these cells exhibit functional reprogramming toward a pro-fibrotic phenotype. CyTOF-based profiling suggests these monocytes may differentiate into SPP1$^+$ macrophages via a CCL2-dependent axis (65). This CCL2-SPP1-ARG1 signalling axis orchestrates a feedforward loop: CCL2 not only recruits monocytes to fibrotic niches but also induces SPP1 expression, which in turn upregulates ARG1 to potentiate collagen synthesis and myofibroblast activation. Both CCL2 and SPP1 are considered to increase in SSc, positioning them as another promising therapeutic target for SSc (66). Parallel to this, an alternative route delineated by Gao *et al*. (67) demonstrates that circulating monocytes activated by tissue-immobilised immune complexes (ICs) differentiate into OPN$^+$ (also known as SPP1$^+$) macrophages via autocrine M-CSF/IL-6 signalling, whereby IC engagement triggers monocyte morphological transformation, sustained SPP1 secretion, correlating with progressive skin and lung fibrosis in SSc patients. These findings align with recent concepts (68, 69). Further integrating platelet-derived signals, trajectory pseudo-time analysis reveals that CXCL4 released from activated platelets serves as a dual regulator: priming pro-fibrotic monocyte activation and instructing SPP1$^+$ macrophage differentiation through platelet-monocyte crosstalk (70). This aligns with clinical observations of elevated CXCL4 in SSc patients, where its levels correlate with inflammatory signatures and macrophage-driven fibrogenesis (71, 72) (Fig. 3). These findings reveal that most pathogenic macrophage subsets in SSc, including SPP1$^+$, FCGR3A$^+$, and TREM2$^+$ populations, predominantly originate from circulating monocyte recruitment rather than tissue-resident progenitors. Targeting monocyte recruitment axes such as CCL2-CCR2, CXCL4, and immune complex signalling therefore represents a promising strategy to interrupt pathogenic macrophage differentiation and halt fibrotic progression in SSc.

Table II. Enriched fibroblast cell populations in SSc and their clinical outcome.

Fibroblast subtypes	Gene signature	Disease specificity	Pathogenic function	Clinical outcome
SCARA5$^+$	SCARA5	Decreased in SSc	Myofibroblast progenitor	Inversely correlated with the skin fibrosis
POSTN$^+$	POSTN, ACTA2	Increased in SSc	Myofibroblast, Promoting the fibrotic process	Positively correlated with the skin fibrosis
SFRP4/SFRP2$^+$	SFRP4, SFRP2	Increased in SSc	Fibrotic tissue remodelling, coagulation	Positively correlated with the local skin score, mRSS, and STPR
PRSS23/SFRP2$^+$	PRSS23, SFRP2	Increased in SSc	Fibrotic tissue remodelling, coagulation	Positively correlated with the local skin score, mRSS and STPR
COMP$^+$	SFRP2, PTGDS, COMP	Increased in SSc	Fibrotic tissue remodelling, coagulation	Positively correlated with the local skin score, mRSS and STPR
COL11A1$^+$	TNMD, MFAP5, GPC3	Increased in SSc	Fibrotic tissue remodelling, response to pro-fibrotic pathways, and coagulation	Positively correlated with the local skin score, mRSS and STPR
MYOC$^+$	MGP, APOE, CFH	Increased in SSc	Metabolism regulation, reactive oxygen species response	Unknown
CCL19$^+$	CCL19, IGFBP3	Increased in SSc	Inflammation	positively correlated with the local skin score, mRSS and STPR
CXCL12$^+$	PTGDS, APOE, CXCL12	Decreased in SSc	Angio-/vasculo-genesis, immature/stem cell division and proliferation	Inversely correlated with the local skin score, mRSS, and STPR
PI16$^+$	SFRP2, PI16, WISP2	Decreased in SSc	cholesterol metabolism	Inversely correlated with the local s kin score, mRSS, and STPR
ANGPTL7$^+$	PTGDS, CYP1B1, ANGPTL7	Decreased in SSc	Angiogenesis-regulating, vascular permeability	Inversely correlated with collagen thickness
COCH$^+$	COCH, ASPN, SPARCL1	Decreased in SSc	Angio-/vasculo-genesis, endothelium development	Unknown

Spatial interplay and reciprocal activation between fibroblast and macrophage

Fibroblast subpopulations in SSc

Fibroblasts are critical drivers of inflammatory and fibrotic processes in SSc, contributing to disease progression through diverse functional roles (73, 74). scRNA-seq has revealed several dermal fibroblast subtypes with disease-specific transcriptional profiles, each characterised by unique gene signatures and pathogenic contributions in SSc samples (Table II). In early SSc, SFRP4/SFRP2$^+$ and PRSS23/SFRP2$^+$ fibroblasts subsets are enriched, promoting inflammation and ECM remodelling. SFRP4/SFRP2$^+$ fibroblasts express pro-inflammatory chemokines, co-localising with immune infiltrates to facilitate recruitment, while PRSS23/SFRP2$^+$ fibroblasts show increased expression of proteases such as PRSS23, contributing to ECM remodelling in early fibrogenesis. SFRP4/SFRP2$^+$, PRSS23$^+$/SFRP2$^+$, and COMP$^+$ fibroblasts are considered a differentiation continuum, as these cells showed more overlap in gene expression and less distinct expression patterns from each other. Pseudo-time analysis shows a linear progression from SFRP2$^+$ to PRSS23$^+$ fibroblasts, culminating in SFRP2$^+$/PRSS23$^+$/SFRP4$^+$ fibroblasts (75). COMP$^+$ fibroblasts, defined by SFRP2, PTGDS, and COMP expression, are increased in SSc skin, driving fibrotic tissue remodelling and coagulation via IL-10 signalling. COL11A1$^+$ fibroblasts, expressing TNMD, MFAP5 and GPC3, are also enriched in SSc and act as myofibroblast precursors. The abundance of COMP$^+$ and COL11A1$^+$ fibroblasts correlates with clinical severity, as measured by mRSS (76). CCL19$^+$ fibroblasts are a proinflammatory fibroblast population, expressing multiple chemokines and cytokines, promoting immune cell recruitment and activation (77). MYOC$^+$ fibroblast signatures showed prominent regulation of processes relevant to vascular homeostasis and alterations in lipid metabolism; meanwhile, another population with major regulatory effects on vasculature is ANGPTL7$^+$ fibroblasts. The signatures of ANGPTL7$^+$ fibroblasts were inversely correlated with the markers of fibroblast activation and collagen thickness (76). CXCL12$^+$ fibroblasts expressed genes related to immature cells and prominent regulation of IL-10 signalling. PI16$^+$ fibroblasts (previously labelled by LGR5 fibroblasts (78)) were characterised by alterations in cholesterol metabolism and multiple terms related to apoptosis. The proportion of PI16$^+$ and CXCL12$^+$ fibroblast signatures was inversely correlated with the local skin score, the total mRSS, and the skin thickness progression rate (STPR), indicating potential antifibrotic effects of PI16$^+$ and CXCL12$^+$ cells. In contrast, the proportions of SFRP4/SFRP2$^+$, PRSS23/SFRP2$^+$, COMP$^+$, COL11A1$^+$, and CCL19$^+$ fibroblast signatures were positively associated with clinical metrics

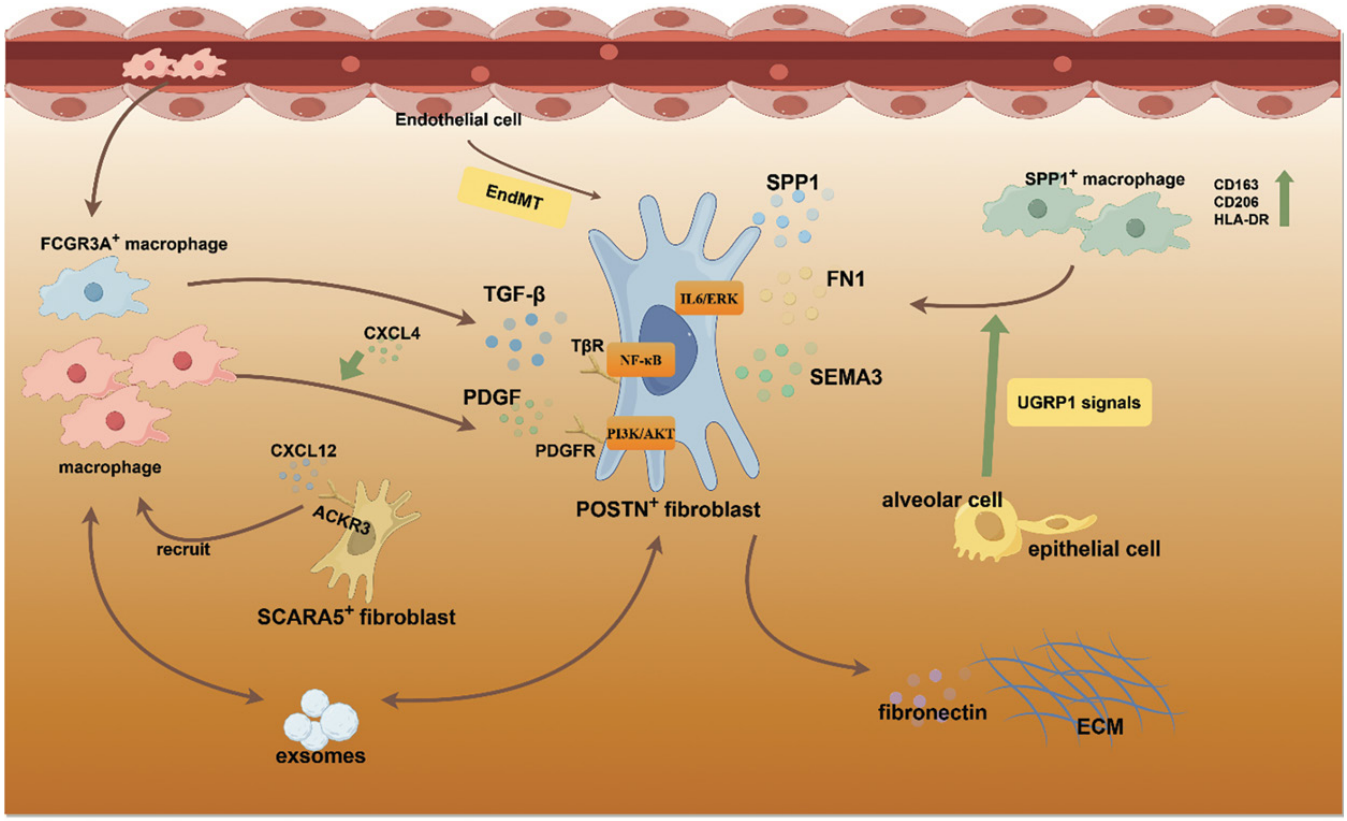

Fig. 4. Once resident in the tissue, these activated macrophages orchestrate a multi-pronged assault on central POSTN⁺ fibroblasts. They secrete canonical pro-fibrotic signals like TGF-β to drive myofibroblast differentiation via pathways including NF-κB, while concurrently releasing PDGF to stimulate fibroblast proliferation through the downstream PI3K-Akt axis. This activation is powerfully amplified by specialised subsets, such as SPP1⁺ macrophages, which release a cocktail of mediators including SPP1, FN1, and SEMA3 to stimulate the IL-6/ERK pathway. The culmination of these convergent signals is the excessive production and deposition of ECM by activated fibroblasts. This pro-fibrotic state is then pathologically sustained through reinforcing mechanisms, including a reciprocal feedback loop mediated by fibroblast-derived exosomes that maintain macrophage activation, and the direct contribution of new myofibroblasts from endothelial cells via EndMT.
PDGF: platelet-derived growth factor; PDGFR: platelet-derived growth factor receptor; PI3K: phosphoinositide 3-kinase; Akt: protein kinase B; CXCL12: C-X-C motif chemokine ligand 12; ACKR3: atypical chemokine receptor 3; TGF-β: transforming growth factor-beta; SPP1: secreted phosphoprotein 1; UGRP1: uteroglobin-related protein 1; FN1: fibronectin 1; SEMA3: semaphorin 3; IL-6: interleukin-6; ERK: extracellular signal-regulated kinase; ECM: extracellular matrix; POSTN: periostin; SCARA5: scavenger receptor class A member 5; FCGR3A: Fc gamma receptor IIIa; EndMT: endothelial-to-mesenchymal transition. (This figure is drafted with Figdraw).

of skin fibrosis, including mRSS and STPR (76). COCH⁺ fibroblasts, seen in both SSc patients and healthy controls (79), expressing COCH, ASPN, and SPARCL1, are decreased in SSc; they show functional profiles in angiogenesis and endothelial development, underscoring their protective role in healthy skin (76). The findings suggest a broad spectrum of fibroblast heterogeneity in SSc, featuring subpopulations with varying pro-fibrotic, inflammatory, or protective functions that align with key clinical indicators like mRSS and skin thickness progression.

Macrophage-fibroblast interactions

In parallel, macrophages are frequently found in close proximity to fibroblasts in affected tissues, suggesting the existence of spatially organised microenvironments where reciprocal interactions between these two cell types may contribute to disease progression (80, 81). While the mechanism of this crosstalk remains to be fully delineated, recent spatial omics studies provide crucial insights by resolving multicellular niches.

Recent advances in spatial omics have begun to illuminate the structure and function of these multicellular niches. A spatial transcriptomics study integrating scRNA-seq profiling and tissue-level mapping identified a macrophage-rich "fibrotic niche" in SSc skin, spatially co-localised with activated fibroblasts and endothelial cells, consistent with results in other pulmonary fibrotic diseases (82, 83).

Within this niche, macrophage-derived PDGF, induced by CXCL4 present in the skin and circulation, appears to engage PDGFR on adjacent fibroblasts (84), further activating PI3K-Akt signalling and forming a reciprocal activation grounded in spatial proximity. This outcome is consistent with the former research (85). In addition, disease-specific SCARA5⁺ fibroblast progenitors were observed in collagen-rich regions alongside proinflammatory macrophages, expressing ACKR3, a decoy receptor for CXCL12, which diminished during differentiation towards mature myofibroblasts (86). This expression pattern may indicate a potentially regulated CXCL12-CXCR4 axis involved in macrophage recruitment/activation and fibroblast differen-

tiation (87). The functional relevance of this pathway is further supported by the observation that PI3K inhibition attenuates dermal thickening in preclinical SSc models (88).

Further *in vitro* studies have explored this reciprocal activation loop. SSc fibroblasts exposed to SSc exosome-activated macrophages produced an increased expression of IL-6, collagen Type I, II, III, IV, and fibronectin, consistent with the proposed role of IL-6 in the early stages of SSc-ILD and the clinical efficacy of IL-6 inhibition. Conversely, macrophages treated with SSc fibroblasts-derived exosomes exhibit elevated surface levels of CD163, CD206, and HLA-DR, along with increased secretion of IL-6, TNF-α, and CCL2 (89). These findings align with reports that $SPP1^+$ macrophage differentiation may be driven by CCL2 signalling (65).

In lung tissue from SSc-ILD patients, $SPP1^+$ macrophages have also been observed adjacent to $POSTN^+$ fibroblasts. In this area, macrophage-derived SPP1, potentially triggered by UGRP1 signals from frontline cells in the lung tissue such as type II alveolar and epithelial cells (34), alongside with FN1 and SEMA3, induced by CXCL4 secretion from platelets, promotes endothelial-to-mesenchymal transition (EndMT) and activation of myofibroblasts, through the IL-6, ERK and downstream PI3K/Akt pathways, inducing myofibroblast expansion and matrix production (70, 90), while inducing B cell autoimmunity (65). This dual pro-fibrotic/autoimmune function is amplified under hypoxia, a signature distinct from IPF, where a similar cluster of macrophages undergoes Th17 differentiation instead (34). Previous studies also demonstrated that ERK inhibition prevents the progression of fibrosis (91).

Notably, both SPP1 and MMP9 serve as key markers of this macrophage population (36, 92) and increased serum levels of MMP-9 and TGF-β have been associated with more severe skin fibrosis in SSc patients (93). Other macrophages have also been shown by spatial transcriptomics to be relevant to fibrosis, for example, $TREM2^+$ macrophages were found to be localised at sites of hepatocellular fibrosis in steatotic liver (59). These findings underscore the dual fibrotic and immune-modulating roles of macrophage-fibroblast interactions within spatially confined niches and suggest that targeting these cellular crosstalk pathways may offer new therapeutic opportunities in SSc (Fig. 4).

Conclusion

In this review we synthesised current insights into macrophage heterogeneity in SSc, with a particular focus on distinct subtypes identified in fibrotic skin and lung tissues. Recent advances in scRNA-seq and spatial transcriptomics have facilitated high-resolution profiling of the myeloid compartment, uncovering a diverse array of macrophage subsets with specialised functions and spatial localisation. Despite their phenotypic diversity, most of these macrophage populations appear to arise from circulating monocytes, highlighting a shared ontogeny across tissues. Notably, $FABP4^+$, $SPP1^+$, and $FCN1^+$ macrophages are predominantly enriched in the lungs of SSc-ILD patients, where they participate in lipid homeostasis, epithelial-mesenchymal transition and inflammatory priming, respectively. In contrast, $CCR1^+$, $MARCO^+$, $TREM2^+$ and $FCGR3A^+$ macrophages are more commonly found in fibrotic skin lesions, contributing to pro-fibrotic remodelling, immune regulation and phagocytic clearance. Among these, $FCN1^+$ and possibly $FCGR3A^+$ macrophages are shared between skin and lung compartments, suggesting they may serve as transitional or migratory subsets. These findings underscore the spatial and functional compartmentalisation of macrophage subsets in SSc and support the notion that tissue microenvironment, rather than origin alone, dictates macrophage phenotype and pathogenic potential. A key challenge in the scRNA-seq analysis is that different studies may use distinct annotations for functionally similar cell populations. After we carefully reviewed the literature, we treated the macrophage subtypes presented herein as unique based on their currently reported gene signatures. Nonetheless, we cannot exclude the possibility that some of these subsets exhibit overlapping gene expression profiles, or that progenitor-mature relationships exist among them. Further studies, particularly those integrating multiple datasets and technologies, will be crucial to fully confirm these potential relationships and establish a unified classification of macrophage heterogeneity in SSc.

One key insight from recent studies is the spatial co-localisation and reciprocal activation of the fibro-immune axis. For instance, the CXCL12-CXCR4 chemokine axis may facilitate targeted recruitment of macrophages into fibrotic niches, while macrophage-derived IL-6 and TGF-β1 drive fibroblast activation and matrix deposition. In this review we highlight that IL-6 is mechanistically linked to specific macrophage subpopulations in SSc pathogenesis: $FCGR3A^+$ macrophages in skin lesions demonstrate NF-κB-dependent IL-6 secretion, while $SPP1^+$ macrophages in lung fibrosis are transcriptionally regulated by IL-6 upstream signalling. These results are consistent with the therapeutic efficacy of IL-6 receptor blockade with tocilizumab in preserving lung function in early SSc-ILD (94). Among all the subpopulations of macrophages in SSc mentioned in this review, $TREM2^+$ macrophages are the only cluster that play a protective role during skin fibrosis in human SSc. However, in other lung fibrosis models such as IPF and bleomycin-induced pulmonary fibrosis model, TREM2 exhibits an opposing role to be strongly negatively correlated with the survival time of IPF patients and blockade/insufficiency of TREM2 protects mice against pulmonary fibrosis (95, 96).

Notably, several problems remain unsolved. Most available data derive from cross-sectional end-stage SSc tissues, limiting our ability to resolve temporal trajectories of macrophage differentiation. For example, recently found $Lyve1^{hi}MHCII^{lo}$ macrophages are not discussed in this review, which is found to play a protective role by maintaining vascular integrity and preventing immune cell infiltration in fibrotic conditions (97). Furthermore, spatial tran-

scriptomics, while powerful, still lacks the resolution to distinguish paracrine from juxtracrine signalling events. While scRNA-seq reveals macrophage heterogeneity, it infers function solely from gene expression, lacking spatial, proteomic and environmental context. Its snapshot nature and computational assumptions limit dynamic insight. Another challenge is the strict requirement for sample quality: fresh tissue samples are deemed appropriate for scRNA-seq, whereas freeze-thaw samples are generally recommended for snRNA-seq, which may not be suitable for subtype identification (98). Correlations with clinical outcomes, such as mRSS and lung function decline, are evident for $SPP1^+$ and $FCGR3A^+$ macrophages, but further studies are needed to validate these associations across larger cohorts. Additionally, widely used murine models only partially recapitulate the complex ontogeny and microenvironment of human macrophages in SSc. Thus, functional roles suggested by scRNA-seq require validation through spatial and *in vivo* studies to ensure biological accuracy. While current research has successfully mapped the transcriptional identities of macrophage subsets in SSc, the upstream regulatory mechanisms that govern their plasticity remain a key area of investigation. Future studies focusing on specific epigenetic modifications, such as histone methylation or chromatin accessibility, will be vital for understanding how pathogenic macrophage phenotypes are established and maintained.

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