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Macrophages as determinants and regulators of fibrosis in systemic sclerosis

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Abstract|

Systemic Sclerosis (SSc) is a multiphase autoimmune disease with a well-known triad of clinical manifestations including vasculopathy, inflammation and fibrosis. Although a plethora of drugs has been suggested as potential candidates to halt SSc progression, nothing has proven clinically efficient. In SSc, both innate and adaptive immune systems are abnormally activated fuelling fibrosis of the skin and other vital organs. Macrophages have been implicated in the pathogenesis of SSc and are thought to be a major source of immune dysregulation. Due to their plasticity, macrophages can initiate and sustain chronic inflammation when classically activated while, simultaneously or parallelly, when alternatively activated they are also capable of secreting fibrotic factors. Here, we briefly explain the polarization process of macrophages. Subsequently, we link the activation of macrophages and monocytes to the molecular pathology of SSc, and illustrate the interplay between macrophages and fibroblasts. Finally, we present recent/near-future clinical trials and discuss novel targets related to macrophages/monocytes activation in SSc.

Graphical Abstract

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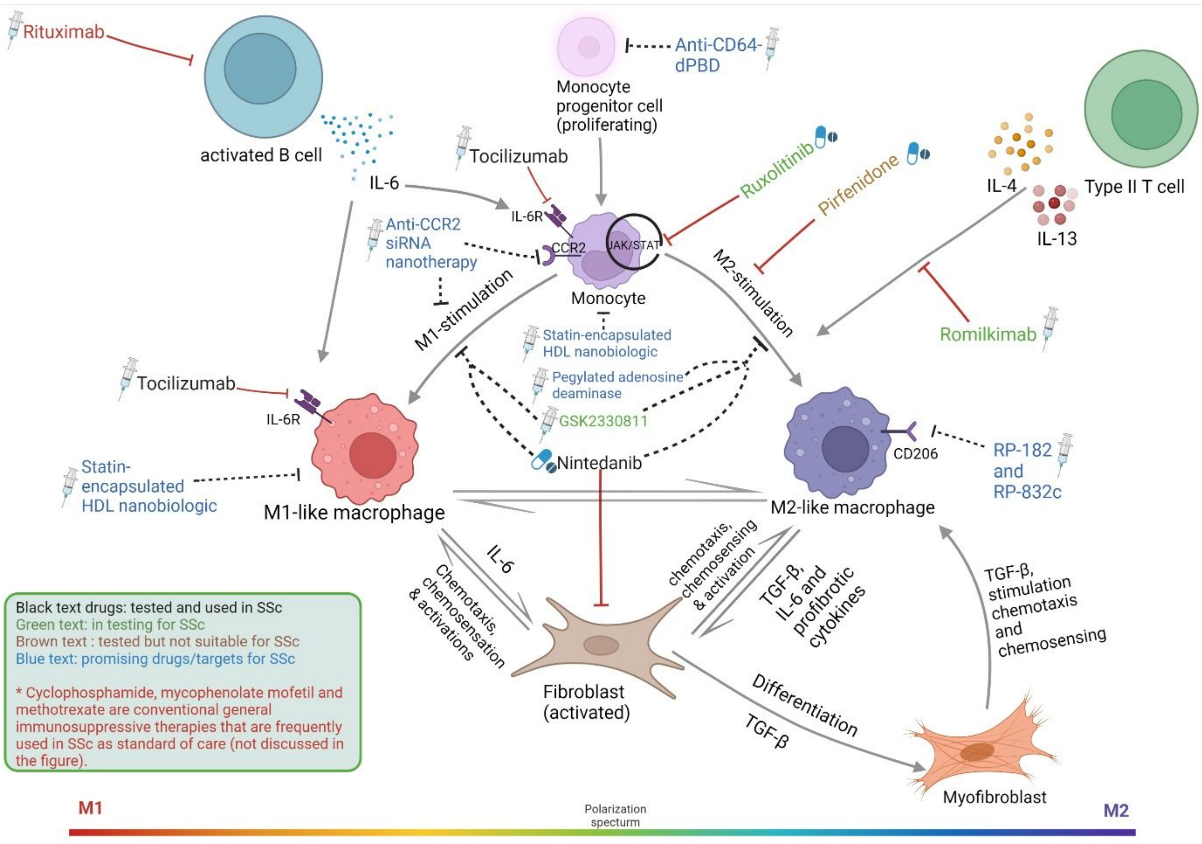

Key words: scleroderma, systemic sclerosis, macrophages, monocytes, fibrosis, potential targeted therapeutics.

Key messages:

- Systemic sclerosis (SSc) is a heterogeneous disease and monocytes/macrophages are central within this heterogeneity.
- Plasticity of monocytes/macrophages allow them to reflect and affect all disease phases of SSc.
- There is an urgent unmet need for personalized medicine to treat SSc patients.

Systemic Sclerosis

Systemic sclerosis (SSc) is an autoimmune disease that involves microangiopathy, early inflammation and progressive fibrosis of the skin and internal organs (1–6). Limited cutaneous (lcSSc), diffuse cutaneous (dcSSc) and sine SSc forms of SSc can present as stable conditions but can also progress to severe disease modes with increased morbidity and mortality (7–9). Although advances have been made in biomarker discovery for SSc, it is still difficult to predict which patients are going to progress to severe fibrotic disease for which no disease-modifying treatment is currently available (9,10). Interstitial lung disease, a result of inflammatory and fibrosing processes, is the leading cause of mortality in SSc patients who develop this complication (~50%) (10,11). Therefore, new treatment strategies are urgently needed to attenuate progression and potentially modify the disease course. Recently, macrophages have captured the interest of the SSc scientific community. This interest is due to the abundance of these cells in affected tissues and their potential in driving both inflammatory, as well as fibrotic processes (12). Macrophages possess great plasticity which allows them to adopt different polarization states.

This review focuses on the polarization dynamics of macrophages and their precursors (monocytes) in SSc, the influence of monocytes and macrophages on the disease course and the role of cytokines in the activation of macrophages in SSc patients. We also discuss the interplay between activated macrophages and fibroblasts. Furthermore, we describe the heterogeneity of the disease and what a multi-phased disease means for the clinic when it comes to treatment. Finally, we discuss how focussing on macrophage polarization could potentially facilitate novel targeted therapy discovery for SSc patients.

Macrophage in health and disease

Macrophages play a principal role in maintaining (physiological) homeostasis by engulfing, degrading and clearing of cellular debris, dead cells, and cancer cells (13). Additionally, macrophages function as reparatory machines, playing an essential role in the wound healing process, allowing quicker post-insult recovery (14). Their capacity of releasing chemoattractants and cytokines to recruit other effector immune cells, make them crucial in terms of host defence response (15).

Macrophage tissue infiltration is a known phenomenon in most autoimmune diseases including SSc (16–24). Accumulating evidence has revealed a crucial role of innate immune reactions in driving not only disease flare ups (causing progression) but also contributing to the ignition processes in such diseases (25,26).

Macrophage polarization

Monocytes are circulating cells, composing around 10% of the cells in healthy peripheral blood, that are known to be precursors of macrophages and dendritic cells forming the mononuclear phagocytic system. CD14 is expressed on the surface of monocytes and is used as a marker to identify them. A complex network of stimuli including cytokines, chemokines and inter-cellular signalling is coordinated in a healthy individual to regulate the differentiation of monocytes to macrophages (27,28).

Historically, macrophages were classified according to their activation pattern into classically activated macrophages (M1) or alternatively activated macrophages (M2). Certain cytokines will polarize/differentiate macrophages into a pro-inflammatory phenotype (classical) which handles pathogen destruction. On the other side of the spectrum, another set of cytokines, chemokines and hormones skew the activation of macrophages into a healing/regenerative phenotype (alternative) which are (pro)fibrotic, anti-inflammatory and in charge of tissue repair (29,30). The contribution of specific factors will be explored later in this review. Recent scientific observations on macrophage classification confirmed that the earlier nomenclature is based on *in vitro* experimentation and does not represent *in vivo* scenarios. Thus, macrophage polarization is rather considered as a continuum than two distinct populations where classical activation is at one end and alternative activation is at the other. Therefore, macrophage phenotypes can slide across this spectrum of the classical/alternative paradigm of activations (31).

Classically activated macrophages maintain inflammation as a defence mechanism to ward off intruders. To be able to function in that manner, classically activated macrophages express a distinct set of surface receptors (see table.1) that allow them to respond adequately to specific stimuli (27,30). An important stimulus for macrophage polarization towards the classical phenotype is interferon-gamma (IFN-γ) through its ability to directly activate effector genes including antiviral proteins, microbicidal molecules, phagocytic receptors, chemokines and cytokines (32). Additionally, IFN-γ can indirectly activate macrophages by enhancing their reaction to other stimuli through what is known as "priming" (32). When macrophages are stimulated with IFN-γ, the result is Janus kinase 1 (JAK 1) and JAK 2 activation, and signal transducer and activator of transcription 1 (STAT1)/interferon regulatory factors (IRF) signalling, leading to differentiation to the classical phenotype as the product (33). This activation pathway is not the only means to produce classical macrophages. During bacterial infections, lipopolysaccharide (LPS) is present abundantly in the body which is well-recognized for stimulating the classical polarization through its binding with toll-like receptors 2 and 4 (TLRs) which in turn initiates nuclear factor-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP-1), IRF and STAT1 signalling (34). Finally, granulocyte-macrophage colony-stimulating factor (GM-CSF) is capable of inducing the classical phenotype through the activation of the JAK2 pathway (35) (Fig.1).

On the other side of the spectrum lies the alternatively activated macrophage phenotype. The central aim of alternative macrophages is to release anti-inflammatory cytokines and recruit specific tissue-regenerating cells (36,37). Activated adaptive immune cells such as mast cells, basophils and type 2 T helper (T_{H2}) cells release IL-4/IL-13 which, in turn, stimulate alternative polarization through the JAK 1 and JAK3/STAT6 pathway. This pathway is considered to be the canonical pathway for alternative activation (38). However, to have a more discrete nomenclature within the alternative phenotype, the IL-4/IL-13-induced activation of macrophages is named M2a (39). Other specific alternative subtypes can be induced by other stimuli such as immune complexes and TLR ligands (40). Such interactions shut down the proinflammatory cytokine IL-12 release and substitute it with the profibrotic cytokine IL-10. This macrophage-activation state involves spleen tyrosine kinase (Syk) and phosphoinositide 3-kinase (PI3K) activation and is known as M2b. The M2b macrophages can release both pro-inflammatory and anti-inflammatory cytokines (41) (see table.1). Both glucocorticoids and IL-10 are able to induce the third subtype of alternative macrophages; M2c through the glucocorticoid receptor (GRC) or IL-10R, respectively. The M2c subtype has a strong fibroproliferative cytokine signature releasing IL-10 and TGF-β cytokines. The fourth subtype is M2d macrophages which are activated through the binding of TLR agonists to the Adenosine 2 receptor. Consequently, significant suppression of pro-inflammatory cytokine release and promotion of anti-inflammatory cytokines production occurs (42) (Fig.1). The detailed macrophage polarization pathways, different cytokine signatures and distinct surface markers have all been previously described elsewhere (43,44).

Monocyte/Macrophage signature in Systemic Sclerosis

Plasticity allows macrophages to influence all phases of SSc. Although limited in number, several studies have investigated the role of macrophage polarization in SSc pathogenesis.

Higashi-Kuwata et al. utilized flow cytometry and immunohistochemistry (IHC) techniques to show that the blood and skin of SSc, respectively, have higher expression of macrophage fibrotic markers (CD163 and CD204) compared to healthy controls. Flow cytometry was used on isolated peripheral blood mononuclear cells (PBMCs) where CD14 surface marker was used to gate for monocytes and CD163 and CD204 surface markers were used to detect the fibrogenic phenotype. Skin biopsies were stained with antibodies against the pan-macrophage surface markers CD68, CD163 and CD204. They found an enhanced expression of CD163 and CD204 on the PBMCs and in skin biopsies from SSc patients compared to controls. Consequently, the authors suggested that the activation status of monocytes/macrophages in SSc patients is profibrotic compared to controls. However, they did not investigate M1 markers to detect classical polarization. (45). Mathai and colleagues showed that CD14 monocytes isolated from PBMCs of SSc-associated interstitial lung disease (SSc-ILD) patients show higher expression of CD163 compared to controls. Interestingly, when CD14+ monocytes were isolated from PBMCs of SSc-ILD patients and treated *in vitro* with LPS, a classical activation inducer, these monocytes were skewed into a more profibrotic pattern in contrast to a proinflammatory profile, with more CD163+, CCL18 and IL-10 expression compared to controls (46). However, as the primary question in this study was whether monocytes from SSc-ILD patients have higher expression of profibrotic markers, inflammatory markers were not studied.

In a monocyte-derived macrophage (MDMs) in vitro transcriptomic study, Morano-Moral et al identified 602 genes that were differentially regulated in SSc patients compared to controls. Upregulated genes were related to hypoxia, glycolysis and mTOR pathways while IFN-γ response pathways were downregulated. This study also highlighted gasdermin A specific variant as an SSc risk factor when upregulated, suggesting that MDMs could be the reason behind dysregulated pyroptosis in SSc. This study robustly links SSc pathogenesis with genetic changes and presents a transcriptomic signature in MDMs of SSc patients (47).

It is known that the affected skin of SSc patients is infiltrated with immune cells, especially T cells and macrophages. To understand the recruitment of macrophages to the skin, researchers have studied the chemokine gene expression in affected skin from SSc patients. RT-qPCR data of homogenised skin showed a higher expression of CCL2, CCL5, CCL18, CCL19 and CXCL13 in dcSSc patients when compared to control skin. Skin biopsies from dcSSc patients exposed a colocalization of CD163+ macrophage subset with CCL19, strongly suggesting the release of CCL19 from the CD163+ macrophage subset. Moreover, not only was CCL2, an important macrophage recruiting chemokine, expression positively correlated with skin thickening in dcSSc skin but also serum levels of CCL2 were elevated in these patients. These localized and systemic correlations are strongly suggestive of the involvement of the alternative macrophage phenotype in the development of skin lesions in SSc. Another group studied patients with SSc-ILD compared to those SSc patients without ILD. There were increased mixed classic (CD80, CD86, TLR2 and TLR4) and alternative (CD206, CD204 and CD163) circulating monocytes in patients with SSc-ILD. (48). Additionally, they found that these markers were significantly elevated on PBMCs isolated from SSc patients compared to healthy controls (49). These results point out that the circulating monocytes from SSc patients have enriched classic and alternative markers compared to controls while this enrichment is even greater when ILD is present.

On a single-cell level, RNA-sequencing of SSc-ILD lung tissue revealed several monocyte/macrophage subgroups in which SPP1^{hi} proliferating macrophages were more predominant in SSc-ILD lungs compared to controls (50). SPP1 macrophages have been attributed to lung fibrosis through the activation of myofibroblasts in idiopathic pulmonary fibrosis (51) which could be having the same role in SSc-ILD. On the same level, RNA-sequencing of dcSSc skin tissues revealed innate immune system activation(52). Specifically, macrophages highly expressing Fcγ receptor IIIA were only associated with dcSSc skin but not in healthy skin. Importantly, the proliferating macrophages were exclusively detected in dcSSc skin but not in healthy ones (52). Thus, it is plausible that proliferating macrophages are fundamental to skin and lung disease progression in SSc.

Due to their plasticity, monocytes/macrophages could be an important link for the transition from the inflammatory to the fibrotic phase in SSc pathology. Perhaps, monocyte/macrophage polarization shifts along the classic/alternative spectrum of activation to a more fibrotic state over time due to intracellular changes and differential presence of cytokines and chemokines in their environment. The change in the cytokine and chemokine profile can be attributed to reactive B cells (IL-6 release) and activated CD4+ T_{H2} cells (IL-13 and IL-4 release) (53,54). Consequently, monocytes/macrophages become profibrotic and start to release fibrotic factors that lead to the activation of more monocyte/macrophages (and of fibroblasts) into the profibrotic phenotype generating an autocrine loop. Moreover, the mounting recruitment of fibrogenesis-effector cells such as fibrocytes and fibroblasts into affected tissues, and their activation by the released fibroproliferative chemokines are key events in SSc- related tissue fibrosis.

<u>The interplay between fibroblasts and macrophages</u>

Activation of monocytes/macrophages is crucial for stimulation of the fibrosis effector cells (fibroblasts) in affected tissues. Bhandari et al. (55) showed that the activation of fibroblasts is dependent on SSc plasma-differentiated macrophages. SSc plasma significantly activated monocytes from both control and SSc groups into the profibrotic (alternative) phenotypes when compared to monocytes cultured with control plasma. Moreover, significantly higher mRNA and protein expression and production of CCL2, IL-6 and TGF-β were reported in SSc plasma-cultured compared to control-cultured monocytes. This experiment illustrated that SSc plasma can differentiate control monocytes into SSc phenotype macrophages. Additionally, RT-qPCR data from dermal fibroblasts revealed overexpression of α-SMA in SSc fibroblasts co-cultured with SSc plasma-differentiated macrophages, compared to healthy dermal fibroblasts co-cultured with SSc plasma-differentiated macrophages. These data indicate that SSc macrophages induce and activate dermal fibroblasts into becoming fibrogenic cells through fibroblast to myofibroblast transdifferentiation (55).

In *in vivo* scenarios, macrophages have to be in close proximity to fibroblasts to stimulate them to myofibroblasts (56). Pakshir et al (57) described how mechanosensation and integrins help myo/fibroblasts attract macrophages to the vicinity of the fibrotic niche. Fibroblasts establish ECM cues through remodeling collagens to form deformation fields in the collagen mesh which guides macrophages to come closer to myo/fibroblasts. Importantly, these ECM alterations have more far-reaching effects than chemotaxis.

Thus, the interaction between macrophages and myo/fibroblasts is necessary to establish a progressive fibrotic niche.

<u>Emerging role of oxidative stress in monocyte/macrophage polarization in SSc</u>

Antioxidant/oxidant imbalance is thought to be connected to SSc pathogenesis (58) The nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf-2) is an important cellular sensing protein for oxidative stress which- in turn- can stimulate the transcription of antioxidants including glutathione (GSH). In an SSc mouse model, Nrf$^{-/-}$ knockdown and wild-type (control) mice were intradermally injected with hypochloric acid (a substance to induce oxidative stress). The Nrf$^{-/-}$ mice showed more severe inflammation and fibrosis than controls. Importantly, in the skin of hypochloric acid-treated mice, the Nrf$^{-/-}$ type had a more pronounced M2 polarization marker profile than the wild-type mice. This indicates that oxidative stress induces a shift towards M2 polarization and suggests a strong link between Nrf-2 function, alternative polarization, fibroblasts activation and fibrogenesis (59).

Systemic Sclerosis is a multi-phase disease – Interventional remarks focusing on targeting monocytes/macrophages (summary of current and prospective/promising SSc therapeutics is stated in table.2)

SSc-investigated targeted therapies

In SSc patients who present early in their disease course, inflammation is generally the predominant process activated, especially in progressive dcSSc. As elaborated, M1 phenotype macrophage activation may be central at this stage, and it would be reasonable to introduce drugs that target the effector pathways early. In patients with SSc-ILD, monocytes are known to produce higher amounts of IL-6 compared to healthy controls (60). This high production is strongly associated with SSc pathogenesis since it leads to the activation, differentiation and proliferation of T lymphocytes. Tocilizumab (anti-IL-6R monoclonal antibody) is of growing interest and use in clinical practice. It is FDA-approved to slow the rate of decline of lung function in adult patients with SSc-ILD (61,62). Moreover, IL-6 is abundantly produced by activated B cells expressing CD20. The DESIRES RCT showed that using rituximab, an anti-CD20 monoclonal antibody, in SSc patients resulted in a significant reduction in mRSS compared to the placebo arm (63). Rituximab's positive results can be indirectly attributed to the blockage of macrophage polarization leading to mitigated skin and lung disease (64,65).

An IFN type I serum profile is related to higher mRSS and HAQ-Disability Index. Researchers have indirectly targeted IFN type I by targeting CD52. In a translational study, transcriptomic analyses of circulating CD14+ monocytes obtained from SSc patients revealed enhanced expression of IFN I-related genes compared to healthy controls. These monocytes also displayed down-regulated CD52 expression which is an important T cell inhibitory antigen. Interestingly, when healthy monocytes were treated with an anti-CD52 antibody, enhanced activation of IFN I pathways were achieved. Consequently, targeting the CD52-IFN I pathway is a promising approach in early SSc patients (66).

Targeting the profibrotic cytokines IL-4 and IL-13 to prevent further activation of monocytes/macrophages to the profibrotic forms has shown promising results. Romilkimab was developed as a humanized bispecific mAB against both IL-4 and IL-13. When neutralizing these serum elevated cytokines, the paracrine and autocrine activation loops of macrophages are blocked. Indeed, a phase 2 RCT in early dcSSc was performed where romilkimab efficacy was tested vs placebo. After 24 weeks, patients who have been treated with romilkimab had a significant improvement in their mRSS compared to the placebo group (67).

Although it is known to work as a multi tyrosine kinase inhibitor, nintedanib also functions by disturbing the expression of surface markers, and/or the chemokine and cytokine signature of monocyte-derived macrophages. It also inhibits the phosphorylation of the colony-stimulating factor 1 receptor in monocyte/macrophages which is essential in activation and polarization of these cells. When monocytes were stimulated to polarize to classical or alternative macrophages -*in vitro*- subsequent to treatment with nintedanib, several alterations were observed. First, classical macrophages continued to express classical surface markers at the same level as untreated macrophages but released significantly less proinflammatory cytokines. Second, the alternatively-stimulated macrophages had a significant decrease in their M2 markers while their profibrotic cytokines and chemokines release remained comparable to untreated alternative macrophages (68,69). Clinically, Azuma et al. (70) performed a phase 3 RCT where SSc patients with at least 10% lung fibrosis on HRCT were included. The primary endpoint was the annual rate of decline in FVC. After 52 weeks, the annual rate of decline in FVC was significantly higher in the placebo arm than in the treatment arm. Based on these data, nintedanib was the first drug to be approved for treating SSc-ILD (71–73).

Pirfenidone has shown inhibitory effects on rat alternatively-activated lung macrophages cultured *in vitro.* This was demonstrated by a significant reduction of TGF-β release and lower expression of M2 surface markers when macrophages were treated with pirfenidone. When the supernatant of the pirfenidone-treated macrophages was used to treat rat lung fibroblasts, suppressed proliferation, and collagen mRNA expression and production were observed in these fibroblasts (74). In light of these data, Khanna et al. (75) and Sharma et al. (76) performed phase 2 clinical trials to assess the efficacy of pirfenidone in SSc-ILD patients. Although the data from these trials have not shown significant differences in lung function decline between the treatment and placebo groups, pirfenidone was well tolerable and appeared likely to maintain lung function better than the placebo.

<u>Current studies</u>

Several studies are currently investigating pharmaceutical agents that could hinder the activation and/or the release of cytokines/chemokines from monocytes/macrophages. For example, in the "Hit hard and early" study [NCT03059979], very early diagnosed SSc (VEDOSS) patients are being treated with high dose methylprednisolone, potentially preventing early vasculopathy by forcing attenuation of inflammation (77). It is also highly plausible that the mechanism behind this strategy is mitigating the polarization of macrophages towards the classical inflammatory phenotype, as this is a known effect of prednisolone (78,79). Another strategy is being investigated using upfront autologous hematopoietic stem cell transplantation (AHSCT) in early dcSSc patients with the aim of resetting the immune system (the UPSIDE study; [NCT04464434]) (80). Monocytes derived from myeloid progenitors are activated and play a role in igniting and perpetuating the inflammatory, and thereafter the fibrosis processes, in SSc patients. Studies show that dcSSc patients have higher expression of CD16+ monocytes which are known to have enhanced pro-inflammatory activation (81). The upfront depletion of these cells, coupled with replacing them with "normal/undiseased" precursors through AHSCT could yield monocytes that can suppress the pathogenetic pathways of SSc by enhancing T_{regs} cell production, inhibiting fibroblast to myofibroblast transdifferentiation and suppressing CD4 T cells proliferation (82).

The SCLERO JAK [NCT04206644] study is investigating the efficacy of the JAK 1/2 inhibitor ruxolitinib in SSc patients. one of the outcomes aims to gather a greater understanding of the impact of this drug on the activation states of monocyte-derived macrophages obtained from SSc patients. It is hypothesized that blocking the JAK-STAT pathway would attenuate the profibrotic properties of monocyte-derived macrophages. This will be tested in an *in vitro* model by measuring CCL18 levels in the culture media of ruxolitinib-treated SSc macrophages compared to untreated (as a primary outcome). In addition, macrophage surface markers studies will be performed.

GSK2330811 is a humanized monoclonal antibody against the oncostatin M (OSM) protein, which is implicated in inflammation, fibrosis and vasculopathy, typical features of SSc pathogenesis. Activated monocytes/macrophages are known to produce OSM which alters fibroblasts -among other connective tissue cells- production of cytokines and chemokines such as MCP-1 and IL-6 which in turn affects the polarization of macrophages (paracrine activation loop) (83,84). After showing a well-tolerated safety profile in phase I clinical trial (85), proof of mechanism phase II randomized clinical trial [NCT03041025] in dcSSc patients is currently being undertaken and it is hoped the data will be available soon.

Future perspectives – promising therapeutics/pathways

SSc upstream processes involve both the innate and adaptive immune systems. repurposing drugs from other medical fields such as oncology and haematology to the field of autoimmune diseases is not unusual. Therefore, we are suggesting the following potential drugs abide the recent understanding of SSc pathogenesis.

Targeting purinergic signalling may ameliorate fibrosis. As explained above, adenosine can skew macrophages towards the alternative phenotype. Degradation of adenosine using pegylated recombinant adenosine deaminase reduced fibrogenesis in SSc preclinical models. Adenosine deaminase has also shown promising results regarding vasculopathy and inflammation in a mouse model of SSc (86). The effects of such a drug should be examined in a clinical trial to better comprehend its potential efficacy in SSc patients.

Direct targeting of proliferating monocyte progenitor cells without affecting other progenitor cells or mature monocytes could be an approach to diminish monocytes' contribution to pathophysiology in SSc patients. Using dimeric pyrrolobenzodiazepine (dPBD)-conjugated anti-CD64 antibody (anti-CD64-dPBD), Izumi et al (87) were able to selectively induce apoptosis in proliferating human monocyte progenitors.

Targeting the migration of inflammatory monocytes to sites of injury using small interfering RNA (siRNA) is another promising approach that could benefit SSc patients. CCR2 chemokine receptor is known to be over-expressed on inflammatory monocytes. Targeting cells with high levels of this receptor with nanoparticles-containing anti-CCR2 siRNA showed promising results in several inflammatory diseases in preclinical settings. In these preclinical models, anti-CCR2 siRNA was able to silence CCR2 mRNA of inflammatory monocytes and consequently reduced migration as well as numbers of monocyte-derived macrophages without affecting other healing, physiologically essential functions of monocytes and associated macrophages (88).

Non-specific memory of the innate immune system, also as known as “Trained immunity”, is thought to be part of the enhanced and continuity of cytokines and chemokines production by monocytes in SSc patients. Pharmacological blocking of upstream processes of trained immunity using NOD2 and dectin 1 inhibitors, GSK669 or laminarin, respectively could be beneficial (89). Additionally, nanomedicine could offer another novel approach for directly targeting and skewing localized inflammatory monocytes to a less inflammatory phenotype through limiting epigenetic and metabolic changes (89). Statin-encapsulated reconstituted high-density lipoprotein (HDL) nanobiologic is a promising tool targeting inflammatory monocytes and macrophages. Such a drug has shown promising results in inflammatory atherosclerotic plaques (90) but has not yet been applied to autoimmune diseases including SSc.

Reprogramming alternatively-activated macrophages towards apoptosis or classical polarization is a well-characterized strategy in tumour research. RP-182 and RP-832c, host immune peptides, can target CD206 alternative MDMs in lung fibrosis leading to alleviation of fibrogenesis. This mechanism could be beneficial for SSc patients, especially for those who are suffering from dermal and lung fibrosis (91,92).

Conclusion

SSc is a multi-organ, multi-phase disease with various potential pharmaceutical interventions. In order to combat its complications, it is first necessary to identify the phase of the disease. Interpretations of literature and previous research highlight monocytes/macrophages as promising biomarkers that dynamically change according to disease progression reflecting disease status. They can also be considered as potential therapeutic targets through modulation of their polarization.

Due to the heterogeneity of SSc pathogenesis, examination of SSc patients must recognise that each patient is a unique case. This is a unique opportunity to address the unmet need for personalized medicine in treating SSc patients. Most SSc complications share similar phenotypical and molecular characteristics, however, several important differences have been observed when it comes to progression and initiating factors. Finally, although this personalized approach is still under development, each SSc patient requires a special set of therapeutics according to their disease phase, active pathogenesis pathway, and number and type of complications.

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Conflict of interest:

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Data availability statement

Data are available upon reasonable request.

Table.1: Differences in surface markers and cytokine signatures in classical and alternative (phenotypes) macrophages

Macrophage phenotype	Surface markers	Cytokine signature
Classical	CD86, CD68, CD80, MHC-II, TLR-2, TLR-4, IL-1R	IL-1β, IL-6, IL-12, IL-18, IL-23, TNF-α
Alternative (M2a)	CD206, CD36, CD163, IL-1R	IL-10, TGF-β
Alternative (M2b)	CD86, MHC-II	TNF-α, IL-1β, IL-6, IL-10
Alternative (M2c)	CD206, TLR-1, TLR-8, CD163	IL-10, TGF-β
Alternative (M2d)	CD206, CD204, CD163	IL-10, VEGF

Table.2: SSc-investigated targeted therapies and suggested novel monocyte/macrophage targeted therapies in the treatment of systemic sclerosis and systemic sclerosis-associated interstitial lung disease

Therapeutic	Target (action)
AHSCT	resetting myeloid progenitor cells including monocytes
Tocilizumab	Anti-IL-6 receptor α-subunit (attenuates monocyte downstream effects)
Rituximab	Anti-CD20 “B cells depletion” (attenuates downstream macrophage polarization)
Romilkimab	Anti- IL-4 and IL-13 cytokines (blocks alternative activation)
Nintedanib	Multi-tyrosine kinase inhibitor (disturbs classical and alternative activations)
Pirfenidone	Blocks alternative activation
Ruxolitinib	JAK 1/2 inhibitor (proposed to attenuate alternative activation)
GSK2330811	Anti-oncostatin M protein (attenuates monocytes downstream effects)
Pegylated adenosine deaminase	adenosine molecules (blocks alternative polarization)
anti-CD64-dPBD	proliferating monocyte progenitors
anti-CCR2 siRNA nanotherapy	inflammatory monocytes migration
Statin-encapsulated HDL nanobiologic	inflammatory monocytes systemically and inflammatory macrophages locally
RP-182 and RP-832c	CD206+ cells “alternatively-activated macrophages”

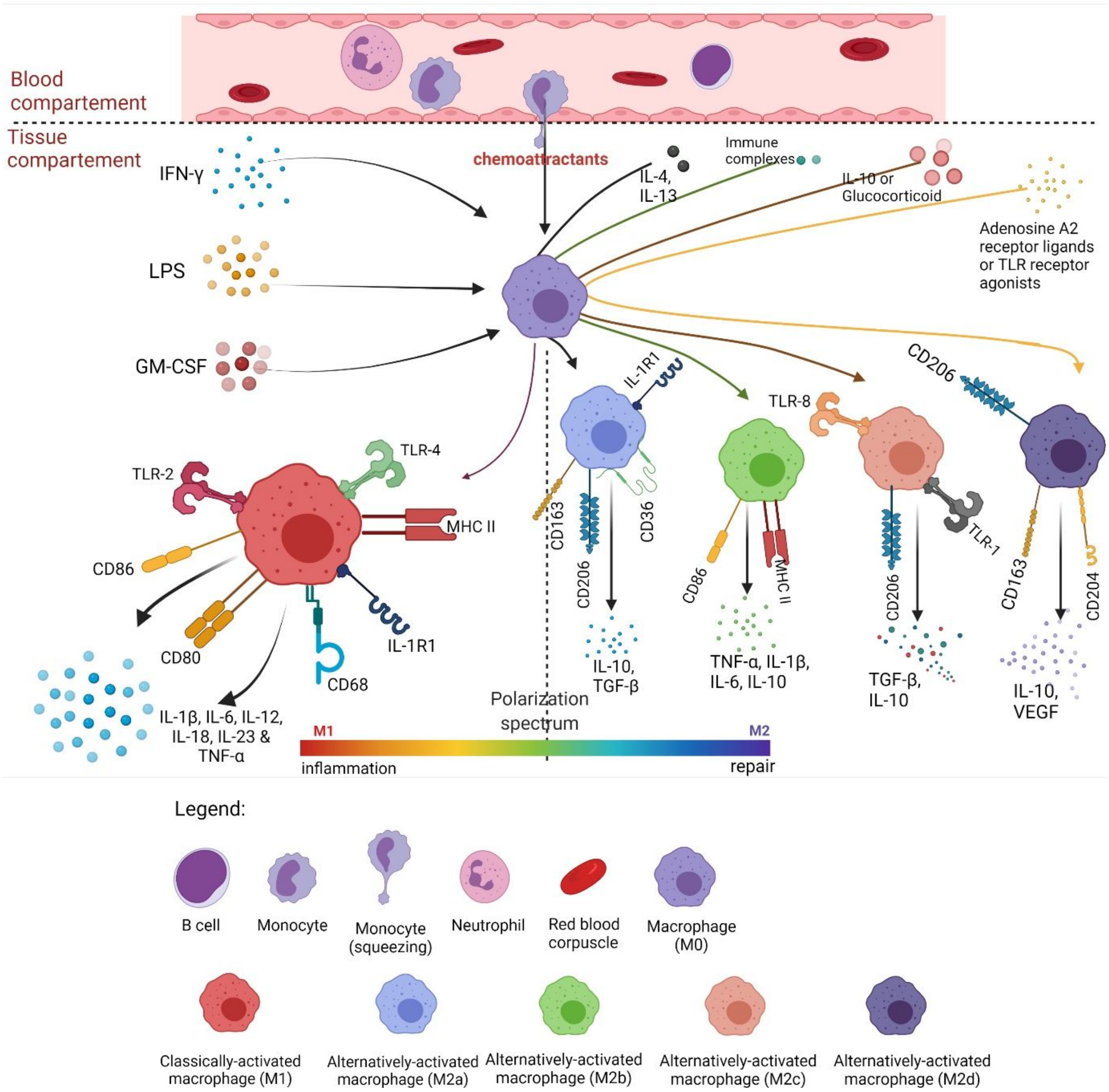

Figure 1: Macrophage polarization.

Monocytes are attracted to injury sites (e.g., infected tissue) by chemoattractants. Thereafter, macrophages are activated dependent on the cytokines/stimuli in the milieu to M1 (classic activation) or M2 (alternative activation). Polarization state of macrophages is rather a dynamic process where macrophages can shift along a polarization spectrum. Activated macrophages express a specific set of (surface) markers and release particular collection of cytokines according to the activation pattern which in turn affects the milieu. CD: Cluster of Differentiation, IL: Interleukin, TGF-β: Transforming Growth Factor-beta, TLR: Toll-Like Receptor, IFN: Interferon, MHC II: Major Histocompatibility Complex class II, VEGF: Vascular Endothelial Growth Factor, TNF-α: Tumor Necrosis Factor-alpha, LPS: Lipopolysaccharide, GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor. Created with BioRender.com

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