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Update on macrophages and innate immunity in scleroderma

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Abstract

Purpose of review—In this review of the literature from 2014 through mid-2015, we examine new data that sheds light on how macrophages and other innate immune cells and signals contribute to inflammation, vascular dysfunction and fibrosis in scleroderma.

Recent findings—Recent human studies have focused on changes early in scleroderma, and linked macrophages to inflammation in skin and progression of lung disease. Plasmacytoid DCs have been implicated in vascular dysfunction. In mice, several factors have been identified that influence macrophage activation and experimental fibrosis. However, emerging data also suggests that myeloid cells can have differential effects in fibrosis. Sustained signaling through different TLRs can lead to inflammation or fibrosis, and these signals can influence both immune and non-immune cells.

Summary—There are many types of innate immune cells that can potentially contribute to scleroderma and will be worth exploring in detail. Experimentally dissecting the roles of macrophages based on ontogeny and activation state, and the innate signaling pathways in the tissue microenvironment, may also lead to better understanding of scleroderma pathogenesis.

Keywords

Macrophage; innate immunity; scleroderma; fibrosis

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Conflicts of interest

None

Introduction

Immune dysfunction of many kinds has been identified in scleroderma, with the innate immune system of significant interest.[1–4] Macrophages in particular have been implicated, and a variety of other innate immune cells including dendritic cells, monocyte-derived cells, innate lymphocytes, natural killer cells, and mast cells, may also be of interest.[5–9*] In addition, innate immune signaling involving chemokines, interferons (IFN), interleukins, pattern recognition receptors and others may contribute to the pathogenesis of scleroderma.[10–12] In this review of the literature from 2014 through mid-2015, we examine new data that sheds light on how macrophages and other innate immune cells and signals contribute to inflammation, vascular dysfunction and fibrosis in scleroderma.

Macrophage ontogeny and activation nomenclature

Macrophages have been studied in scleroderma and tissue fibrosis, and understanding of this complex cell type is rapidly evolving.[1,5,13–15] There has been a recent burgeoning of the understanding of macrophage ontogeny. Tissue resident macrophages are now known to originate from progenitors in the yolk sac and fetal liver; additionally, they self renew *in situ* independently of hematopoiesis in most tissues examined, even under certain inflammatory conditions.[9*,15–20] Murine dermis is an important exception, with a significant proportion of macrophages arising from circulating progenitors.[16**] In contrast, other myeloid cells are derived from hematopoietic stem cells via a well-characterized differentiation program.[9*,17,18,21] Thus, each tissue has a unique and complex composition of macrophages of embryonic and adult origin, and studies that examine how ontogenic origin impacts macrophage function may be useful in understanding scleroderma pathogenesis.

In addition to advances in understanding macrophage ontogeny, the biology of macrophage activation is also progressing. Macrophage activation is now thought to be a continuous spectrum of activation states, rather than two opposing phenotypes: M1 (classically activated) versus M2 (alternatively activated).[22] To effectively communicate this complexity, new nomenclature has been proposed for describing macrophage activation based on reference to known phenotypes along the spectrum: at one end, macrophages activated with IFNγ (prototypical M1) would be now be termed M(IFNγ), while macrophages activated with IL-4 (prototypical M2) at the other end of the spectrum would be M(IL-4). *In vivo*, macrophage activation state may be evaluated with markers that are already widely used in the study of macrophages and in scleroderma, and compared to reference points.[22] One such marker is Arginase 1 (Arg1), which is commonly used as a marker of M(IL-4)-type macrophage activation; however, it may be expressed by macrophages throughout the activation spectrum.[22] Similarly, CD163 is a scavenger receptor associated with scleroderma and M(IL-4)-like activation, though it is also upregulated upon IL-10 or glucocorticoid stimulation.[22–24] Thus, describing several features of a particular population may be needed to fully phenotype macrophages *in vivo*.

Macrophage association with inflammation and early fibrosis

Recent studies of human scleroderma patients early in disease course have demonstrated an association between macrophages, inflammation and fibrosis in lung and skin. In lung tissue obtained via open biopsy, Christmann and colleagues identified genes that distinguished SSc from normal control, then correlated expression changes with progression of lung disease as determined by high-resolution computed tomography and pulmonary function tests.[25**] In SSc patients with non-specific interstitial pneumonia, collagen, IFN-regulated and macrophage-associated gene clusters were positively correlated with progressive lung disease.[25**] The macrophage-associated gene cluster included transcripts associated with M(IL-4) activation.[22,25**] Notably, this study evaluated lung tissue before end-stage fibrosis, and thus may implicate macrophages and IFN in development or progression of SSc lung fibrosis.

In a microarray study designed to identify chemokine changes in scleroderma skin, Mathes and colleagues identified CCL19 as the only one of 6 chemokines upregulated in dsSSC skin that is positively correlated with perivascular inflammation, as well as with markers of vascular injury.[26*] CD163+ macrophages, but not fibroblasts, co-localized with CCL19 in dsSSC skin.[26*] CCL19 along with CCL21 interacts with receptor CCR7 on antigen presenting cells, and is known to play a key role in recruitment of immune cells to lymph node and inflamed non-lymphoid tissues.[26*–31] Together, this suggests that CD163+ macrophages were key expressers of CCL19, and implicates them in the recruitment of inflammatory cells to the perivascular environment in skin fibrosis.

Intriguingly, the chemokines found to be upregulated in this study are associated with a range of macrophage activation states.[22,26*] Novel upregulated chemokines identified by the study were CCL18, CCL19 and CXCL13, while the upregulation of CCL2, CCL5 and CXCL9 noted in previous studies was confirmed.[26*,32–36] CCL18 and CXCL13 are associated with M(IL-4) activation, while CCL5 and CXCL9 expression are more typical of M(LPS + IFNγ) inflammatory macrophages towards the other end of the activation spectrum.[22] While these chemokines may be expressed by many cell types, this pattern of expression could potentially reflect the involvement of multiple differentially activated myeloid populations in modulating the local immune environment in scleroderma skin.

Macrophage activation and function in fibrosis

Identification of macrophages resembling an M(IL-4) phenotype has been recognized in scleroderma, and several recent studies delineate upstream changes affecting macrophage activation that could contribute to fibrosis.[5,14,23,37,38] Wu and colleagues found a role for Cadherin11, which has been implicated in both skin and pulmonary fibrosis, in TGFβ1 production by M(IL-4) macrophages.[39*–42] Cadherin11 mRNA positively correlated with MRSS in patients with disease duration <4 years, and with TGFβ-induced genes in early diffuse SSc.[39*] Data from individual cultures of macrophages or fibroblasts showed that Cadherin11 is important for macrophage secretion of TGFβ1 upon IL-4 stimulation, but not for TGFβ1 signaling in fibroblasts.[39*] As anti-Cadherin11 blockade led to decreased dermal thickness and fewer myofibroblasts in established fibrosis, further study is warranted

to determine if Cadherin11 blockade may be therapeutically useful, and to understand how Cadherin11 expression in macrophages affects TGFβ1 secretion.[39*]

The enzyme N-acetylglucosaminyltransferase-V (GnT-V) that forms β1,6 GlcNAc-bearing oligosaccharides is important for negative regulation of T cells and suppressing autoimmunity.[43,44] In a small group of lcSSc skin biopsies, GnT-V was partially co-expressed with CD163+ macrophages.[43] Experiments in the bleomycin model suggest a role for GnT-V in promoting CD163+ macrophage accumulation in murine skin, while *in vitro* data points to a possible role for GnT-V in establishing an M(IL-4) phenotype.[43] It may be useful to understand how oligosaccharides influence macrophage phenotype, perhaps by interaction with galectin-3, a known binding partner of glycosphingolipids that is implicated in macrophage activation.[45,46] Alternatively, oligosaccharide structures may influence macrophage phenotype by altering integrin-mediated cell adhesion. GnT-V-driven β1,6 GlcNAc branching is known to influence integrin-mediated cell adhesion, which in turn affects macrophage phenotype.[47–51]

Enhanced M(IL-4)-type macrophage activation was also found in Fli1 haploinsufficient mice, with more Arg1+ macrophages present in bleomycin treated skin and cultured peritoneal macrophages upregulating *Arg1, Relma* (*Fizz1)* and *Chi3l3* (*YM1*) after IL-4 or IL-13 stimulation.[52] Fli1 is a transcription factor that is down-regulated in SSc skin, and insufficiency of Fli1 has been linked to SSc-related changes in fibroblasts and endothelial cells.[53–57] Fli1 may have a broader role in skin than previously hypothesized, influencing macrophage activation in addition to effecting pro-fibrotic changes in stromal cells.

Differential roles for myeloid subpopulations in fibrosis

In recent years there has been a growing appreciation of the diversity of macrophages in peripheral tissues, and the pleiotropic effects of macrophages and dendritic cells on tissue fibrosis have been reviewed.[3,9*,14–20,22,58–62] New data from the study of heart and liver further support the hypothesis that separate myeloid lineages play differing roles in fibrosis. In schistsomiasis-associated liver fibrosis, there are two populations of macrophages that express the M(IL-4)-like transcripts *Arg1*, *Relma*, *Chi3l3* and *Mrc1*.[63*] These populations consist of immature macrophages (either proliferating from resident macrophages or monocyte-derived) that slow the progression of liver fibrosis, whereas a mature and perhaps resident macrophage population limits inflammation.[63*] Similarly, monocytes can also have multiple functions in fibrosis. In a study of hypertensive myocardial fibrosis induced with angiotensin II, depletion of all monocytes using clodronate liposomes was protective, while *Cx3cr1*$^{-/-}$ mice, in which CX_3CR1^{hi} but not CX_3 $CR1^{lo}$ monocytes are reduced, developed more severe disease.[64,65] While the interpretation of this study is complicated by the possibility that CD11b+ CX_3CR1+ dendritic cells may also be functionally altered in *Cx3cr1*$^{-/-}$ mice, the data nonetheless suggest that at least 2 populations of myeloid cells are exerting opposing actions in hypertensive myocardial fibrosis.[9*,66]

These studies add to the accumulating literature that describes pleiotropic functions of myeloid cells in fibrosis. Studying individual populations of monocytes, macrophages and dendritic cells may lead to disambiguation of their roles in this complex disease process.

Plasmacytoid DC as mediators of inflammation and endothelial damage

Plasmacytoid dendritic cells (pDC) have been implicated in the tight skin genetic model of skin fibrosis, and VanBon and colleagues recently delineated a role for these cells in human scleroderma.[67,68**] The investigators found that the chemokine CXCL4 is highly secreted by circulating pDC of SSc patients, but not healthy donors.[68**] CXCL4 levels in plasma were especially high in early diffuse SSc, and correlated with the extent of skin fibrosis in both limited and diffuse SSc.[68**] In SSc lesional skin, CXCL4 co-localized with pDC; Mathes and colleagues identified the CXCL4 receptor CXCR3 as upregulated >1.5-fold in dsSSc skin.[26*,68**–70] CXCL4 in SSc plasma was sufficient to induce endothelin-1 and decrease Fli1 in cultured endothelial cells, both associated with endothelial damage in fibrosis.[57,68**,71–73] CXCL4 has already been described as having angiostatic properties and this study reports that CXCL4 inhibited endothelial cell proliferation *in vitro*.[68**,74] CXCL4 drove hyper-secretion of type I IFN by SSc pDC, and as type I IFN is known to be angiostatic, these findings imply both direct and indirect mechanisms for CXCL4-induced vascular dysfunction.[68**] Interestingly, elevated CXCL4 was also identified in patients with Raynaud's Syndrome, most of whom do not develop SSc despite the vascular dysfunction underlying Raynaud's.[1,68**,75] In healthy mice, CXCL4 administration increased mRNA of the anti-angiogenic molecule thrombospondin-1.[68**] Together, these data suggest a potential novel role for pDC and pDC-derived CXCL4 in vascular dysfunction in SSc.

Toll-like Receptor signaling as a driver of inflammation and fibrosis in scleroderma

Toll-like Receptors (TLRs) are a family of pattern recognition receptors that relay exogenous and endogenous danger signals, and have become a topic of significant interest in scleroderma.[12,76,77] Several recent studies place TLR signaling upstream of inflammatory and pro-fibrotic changes in skin.

TLRs 3,7,8 and 9 are localized to intracellular organelles where they recognize nucleic acids; recent findings have implicated this group of TLRs in driving inflammation associated with scleroderma.[68**,76,78] The TLR9 agonist CpGB DNA delivered via osmotic pump led to inflammation but not fibrosis.[78] Consistent with this, in their study of pDC in scleroderma, vanBon et al demonstrated that stimulation of TLR7/8 or TLR9 led to CXCL4 expression upstream of type I IFN release.[68**] In mice, delivery of CXCL4 led to increased inflammation and IFN-inducible genes, but was not sufficient to induce fibrosis.[68**] Thus, stimulation of nucleic acid-responsive TLRs in isolation induced inflammation, but were not sufficient to drive fibrosis. Interestingly, CXCL4 has been shown to be protective in HIV-1 infection.[79] High CXCL4 in scleroderma may therefore be a host protective mechanism, and the triggers for CXCL4 secretion from pDC, whether endogenous or exogenous, may be a significant topic for future study.

In contrast, TLR4, which is expressed on the cell surface and may bind bacterial, viral and host proteins and lipoproteins, has been implicated as a driver of fibrosis.[76] Stifano and colleagues observed increased mRNA for TLR4 and its co-receptors MD2 and CD14 in

lesional SSc skin, the latter correlated with worsening Modified Rodnan Skin Score (MRSS) over 6 months.[80*] This is echoed by Mathes and colleagues data suggesting that TLR4 stimulation may be a potential inducer of CCL19 expression in human CD14+ monocytes.[26*] In mice, LPS stimulation and signaling through the TLR signal transducing protein MyD88 was sufficient to upregulate TGFβ1-induced and fibrosis-related genes in skin.[80*] Using CD11b+ cell depletion and TGFβ1 blockade, the authors delineated a pathway in which CD11b+ cells were necessary for LPS-induced fibrotic change upstream of TGFβ1.[80*] CD11b, which in conjunction with CD18 forms complement receptor 3, is expressed on the surface of neutrophils, monocytes, macrophages and dendritic cells, and it will be interesting to dissect the contributions of these populations.[16**,81]

LPS is a canonical TLR4 ligand, thus it was surprising that constitutive TLR4 knockout was only partially protective from LPS-induced gene expression changes in the study by Stifano et al, suggesting a TLR4-independent action for LPS in this model.[80*] TLR4-independent LPS signaling has been described in macrophages, and demonstrates the complexity of innate immune signaling.[82] Consistent with these findings, constitutive loss of TLR4 was partially protective from bleomycin-induced inflammation and fibrosis murine in skin and lung.[83,84]

TLR4 may also respond to endogenous signals, such as the fibronectin splice variant fibronectin extra domain A (Fn^{EDA}) that is associated with tissue remodeling and inflammation.[85,86**] Bhattacharyya and colleagues demonstrated that Fn^{EDA} is an abundant endogenous TLR4 ligand in scleroderma and is capable of driving fibrotic changes that characterize skin fibrosis *in vitro* and *in vivo*, while antagonism of TLR4 signaling *in vivo* led to attenuated experimental fibrosis.[86**] In contrast to Stifano and colleagues, this study identified fibroblast-intrinsic TLR4 signaling *in vitro*, as previously described in skin and other tissues.[83,87–90] Future studies to discriminate between the effects of TLRs on immune cells versus stromal cells in scleroderma will be of interest.

O'Reilly and colleagues identified TLR2 overexpression on SSc fibroblasts, and demonstrated *in vitro* that the acute phase reactant serum amyloid A is capable of stimulating IL-6 from fibroblasts in a TLR2-dependent manner.[91] IL-6 has been implicated in SSc, and this study provides another example of how endogenous signals may drive TLR signaling and fibrosis-associated responses.[92]

Other innate signals and cells in skin fibrosis

Type I and II IFN have been studied in the pathogenesis of SSc, while little data has emerged regarding the role of type III IFN in scleroderma.[10] This year Dantas and colleagues published a brief report describing a two-fold increase in circulating type III IFN in SSc.[10,93] Type III IFN (also called IFN-λ or IL-28A, IL-28B and IL-29) is involved in antiviral immunity, similar to type I IFN.[94,95] However, type III IFN signals through a distinct receptor, which, unlike IFNAR, is largely restricted to the epithelium.[94] Further investigation may elucidate a role for type III IFN in scleroderma, as the epithelial tissues skin and lung are particularly affected.

IL-17C is another cytokine known to regulate the innate immune function of epithelial cells, and was recently identified by Lonati and colleagues to be decreased in both lesional SSc and morphea compared to healthy skin.[96–98] Consistent with previous reports, the authors found more IL-17A+ cells in SSc skin; along with fewer IL-17F+ cells, this feature distinguished SSc lesional skin from morphea and healthy donors.[96,99–101] Interestingly, mast cells were a more abundant source of IL-17A than T cells.[96,99–101] A similar finding has been reported in psoriatic skin, and underscores the importance of examining cellular sources of signaling molecules.[96,102]

Mast cells are pivotal for innate defenses against pathogens and are key players in allergy, while a role for these cells in scleroderma tissue fibrosis has been debated.[103,104] Two groups have used mice congenitally lacking mast cells with a mast cell replacement strategy to show that mast cells are required for induction of bleomycin lung fibrosis.[105,106] In contrast, a 1999 study by Yamamoto and colleagues suggested that mast cells are not necessary for induction of bleomycin skin fibrosis, but may hasten progression of disease.[107] Recently, a model of inducible mast cell depletion from connective tissues has confirmed that mast cells are not required for the development of bleomycin skin fibrosis.[108] Thus, mast cells may play a redundant role in fibrosis induction in skin, whereas they appear to be indispensible for the development of bleomycin lung fibrosis.

Conclusions

Recent human studies have focused on changes early in scleroderma, and linked innate immunity to vascular dysfunction and disease progression, perhaps suggesting a pathogenic role. However, different myeloid cell populations have separate, and sometimes opposing, actions in murine fibrosis. Similarly, nucleic acid-responsive TLRs may drive inflammation, while TLR4 responses to endogenous or exogenous signals drive fibrosis. Other innate cells such as conventional dendritic cells, natural killer cells and innate lymphoid cells have not been fully explored in scleroderma, though their biology is being rapidly elucidated in normal tissue, and they are implicated in other forms of fibrosis.

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Abbreviations

Arg1	arginase 1
Cad11	cadherin 11

dcSSc	diffuse cutaneous systemic sclerosis
Chi3l3	chitinase 3 like 3
Fli1	Fli-1 proto-oncogene, ETS transcription factor
Fn^{EDA}	fibronectin extra domain A
GlcNAc	N-acetylglucosamine
GnT-V	N-acetylglucosaminyltransferase-V
IFN	interferon
IFNAR	interferon α/β receptor
lcSSc	localized cutaneous systemic sclerosis
LPS	lipopolysaccharide
Mrc1	mannose receptor, C type 1
pDC	plasmacytoid dendritic cell
Relma	resistin-like molecule α
SSc	systemic sclerosis
TGFβ1	transforming growth factor β1
TLR	toll like receptor

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Key Points

1. New nomenclature to describe the state of macrophage activation will be useful in understanding and communicating about these complex populations in scleroderma.
2. Macrophages and pDC have been implicated in vascular dysfunction and in sustaining inflammation in early disease.
3. Multiple subpopulations or activation states of macrophages and other myeloid cells can have differential effects in fibrosis.
4. Sustained signaling through different TLRs can lead to inflammation or fibrosis.
5. There are many types of innate immune cells that can potentially contribute to scleroderma and will be worth exploring in detail.