

## CHEMOKINES IN SYSTEMIC SCLEROSIS

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### ***Scleroderma as an immune fibrotic disorder***

Systemic sclerosis (scleroderma, SSc) is a connective tissue disease in which a dysregulated immune process in the involved tissues is linked to persistent myofibroblast activation, resulting in skin and organ fibrosis of varying severity (Allanore et al., 2015). Current concepts indicate that the disease onset is triggered by environmental factors in individuals predisposed by genetic polymorphisms in immune-regulatory genes (Mayes et al., 2014). Immune cells, including macrophages, mast cells, as well as T helper cells, are recruited into the skin lesions at an early stage of the disease (Fleischmajer et al., 1977, Roumm et al., 1984, Hawkins et al., 1985). It is believed that the aberrant local immune response involving T helper cells and alternatively activated macrophages contribute to the pro-fibrotic microenvironment (Wermuth and Jimenez, 2015, O'Reilly et al., 2012). Local resident fibroblasts are activated to become myofibroblasts and other myofibroblast precursor cells derived from circulating monocytes (fibrocytes) as well as cells undergoing EndoMT and possibly EMT are drawn into the fibrotic lesions (Postlethwaite et al., 2004, Nikitorowicz-Buniak et al., 2015, Good et al., 2015, Mathai et al., 2010). Mechanical stress in the extracellular matrix, autocrine stimulation and epigenetic changes result in persistence of myofibroblasts through failure of apoptosis and ongoing extracellular matrix (ECM) overproduction (Shiwen et al., 2015, Altorok et al., 2014). Current strategies to treat this condition include immunosuppression or specific targeting of pathogenic cytokines and growth factors, but none of these approaches are curative and all have undesirable off-target effects (Khanna et al., 2016a, Lafyatis, 2014, Tashkin et al., 2016). Autologous stem cell therapy may achieve remission in some patients but has a 10% risk of mortality in the first year after treatment (van Laar et al., 2015). It is possible that improved understanding of the factors recruiting immune and fibrotic cells into the lesions could lead to more specific therapies with fewer off-target effects. Furthermore, the local production of chemo-attractants in the fibrotic lesions could be manipulated to recruit cell based therapies into the lesions (Vignali and Kallikourdis, 2017) (<http://fibrocell.com/pipeline-clinical-trials/fcx-013-gene-therapy-for-linear-scleroderma>).

### ***Chemokine background***

Chemokines are small proteins of 8-10 kDa having two cysteine residues in conserved sites in the N terminus (Le et al., 2004, Arimont et al., 2017). The conformation of the cysteine residues has been used to classify these proteins as CC, having adjacent cysteine residues and typically involved in macrophage and T cell recruitment, CXC having an intervening amino acid between cysteines and typically involved in neutrophil recruitment, as well as CX3C which has 3 interposed amino acids and

comprises a single member, fractalkine. Furthermore, the C subtype has a single cysteine residue and includes lymphotactin- $\alpha$  and  $\beta$ . In general chemokines function as soluble factors which recruit innate and adaptive immune cells into sites of injury or pathogen invasion, acting via seven transmembrane receptors coupled to heterotrimeric G proteins which signal via phospholipase C and alter migration through elevation of intracellular calcium (Kufareva, 2016). The chemokine-chemokine receptor system has a high degree of redundancy so that multiple chemokines can act at each receptor. Overall this leads to a highly complex network of chemokine related pathways including at least 28 CCL proteins, 17 CXCL ligands each with overlapping effects on receptors CCRs 1-10 and CXCRs 1-6 as well as the fractalkine receptor CX3CR1.

Furthermore, other secreted proteins can act as important chemo-attractants involved in the response to tissue injury leading to optimal tissue repair, but induced in pathologic conditions, including PDGF, which recruits myofibroblast precursors into wounds and fibrotic lesions (Seppa et al., 1982), as well as stem cell factor (SCF) (Yamamoto et al., 2001), an attractant for c-kit positive mast cells and progenitor cells, plus stromal cell-derived factor 1 (CXCL12) implicated in recruitment of fibrocytes into lesions (Seppa et al., 1982, Bucala et al., 1994, Tourkina et al., 2011). As discussed below it is likely that chemokines and these other chemo-attractants are involved at multiple steps in the scleroderma disease process, following some disease initiating insult leading to their expression in the damaged tissues. It is possible that therapeutics targeting the chemokines could be of benefit overall in scleroderma, or in pathogenic subgroups of the disease. Limitations include the multiple chemokines and chemoattractants involved and the molecular promiscuity of the chemokine receptors.

### ***Initiation of tissue injury in scleroderma***

Scleroderma onset is linked to occupational exposure including exposure to organic solvents such as trichloroethylene, working with polyvinyl chloride, and silica dust exposure (Stephens et al., 1994, Englert et al., 2005, Marie and Gehanno, 2015). Furthermore certain geographical clusters have been described in keeping with an environmental initiating factor. Concordance between siblings or first degree relatives is often near-synchronous, implicating some common environmental exposure (Arnett et al., 2001, Feghali-Bostwick et al., 2003). As with most complex autoimmune disorder, viral exposures have also been implicated in triggering the onset of the disease, and we have experience of patients triggered by proven acute CMV infection for example (Goulabchand et al., 2014, Arnson et al., 2009). Damage to and activation of environment-exposed integument cell layers including epithelial and endothelial cells is seen at early stage in SSc (Aden et al., 2008, Aden et al., 2010, Kahaleh and LeRoy, 1999). Multiple studies indicate that microvascular endothelial cell

damage is present at clinical onset in the majority of cases (Saito and Ishikawa, 1976, Freemont et al., 1992). Microvascular endothelial cell apoptosis, adhesion molecule expression and elevated reactive oxygen species (ROS) production are all seen, leading to enhanced adhesion and migration of circulating immune cells (Gabrielli et al., 2009, Svegliati et al., 2014, Kuwana et al., 2004, Denton et al., 1995). Recruitment of immune cells into the dermis or affected organs is likely to be driven by local overproduction of chemokines and a gradient between the involved tissues and circulating plasma.

### ***Chemokines induced in the disease microenvironment in early and late stage disease***

Monocyte chemoattractant proteins (MCP) are a subfamily within the CC chemokines strongly implicated in scleroderma. MCP-1 (CCL2) was first identified as a monocyte-specific chemoattractant secreted by glioma cells (Yoshimura et al., 1989). Overexpression of MCP-1 by scleroderma skin fibroblasts was first shown to induce migration of macrophages across an endothelial monolayer towards scleroderma fibroblasts overproducing the chemokine, and furthermore elevated MCP-1 in scleroderma tissues has been demonstrated in multiple studies (Denton et al., 1998, Matsushita et al., 2006, Hasegawa et al., 2011). In these analyses it was shown that multiple cell types within the lesions are responsible for production of MCP-1, including fibroblasts, endothelial cells, keratinocytes as well as mononuclear cells. The mechanistic importance of these findings is supported by demonstration of co-localisation of macrophages expressing CCR2, the receptor for MCP-1, with the cells expressing the chemokine in scleroderma tissues (Carulli et al., 2005). Furthermore scleroderma fibroblasts continue to overexpress CCR2 in tissue culture, and induce MCP-1 following stimulation by IFN $\gamma$  plus TNF $\alpha$ . Strong elevation of MCP-1 correlates with the more severe diffuse cutaneous disease subset as well as with the development of pulmonary fibrosis, indicating a role in the development of major fibrotic features (Carulli et al., 2008). Furthermore MCP-1 has a role in Th2 polarisation consistent with a pro-fibrotic mechanism (Karpus et al 1997).

There are four further members of the MCP family which have a high degree of homology between family members, of which MCP-3 (CCL7) has been studied in scleroderma (Ong et al., 2003, Yanaba et al., 2006, Distler et al., 2009). MCP-3 binds to the same receptor as MCP-1, CCR2, but is also a ligand at CCR1 and 3. In scleroderma tissues MCP-3 is overexpressed by basal layer keratinocytes, dermal fibroblasts as well as mononuclear cells. MCP-3 has been shown in systematic studies to be overproduced in scleroderma lesions and is believed to promote monocyte and T cell recruitment, and to be a direct stimulant to fibroblasts leading to collagen I induction (Ong et al., 2003). MCP-3 is implicated in mouse models of fibrosis and is induced in fibroblasts by the pro-fibrotic growth factor TGF $\beta$ .

Furthermore, other chemokines have been shown to be elevated in the disease tissue, including RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$  as well as fractalkine and IP10, and the data are summarized in Table 1 (Hasegawa et al., 2005, Rabquer et al., 2011, Bandinelli et al., 2012, Corrado, 2014).

RANTES (CCL-5) interacts with CCR1, CCR3 and CCR5, and is chemotactic for T-cells, eosinophils and basophils (Schall et al., 1988). MIP-1 $\alpha$  (CCL-3) and MIP-1 $\beta$  (CCL-4) are structurally similar chemokines produced by many cells, particularly macrophages, dendritic cells and lymphocytes (Sherry et al., 1988). MIP-1 $\alpha$  interacts with CCR1, CCR4, and CCR5, whereas MIP-1 $\beta$  shows specificity for CCR5. Both MIP factors are involved in the recruitment and activation of many pro-inflammatory cells, including polymorphonuclear cells, as well as CD4 and CD8 T-cells. MIP-1 $\alpha$  and MIP-1 $\beta$  are considered important chemo-attractants for activated T cells. This was demonstrated in tissue culture where recombinant MIP-1 proteins only attracted T cells which had been activated by monoclonal antibody to CD3. In fact recombinant MIP-1 $\beta$  attracted CD4+ T helper subset whereas recombinant MIP-1 $\alpha$  attracted predominantly CD8+ T lymphocytes. Furthermore, these factors increased T cell adhesion to endothelial cells in tissue culture (Taub et al., 1993).

Elevated serum levels of RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  has been observed in scleroderma patients compared to controls (Bandinelli et al., 2012, Hasegawa et al., 2013) and increased production of MIP-1 $\alpha$  and MIP- $\beta$  by peripheral blood derived monocytes has been demonstrated (Hasegawa et al., 2013). Furthermore, increased cutaneous expression of RANTES and MIP-1 $\alpha$  mRNA precedes skin thickening in a murine model of scleroderma (Zhang et al., 2002), with RANTES showing greatest early increase in expression and then falling more rapidly compared to MIP-1 $\alpha$ .

Presence of these chemokines has also been demonstrated in tissues of organ affected in scleroderma. Increased levels of RANTES and MIP-1 $\alpha$  have been detected in the bronchoalveolar lavage (BAL) fluid of scleroderma patients (Bolster et al., 1997). In this study, BAL fluid concentrations of MIP-1 $\alpha$  were significantly higher in scleroderma patients with alveolitis compared to those without alveolitis and compared to healthy controls, whereas BAL fluid concentrations of RANTES were significantly higher only in scleroderma patients without alveolitis compared to healthy controls. Furthermore, abundant expression of RANTES mRNA has been observed in the skin of scleroderma patients, with no expression seen in healthy controls in the same study (Anderegg et al., 2000).

In one study MCP-1 was found to correlate with Modified Rodnan Skin Score (Bandinelli et al., 2012), whereas RANTES and MIP-1 $\alpha$  failed to correlate with diffuse or limited subgroups. The overall abundance of MIP-1 $\alpha$  was very low relative to RANTES and MCP-1.

### ***Role of fractalkine: a unique class of chemokine which can be membrane bound or soluble***

In 1997 Bazan reported a unique chemokine with CX3C structure, with unique tissue distribution, termed fractalkine/CX3CL1 (Bazan et al., 1997). Fractalkine is synthesized as a transmembrane molecule consisting the extracellular domain and a mucin-like stalk which can be cleaved from the cell surface by metalloproteinases ADAM17 and ADAM10, and so can act both as an adhesion molecule and soluble chemoattractant (Bourd-Boittin et al., 2009). Fractalkine acts as a chemoattractant for monocytes, NK cells and T cells (Imai et al., 1997). Membrane bound fractalkine can be induced on endothelial cells by inflammatory cytokines.

Fractalkine has been studied in scleroderma by Hasegawa et al 2005, showing enhanced expression in the skin and lungs of patients. This increased expression augments the recruitment of mononuclear cells into the affected tissue leading to inflammation (Hasegawa et al., 2005)(Hasegawa et al., 2005). Polymorphisms of the fractalkine receptor CX3CR1 are associated with susceptibility to scleroderma and scleroderma related complications (Marasini et al., 2005). The 429I and 480M alleles are associated with the development of pulmonary hypertension in scleroderma, whereas overall increased susceptibility is associated with the 249II polymorphism (Marasini et al 2005). Furthermore use of prostanoid infusions, which are a therapeutic for vascular manifestations, were found to reduce serum fractalkine levels in scleroderma patients (Sicinska et al 2008).

### ***Chemokines can also regulate angiogenesis***

Chemokines, in addition to their immune chemoattractant properties, can act as regulators of angiogenesis, a process which is significantly dysregulated in scleroderma (Kahaleh and LeRoy, 1999). Chemokines acting as inhibitors of angiogenesis include monokine induced by interferon- $\gamma$  (IFN- $\gamma$ ) (MIG/CXCL9) and IFN-inducible protein 10 (IP-10/CXCL10). These factors inhibit angiogenesis through binding to CXCR3, whereas CXCL16 is believed to be pro-angiogenic acting through CXCR6. This system has been studied in scleroderma by Rabquer and colleagues, indicating that the anti-angiogenic factors IP-10 and MIG were elevated in serum of patients (Rabquer et al., 2011). A complex picture emerged from these studies indicating that IP-10 and MIG/CXCL9 were elevated but the CXCR3 receptor is decreased. Furthermore, the pro-angiogenic CXCL16 is overexpressed as is its receptor in the scleroderma endothelial cells. These findings support a model of vascular injury and dysregulated repair in scleroderma and parallel the findings with VEGF which is increased in the disease but fails to restore vascular integrity. These aberrant vascular repair mechanisms may

explain chaotic vascular lesions seen in patients such as skin telangiectasia and gastric antral vascular ectasia (GAVE).

Many studies suggest that the IP-10/CXCR3 axis has a role in autoimmune conditions and in fibrosis in patients with scleroderma. A longitudinal study of IP10 plus MCP-1 have shown high levels of both chemokines in recent onset scleroderma. Subsequently IP-10 declined during follow up whereas the MCP-1 levels remained high. Furthermore, high baseline IP-10 correlated with severe clinical involvement such as pulmonary and renal involvement. One possible interpretation of these data is that IP10 is being released by Th1 cells in severe early disease, which switch to a Th2 response in ongoing later stages of disease, indicating an inflammatory initiation phase and pro-fibrotic downstream phase. Other studies have shown that IP-10 is a marker of a more aggressive autoimmune process involving organs such as thyroid or lung. Fibroblasts from scleroderma patients overproduce IP10 which can be induced further by IFNs. Furthermore, it has been suggested that the IFN-inducible chemokine IP-10 is a stable serologic marker of a more severe form of scleroderma and may be useful for risk stratification of patients, regardless of disease type (limited or diffuse).

### ***Multiplex analysis of chemokines in tissue fluid: chemokine levels in early and late stage disease***

In our own studies we have used dermal suction blister fluid sampling as a minimally invasive method to gain insight into the levels of soluble chemokines present in the disease microenvironment. These samples can be analysed by Luminex Multiplex analysis, or by further technologies which are expanding in the capacity for analysis of multiple factors. When unbiased hierarchical clustering was applied patients and chemokines were found to cluster into groups. The patients were found to cluster into 3 clinically distinct groups; firstly a group with relatively early diffuse scleroderma (mean disease duration 5 years, skin score 27), secondly a late stage diffuse scleroderma group (late stage disease duration 14 years, skin score 21), and thirdly a group with limited or mild diffuse with bland expression of chemokines and growth factors (mean disease duration 7.5 years, skin score 10). The heat-map is shown in Figure 1. Based on this analysis a cluster of chemokine expression in early disease was seen indicating high levels of MCP-1, fractalkine, RANTES and MIP-1 $\alpha$  and 1 $\beta$  which co-clustered with innate inflammatory cytokines such as TNF $\alpha$ , IL-6 and IL-1 $\alpha$ . In the late stage diffuse group MCP-3 and IP-10 were elevated and co-clustered with Th2 cytokines IL-4, 5 and 13. Limitations of the study are the small number of patients included who were untreated and in the very earliest stages.

The question of an early versus late role of these chemokines in fibrosis has been addressed in mouse models of scleroderma. In these studies MCP-1, RANTES, and MIP-1 $\alpha$  were all elevated in early stages of the fibrotic model, with RANTES having the earliest role, whereas MCP-1 and MIP-1 $\alpha$  persisted while RANTES declined rapidly (Ugucioni et al 1995).

Studies from Japan have assessed the expression levels of chemokines longitudinally in scleroderma patients. In one study annual plasma samples were assayed for 3 years, measuring IP-10, MIG (CXCL9), IL-8 and correlation sought with clinical parameters. IP10, MIG and IL-8 were significantly elevated in scleroderma versus controls. MCP-1 correlated with skin score and lung involvement as assessed by MRSS and FVC, indicating that MCP-1 was the most useful biomarker (Hasegawa et al., 2011). In one further longitudinal study levels of these chemokines plus RANTES, were examined in scleroderma patients, where annual blood samples were assayed longitudinally over a four year period and compared to outcome including disability index HAQ-DI. Of the chemokines studied baseline CXCL8 (IL-8) levels correlated with HAQ-DI at year 4 (Hasegawa et al., 2013).

### ***Role of atypical chemokine receptor D6 in scleroderma***

More recent evidence has indicated an atypical family of chemokine receptors which fail to signal and act as an anti-inflammatory regulatory mechanism binding excess chemokines present in the extracellular environment. Such receptors would be of therapeutic interest, since they bind multiple agonists and could be manipulation to reduce chemokine levels in disease. D6 is one such 'scavenging' receptor, which binds CC chemokines and is thereof reo fin interest in systemic sclerosis. An interesting study from Glasgow examined D6 expression levels in peripheral blood mononuclear cells in scleroderma, and attempted to distinguish between D6 high expressing and low expressing individuals (Codullo et al., 2011). D6 was found to be 10 fold elevated in scleroderma peripheral blood mononuclear cells. Also, these studies confirmed elevation of MCP-1, IL-8, MIP-1 $\alpha$ & $\beta$ . In general inflammatory cytokine levels did not differ between D6 high and low groups, however MIP-1 $\alpha$  levels were lower in D6 high patients consistent with absorption/neutralization by D6.

### ***Chemokines in scleroderma: implications for therapy***

In recent years development of specific biologic therapies has enabled clinical trials in scleroderma which not only inform potential therapies but also give the clearest experiment to test the importance of the growth factors or cytokines targeted (Khanna et al., 2016b, Rice et al., 2015). In this phase it might be possible to evaluate specific therapies blocking chemokines or



chemoattractants in order to test efficacy and determine the overall importance of the chemokine or chemokine receptor involved.

Because of their implication in multiple immune and inflammatory conditions a very large number of therapeutics targeting chemokines or their receptors have been developed. Examples include an MCP-1 mimetic which blocks the effect of MCP-1 at CCR2, as well as orally active CCR2 signalling inhibitors which have been shown to inhibit monocyte recruitment in mouse models. Monoclonals against MCP-1 led to the unexpected greatly elevated plasma levels of MCP-1 and worsening systemic inflammation.

Antagonism of the protein-protein interactions between chemokines and their receptors by small molecules are considered challenging. However G-protein coupled receptors such as the chemokine receptors are drugable targets, and multiple small molecule inhibitors have been developed. Furthermore, elucidation of the 3-D structure of the chemokine receptors has enabled effective inhibitors to be developed, leading to a large range of potential therapeutics. Of note in scleroderma CCR2 antagonists are available (INCB3284 Incyte, BMS-741672 Bristol-Meyers Squibb, BMS-741672 Bindarit Angelini, Bristol-Meyers Squibb, JNJ-17166864 Johnson & Johnson) as well as antagonists of MCP-1, although none of these drugs has reached the clinical market. Clinical failure has been for a variety of reasons but further drug development is ongoing. A CCR2 antagonist, CCX-140, is in a Phase 3 trial for Type 2 diabetes with diabetic nephropathy (Hanefeld et al., 2012; Sullivan et al., 2013) and also a CCR9 antagonist CCX282 is in Phase 2 trials for inflammatory bowel disease (Keshav et al., 2013). Furthermore the CCR1 inhibitor CCX354, has completed a Phase 2 trial for rheumatoid arthritis being well tolerated and exhibiting possible efficacy at higher dose (Tak et al., 2013). Furthermore, FX-125L is an orally-active small molecule developed by Funxional Therapeutics, described as a somatotaxin acting through the type-2 somatostatin receptor and currently being evaluated by Boehringer Ingelheim as a treatment for COPD, asthma and rheumatoid arthritis.

Chemokine receptor antagonist nanobodies have also shown efficacy in pre-clinical models, broadening the repertoire of therapeutic approaches (Maussang et al., 2013).

Through these multiple potential strategies it might be possible to inhibit cellular recruitment into the immune-fibrotic disease microenvironment in scleroderma patients (Figure 3). Furthermore, it should be possible to individualise tailor-made therapy based on the individual chemokine environment. We propose that the dermal blister fluid profiling method will be a key part of these

treatment approaches, enabling tailor made therapy. Repeat sampling after chemokine inhibitor therapy could be used to demonstrate biologic effect and or silencing of the specific target.

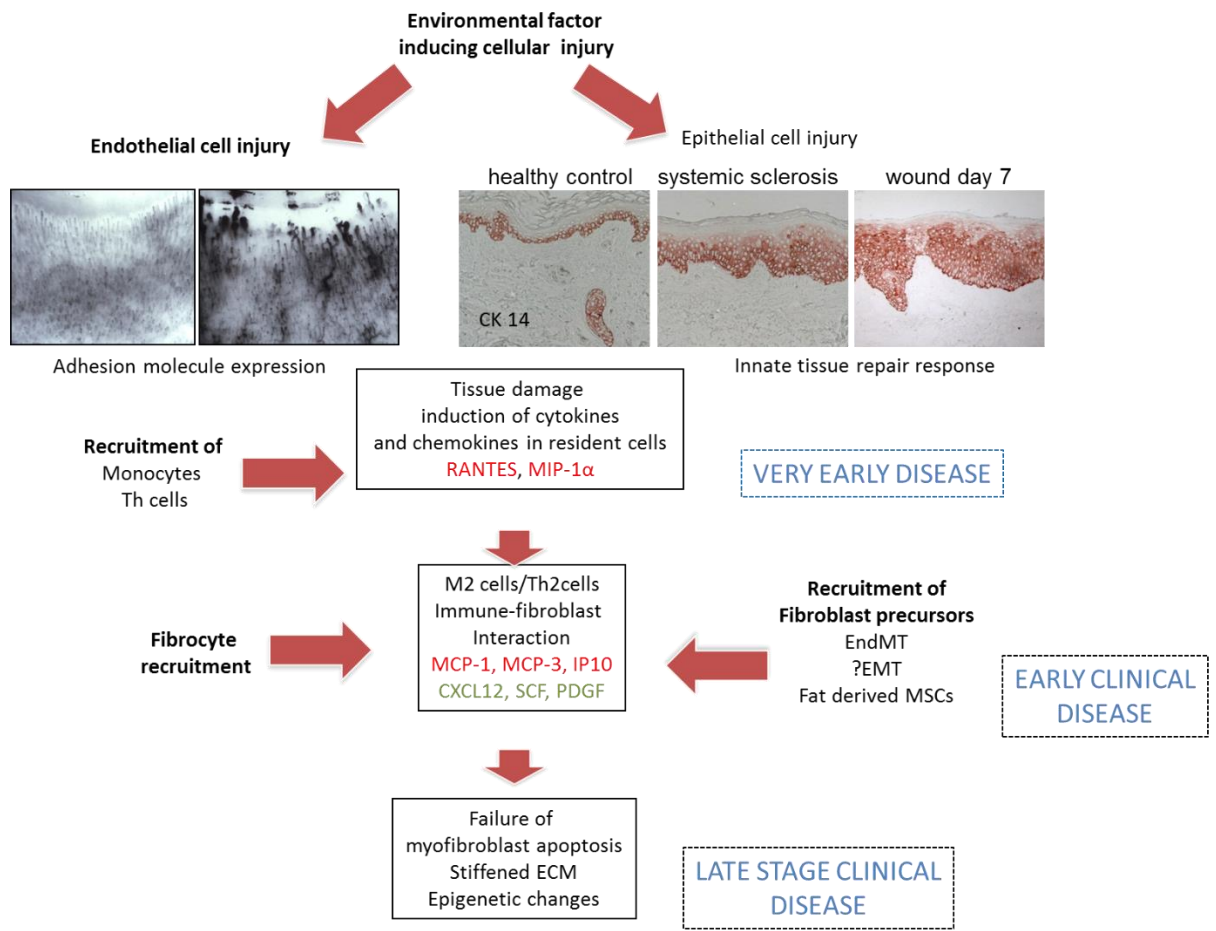
### ***Conclusions and future perspectives***

Chemokines and their cognate receptors are important modulators of scleroderma. In particular their altered expression, inappropriate activation and utilization are likely to play important roles at all stages of the disease process including the early vasculopathy, developing inflammatory phase leading to autoimmunity, and the late stage of established scarring and fibrosis (Figure 3). These molecules and their receptors have therefore in the last decade become highly attractive targets for multiple therapeutic approaches (Proudfoot, 2002, Horuk, 2009, O'Hayre et al., 2010, Klarenbeek et al., 2012, Blanchetot et al., 2013, Rees et al., 2015). The development of antibodies, antagonists and chemokine binding proteins have all advanced the field, but have also highlighted the challenges including chemokine and receptor redundancy, correct target selection, differential expression and diversity (Klarenbeek et al. 2012). Effective application of chemokine/receptor therapies in scleroderma will require a more thorough understanding of the complex interactions and involvement of these factors in pathogenesis. This will no doubt require the use of these sophisticated therapies within disease endotypes, targeting perhaps multiple or sequential chemokine/receptor pathways along the natural history of the disease. Nonetheless, and despite these clear hurdles, chemokines and their receptors are likely to remain critical targets and play a pivotal role in therapeutic strategies in the future.

Chemokines	Receptors	Cellular origin	Target cells	Putative role in scleroderma	Early versus late	Therapeutic antagonists
<b>MCP1 (CCL2)</b>	CCR2	Fibroblasts, endothelium, keratinocytes, monocytes.	T cells and Monocytes	Correlation with severity of skin and lung fibrosis	Early and then maintained	CCR2 antagonists
<b>MCP3 (CCL7)</b>	CCR1,2 &3	Keratinocytes, fibroblasts, monocytes	T cells and Monocytes	Recruitment of immune cells in early disease	Early	
<b>Fractalkine</b>	CX3CR	Activated endothelium	Adhesion/recruitment of monocytes, NK cells and T cells	Pulmonary hypertension risk via SNPs	Early	
<b>IP10</b>		Monocytes, endothelium, fibroblasts		Severe disease biomarker	Early then declining	
<b>RANTES</b>	CCR1,3&5	T cells	T cells, mast cells, eosinophils	Cellular recruitment into lungs	Very early (mouse models).	
<b>MIP1<math>\alpha</math></b>	CCR1,4&5	Macrophages	Activated CD8+ T cells		Very early (mouse models)	
<b>MIP1<math>\beta</math></b>	CCR5	Macrophages	Activated CD4+ T cells			
<b>SCF</b>	c-Kit	Keratinocytes, scleroderma fibroblasts	Mast cells, stem cells	Recruitment of mast cells and progenitor cells	Not known	Tyrosine kinase inhibitors
<b>CXCL12 (SCDF)</b>	CCR4		Fibrocytes	Recruitment of fibrocytes into areas of lung fibrosis	Not known	
<b>PDGF</b>	PDGFR1-4	Platelets, macrophages	Fibroblasts, fibroblast precursors	Recruitment of fibroblasts and fibroblast precursors.	Not known	Tyrosine kinase inhibitors

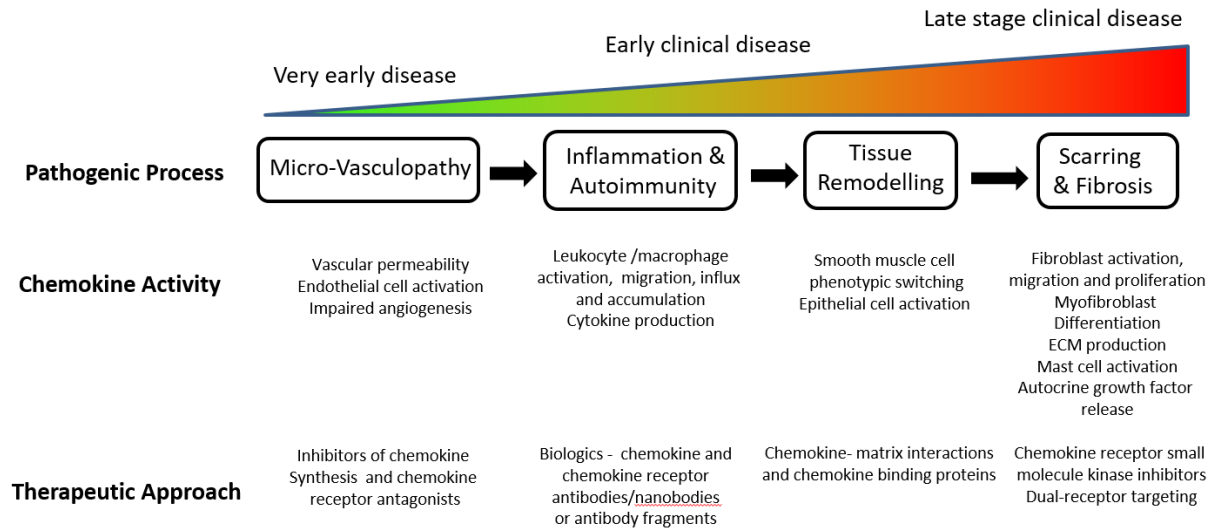
**Table 1 Summary of the role of chemokines and other chemoattractants in scleroderma**





**Figure 2 Expression of chemokines and chemo-attractants in the involved tissues of scleroderma patient**

### Potential impact of chemokines in scleroderma and therapeutic options



**Figure 3. Potential impact of chemokines in scleroderma and therapeutic options**

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