



# Type 1 interferon activation in systemic sclerosis: a biomarker, a target or the culprit

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## Purpose of review

Activation of the type 1 interferon (T1 IFN) pathway has been implicated in the pathogenesis of systemic sclerosis (SSc) by an increasing number of studies, most of which share key findings with similar studies in systemic lupus erythematosus (SLE). Here we will focus on the evidence for T1 IFN activation and dysregulation in SSc, and the rationale behind targeting the pathway going forward.

## Recent findings

An increased expression and activation of T1 IFN-regulated genes has been shown to be present in a significant proportion of SSc patients. T1 IFN activation markers have been found to predict and correlate with response to immunosuppressive treatment as well as severity of organ involvement. As inhibition of the IFN- $\alpha$  receptor has been proven to be effective in active SLE, benefit may be seen in targeting the IFN pathway in SSc.

## Summary

The role played by T1 IFN and its regulatory genes in SSc is becoming increasingly evident and strikingly similar to the role observed in SLE. This observation, together with the benefit of type 1 IFN targeting in SLE, supports the notion of a potential therapeutic benefit in targeting T1 IFN in SSc.

## Keywords

interferon regulatory factors, plasmacytoid dendritic cells, systemic sclerosis, Toll-like receptors, type 1 interferon

## INTRODUCTION

Systemic sclerosis (SSc) is a progressive, heterogeneous multisystem autoimmune disease, which is characterized by autoimmune activation as well as a pathognomonic tissue and vascular fibrosis [1,2]. It has the greatest mortality amongst the major rheumatic diseases [1,3,4<sup>\*\*\*</sup>]. Genetic predisposition combined with triggers activating a persistent immune response at the level of the tissue is thought to drive the pathogenetic process in SSc. Type 1 interferons (T1 IFNs) are a family of cytokines playing a key role in response to viruses and a variety of danger and damage signals, triggering innate immune activation. The dysregulation in T1 IFN signalling has now been implicated in the pathogenesis of certain autoimmune diseases, including SSc and systemic lupus erythematosus (SLE) [4<sup>\*\*\*</sup>,5,6]. Clinical evidence of the harmful effects of T1 IFN in SSc, is provided by a randomized, placebo-controlled trial of IFN- $\alpha$ , in patients with early diffuse SSc, where the trial had to be stopped early because of a deleterious effect seen in the lung function of the treatment group. The withdrawal and serious adverse event rates were also

greater in the treatment group than in the placebo group [7].

Here we will focus on the evidence for T1 IFN activation in SSc, the potential mechanisms leading to its dysregulation, the predictive role on disease progression and the rationale to target the pathway going forward.

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## KEY POINTS

- The activation of T1 IFN has been implicated in the pathogenesis of SSc and SLE, with inhibition of the IFN $\alpha$  receptor being recently shown to be effective in active SLE.
- An increased serum concentration of ISGs is detectable in a variable proportion of patients with SSc, even at the very early stages of the disease, before onset of clinically detectable damage.
- The origin and triggers of T1 IFN, and how the interactions between genetic and environmental factors, leads to dysfunction in the T1 IFN response remains unclear.
- IFN activation markers have been found to predict and correlate with response to immunosuppressive treatment as well as in the severity of organ involvement.
- Clinical trials of T1 IFN antagonists in carefully selected SSc patients would lead to a better understanding of the role T1 IFN plays in SSc pathogenesis, potentially improving outcomes in certain SSc patients.

## FROM DANGER SENSORS TO INTERFERON-STIMULATED GENES: A MULTIFACETED DEFENSE MACHINERY THAT CAN LEAD TO IMMUNE-MEDIATED TISSUE DAMAGE

T1 IFNs are a heterogeneous family of cytokines, which provide a robust first line of antiviral defence. Type 2 and 3 interferons have partially different roles, which are outside the scope of this review and have been summarized elsewhere [8,9].

T1 IFN can be divided into five classes in humans:  $\alpha$ ,  $\beta$ ,  $\omega$ ,  $\epsilon$  and  $\kappa$ . All five, T1 IFN classes signal through the same type 1 IFN heterodimeric receptor complex constituting IFN- $\alpha$  receptor 1 (IFNAR1) and IFNAR2 subunits.

Secretion of T1 IFN in the extracellular space is the terminal event of an 'innate' response mechanism to a variety of danger and damage stimuli. The detection of repetitive molecular patterns displayed by a pathogen (pathogen-associated molecular patterns or PAMPs) is one of the stimuli, which is 'sensed' by the pattern recognition receptors (PRRs) [10]. There are four classes of PRRs – the Toll-like receptors (TLRs), the nucleotide-binding oligomerization domain-like receptors (NLR), the retinoic acid inducible gene I (RIG-I) and the C-type lectin receptors [11]. They all differ in ligand recognition, signal transduction and cell localization.

TLRs are the most extensively studied class of PRRs and consist of 10 types (TLR 1–10) [10,12,13]. TLRs are expressed on most nucleated cells, and

once they are engaged with their ligand, they lead to T1 IFN pathway activation. While this is true in most cells, plasmacytoid dendritic cells (pDCs) are the cells that are 'professionally' differentiated to secrete vast amounts of T1 IFN in response to TLR engagement. For this reason, they are believed to play a central part in the T1 IFN-mediated immune response and their role has been implicated both in the pathogenesis of SLE [14] and SSc [15<sup>■</sup>,16].

The first indirect evidence of a putative involvement of pDC in the aberrant T1 IFN activation in SSc was suggested by a proteome-wide analysis showing that CXCL4 in the plasma of SSc patients was substantially higher than healthy controls, and it predicted the presence and worsening of lung fibrosis and pulmonary hypertension. In the same study, the authors implicated pDC as one of the potential sources of CXCL4 [17]. More recently, CXCL4 has been found to function as a Damage Associated Molecular Pattern (DAMP) sensor. Lande *et al.* observed that CXCL4 organized microbial and self-DNA into liquid crystalline complexes that amplified TLR9-mediated IFN- $\alpha$  production in pDCs. Importantly, CXCL4-DNA complexes were present *in vivo*, and correlated with T1 IFN in SSc blood and skin, revealing a direct link between CXCL4 overexpression and T1 IFN production in patients with SSc [18]. Another study also indicated the infiltration of SSc skin by pDCs, where they were chronically activated, producing high levels of IFN- $\alpha$  and CXCL4. CXCL4 was under the control of phosphatidylinositol 3-kinase  $\delta$ , which was linked to the aberrant presence of TLR8 on pDCs in SSc patients. CXCL4 was also found to potentiate the activities of TLR8-induced and TLR9-induced IFN production in SSc pDCs [16].

Importantly, Ross *et al.* [15<sup>■</sup>] have shown that functional inhibition of pDCs was effective in preventing skin activation and fibrosis in preclinical models of SSc, similar to what has been observed in SLE [14].

In another study, anti-CXCL4 antibodies were shown to be present in at least half of SSc patients and correlated with serum/plasma IFN- $\alpha$  levels. Recently, CXCL4 itself was found to behave as a self-antigen, maintaining a vicious cycle by promoting T1 IFN activation via pDCs and anti-CXCL4 antibodies by B cells, sustaining the SSc IFN signature [19]. Further work with CXCL4 has interestingly shown that the anti-CXCL4 antibodies were present in patients with VEDOSS (very early diagnosis of systemic sclerosis), suggesting that this mechanism can intervene very early in the pathogenesis of disease, before clinically apparent tissue damage [20].

Activation of TLRs have also been found to play a role in interstitial lung disease (ILD). TLR3 activation by poly I:C has been reported to increase lung

inflammatory proteins including the cytokines CCL3, CCL5 and CXCL10, in airway epithelial cells. Importantly, TLR3 knockout mice showed protection against the inflammatory response [21]. TLR4 has also been implicated in pulmonary and skin fibrosis, with the ability to activate IRF5 [22].

Pathogens have long been proposed as a trigger for autoimmune illnesses, and one mechanism for this is ‘molecular mimicry’ between self-derived and pathogen-derived molecules. Another mechanism, occurs through the inability to clear the pathogen, resulting in infection persistence, and repeated stimulation of the innate immune cells via TLRs [23,24]. Farina and colleagues have shown evidence of infectious Epstein–Barr virus (EBV) in monocytes triggering SSc. Induction of EBV viral lytic genes resulted in the induction of TLR8 expression in both healthy control and SSc monocytes infected with EBV [25]. Further, Farina *et al.* [26] have shown that EBV can infect endothelial cells and fibroblasts in SSc skin, leading to an aberrant TLR activation. A novel mechanism has also now been demonstrated by which human monocytes bound to EBV recombinant virus are capable to transfer EBV to the endothelial cells. In the same study, EBV lytic antigens in scleroderma dermal vessels were detected, suggesting EBV could target endothelial cells in SSc skin, activating TLR 9 in the process and possibly contributing to the vascular injury seen in SSc [27].

Beyond classic pathogens, there is increasing evidence for an important role played by mitochondria, in the events driving T1 IFN activation and subsequent autoimmunity. It is widely accepted that fragmentation in mitochondrial DNA (mtDNA), can lead to the activation of T1 IFN pathway, through cGAS (cytosolic cyclic GMP-AMP synthase), a specific cytosolic receptor for free DNA, which, in turn, activates the endoplasmic reticulum membrane protein, stimulator of interferon genes (STING). cGAS-STING activation by mtDNA was shown to be positively associated with T1 IFN and IL-6 expression in SSc as well as in SLE [28,29]. Consistent with these findings, mtDNA has been found to be at increased concentration in SSc plasma, with the ability to function as DAMPs and interact with PRRs [30]. This is one of the putative mechanisms by which necrotic cells or those under stress have been found to activate TLR 9 and the double-stranded DNA sensor, cGAS.

Interestingly, it has been also proposed that mtDNA could be damaged as a consequence of oxidative stress because of high exposure to reactive oxidative species (ROS) produced by the mitochondria itself [28].

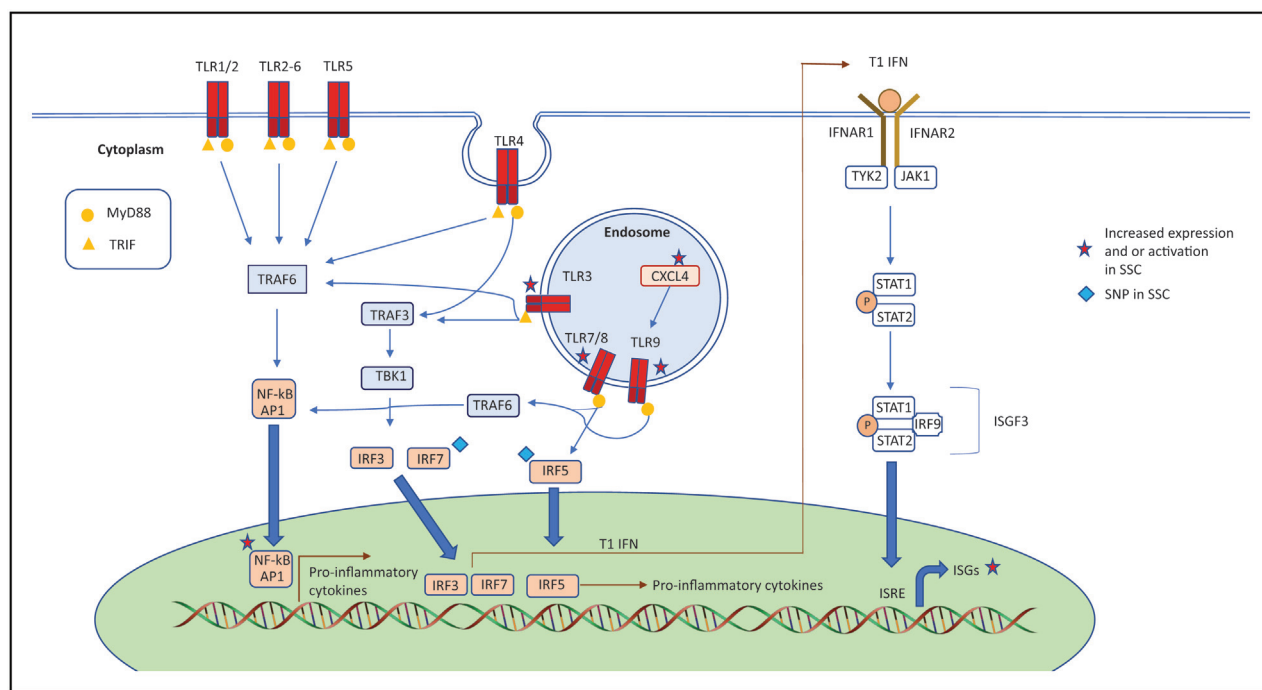
Regardless of the source of its secretion, T1IFN signal through IFNAR1 and IFNAR2, which in turn activate Janus kinase (JAK)-signalling pathway

downstream [4<sup>\*\*\*</sup>,8]. This consists initially with phosphorylation of pre-associated JAK1 and tyrosine kinase 2 (TYK2), which triggers kinase activity of signal transducers and activators of transcriptions 1 and 2 (STAT1 and 2) via cross-phosphorylation. This leads, in turn, to the recruitment of IFN-regulatory factor 9 (IRF9), a member of the family of transcription factors called IFN Regulatory Factors (IRFs), for their ability to regulate the expression of T1 IFN and its effects on target gene expression. IRF9 together with STAT1 and 2 form a complex known as the IFN-stimulated gene factor 3 (ISGF3). This complex translocates to the nucleus to bind to IFN-stimulated response elements (ISRE) in order to induce a family of genes that for this reason are called interferon-stimulated genes – ISGs [4<sup>\*\*\*</sup>,8]. A summary of the different pathways and key factors mentioned above leading to T1 IFN activation is shown in Fig. 1.

## GENETICS AND EPIGENETICS OF TYPE 1 INTERFERON DYSREGULATION IN SYSTEMIC SCLEROSIS

Familial association studies have previously shown that family history appears to be the strongest known risk factor for SSc. It was found that amongst first-degree relatives of SSc patients, the prevalence of the disease was 0.33%, with a relative risk factor of 13 when compared with the general United States population, which had a prevalence of 0.026% [31]. A twin study including 42 twin pairs (24 monozygotic and 18 dizygotic), found that the overall concordance of SSc was only 4.2% (1 out of 24) in monozygotic twins and 5.6% in dizygotic twins. The concordance, however, of antinuclear antibodies (ANAs) was significantly higher in monozygotic twins vs. dizygotic twins (90% vs 40%), suggesting that concordance for autoimmunity was much higher than the one for clinical disease phenotype. Consistent with these findings, a study in 4612 first-degree relatives of 1071 probands revealed an increased risk for familial autoimmunity among subtypes of SSc, with thyroid diseases and SLE showing the most significant increased prevalence when compared with control families, together with Raynaud’s phenomenon and ILD [32].

The most frequent form of genetic variation in humans is the single-nucleotide polymorphism (SNP), which influences protein function and is key to personalized medicine [33]. In a recent meta-analysis of Genome-Wide Association Studies (Meta-GWAS), which included 26 679 individuals, 27 independent genome-wide associated signals were identified, which included 13 new-risk loci, and nearly doubled the number of genome-wide hits



**FIGURE 1.** Different pathways and key factors leading to T1 IFN activation and ISG release.

previously reported in SSc [34]. This meta-analysis has suggested a variety of IFN-signalling loci, including T1 IFN regulatory factors IRF4 [35], IRF5 [36,37], IRF7 [34,38] and IRF8 [34,39,40]. (Fig. 1) Interestingly, apart from SSc, the genes have also shown an association with SLE [41–44]. Tyrosine kinase 2 (TYK2) [45], and STAT4 [34,46] are genes that have also been linked to SSc genetic susceptibility.

A shared genetic background of autoimmune diseases is clearly seen in GWAS, but additionally a vital role played by environmental factors (air pollution, infection and chemical substances, such as silicon) [47], and epigenetic influences in the pathogenesis of SSc has been suggested. Links to the pathogenesis of SSc have been previously reported for all the major epigenetic alterations, including DNA methylation [48–50], histone modifications [51,52], noncoding small (miRNA) and long (lncRNA) RNA transcript expression [53–56]. For instance, MiR-618 was found to be significantly overexpressed in SSc pDCs, causing an IRF8-dependent inhibition of pDC differentiation and activation, as well as increased production in IFN- $\alpha$  upon TLR9 stimulation [57]. lncRNAs are a larger class of transcribed RNA molecules, that are not translated but regulate gene expression [58]. It has recently been shown that a group of lncRNAs were modulated in a T1 IFN-dependent manner in human monocytes in response to TLR4 activation [59]. Among the lncRNAs, the negative regulator of the IFN response (NRIR) was found significantly upregulated in-vivo in SSc monocytes, and affected

the expression of the ISGs, CXCL10 and CXCL11. Therefore, dysregulation of NRIR in SSc monocytes may play a part in contributing to the aberrant IFN response present in SSc patients [59].

### EVIDENCE OF INCREASED TYPE 1 INTERFERON ACTIVATION IN SYSTEMIC SCLEROSIS

Due to the difficulty of directly measuring T1 IFN levels from human samples, an ‘interferon signature’ including the levels of expression of the transcript levels of multiple known ISGs has been widely used for this purpose. This method established the presence of increased T1 IFN in SLE, and more recently in other rheumatic diseases [60]. The first reported finding of an IFN signature in SSc dates back to 2006 [61]. Since then, it has been shown that an IFN signature in blood is found in a large proportion of SSc patients [5,62,63]. It has been also shown that activated monocytes and macrophages can be a potent source of T1 IFN and other profibrotic factors, stimulating the proliferation of fibroblasts and extracellular matrix accumulation [64]. An IFN signature in monocytes has even been found at the earliest phases of SSc, before overt fibrosis, suggesting of this being an early event in SSc pathogenesis [10].

A higher IFN signature in SSc whole blood or plasma has been found to correlate with the antibody profiling, where antitopoisomerase and anti-U1-RNP antibodies were associated with a higher IFN

signature [5,65]. Correlation of this higher IFN signature was also seen in more severe vascular manifestations and lung involvement [65–68]. Organs known to be targeted in SSc such as the skin and lung, have also demonstrated an overexpression of ISGs in SSc patients [69,70].

Upregulation of ISGs in the skin of SSc patients was also demonstrated in skin biopsy gene expression studies [70,71]. A study performing microarrays from lung tissue revealed upregulation of ISGs in addition to TGF- $\beta$ -regulated genes in SSc patients with ILD, with an increased expression of ISGs, associated with a higher rate of progression in ILD [69]. Interestingly, a recent multiomic comparative analysis of the serum profile, peripheral blood cells and skin ISG expression in SSc patients showed that the serum protein profile correlated more closely with the transcriptome of the skin than that of the PBMCs. This may be because of a spill-over effect from diseased end organs and suggests that IFN-inducible chemokine concentration may be a better predictor of tissue IFN activity than PBMC ISG expression levels [72,73].

Apart from the trial in IFN- $\alpha$  mentioned in the introduction of this review, case reports have been documented of the development of SSc in individuals treated with T1 IFN for other conditions. Interestingly, Anifrolumab (anti-IFNAR1 monoclonal antibody) in a phase 1 trial of SSc patients led to the suppression of the IFN signature and TGF $\beta$  signalling in SSc skin [74]. Additionally, in a graft-versus-host disease (GVHD) mouse model of SSc, neutralization of IFNAR1, and consequent normalization in the overexpression of T1 IFN-inducible genes, led to a marked reduction in the dermal fibrosis [75]. Consistent with these findings, in SSc patients treated with high-dose cyclophosphamide followed by rescue autologous hematopoietic stem cell transplantation, clinical response strongly correlated with normalization in T1 IFN module by RNAseq of peripheral blood cells [76].

The close mirroring of disease activity of T1 IFN activation has also been shown in the analysis of the SLS2 trial. Assassi *et al.* [77] have shown that higher serum IFN-inducible chemokine score predicted a better clinical response in both the cyclophosphamide and the mycophenolate mofetil arms. Importantly during the second year of the study, higher serum IFN score predicted worse clinical course in patients put on placebo, supporting the notion that IFN activation in SSc is deleterious, unless immunosuppressive treatment is initiated.

Vascular injury plays an important role in organ dysfunction in SSc, and it is the main driver of disease in patients with the limited cutaneous subset (LcSSc) of SSc. T1 IFN has been implicated in the

dysregulation of the vascular remodelling process in SSc. Myxovirus-resistance protein A (MxA), which is induced by T1 IFN, was found to correlate with digital ulcerations and lower pulmonary forced vital capacity in SSc [78]. T1 IFN has also been shown to contribute to the increased vascular permeability in SSc through downregulation of Fli1 (friend leukemia integration 1 transcription factor) and vascular endothelial cadherin (VE-cadherin) in endothelial cells and fibroblasts [79]. Features of SSc vasculopathy were also seen in mice with conditional deletion of Fli1 in endothelial cells confirming that T1 IFN-mediated downregulation of Fli1 enhanced the development of SSc [80].

Consistent with these observations, IFN-inducible chemokines were found to predict progression of patients with LcSSc as far as a multi-morbidity score including skin, lung, vascular and gastrointestinal progression [81].

Taken together, these observations suggest that T1 IFN is involved in both tissue and vascular fibrosis in SSc, strongly supporting the rationale for a direct therapeutic approach targeting the pathway.

## CURRENT EXPERIENCE IN TYPE 1 INTERFERON TARGETING FOR DISEASE MODIFICATION

Dysregulation in the T1 IFN response has been shown to contribute to the development of autoimmunity. Although the clinical manifestations vary amongst the different types of autoimmune diseases, T1 IFN protein or transcript signatures have now been identified in many of them (SSc, SLE, dermatomyositis and Sjogren's disease) [5,10,82–85].

In SLE, up to 80% of patients were shown to have a T1 IFN signature, with around 50% having chronically elevated T1 IFN levels, detectable in blood [86,87]. SLE patients with high T1 IFN activity, also tend to have higher disease activity scores with a greater tendency to relapse whilst in remission and a lower response rate to placebo medication [88–90]. Similarly to what has been observed in SSc, deranged pDC activation also occurs in SLE, and monoclonal antibodies against pDC have recently shown benefit on cutaneous and musculoskeletal lupus [91–93].

The effectiveness of blocking IFNAR, which plays a critical role in T1 IFN signalling, has now been concretely demonstrated in SLE patients with the monoclonal antibody Anifrolumab. The phase III Tulip-2 trial met its primary end-point, with an improvement in overall disease activity vs. placebo [94], leading to Food and Drug Administration (FDA) and European Medicine Agency (EMA) approval for treatment in SLE.

The similarities of T1 IFN activation in SSc, therefore, informs the rationale to block IFNAR in SSc and determine its therapeutic effectiveness [4<sup>■</sup>]. As mentioned above in this review, early phase 1 study of 34 SSc patients, showed that anifrolumab was well tolerated and showed peak inhibition of the T1 IFN signature in blood [95]. A follow-up mechanistic study showed that treatment with anifrolumab led to the reduction of the T1 IFN signature in whole blood and skin biopsy samples, demonstrating the suppressive effects of the anti-IFNAR1 antibody [74]. These findings provide further support for future larger double-blind, placebo-controlled trials of Anifrolumab in early SSc.

## CONCLUSION

Over the past few years, substantial progress has been made in deconvoluting the immune complexity of SSc, which has led to identify key molecular and cellular components of T1 IFN signalling involved in disease pathogenesis. In spite of the progress made, many unanswered questions in the pathogenesis of SSc remain. The origin and triggers of T1 IFN, and the interactions played between genetic and environmental factors, leading to dysfunction in the T1 IFN response still remains a grey area. However, newly discovered function of molecules such as CXCL4, start to lead towards a better understanding of the connections between pDCs, the IFN continuum and the fibrotic process. Further studies are also needed to elucidate downstream processes linking the T1 IFN activation to the exaggerated fibrotic response in fibroblasts and other key effector cells implicated in SSc pathogenesis.

Specifically, the identification of specific ligands and signalling pathways driving T1 IFN signalling in SSc will need further investigation with in-vivo and in-vitro studies. This will improve our understanding of SSc pathogenesis, and will increase the armamentarium of the therapeutic targets that could be exploited to improve patient outcome.

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- of special interest
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