The receptor for advanced glycation endproducts: A potential role in systemic sclerosis

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Chapter 4

THE ROLE OF ADVANCED GLYCATION ENDPRODUCTS AND HIGH MOBILITY GROUP BOX 1 IN SYSTEMIC SCLEROSIS SKIN FIBROSIS

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Chapter, abstract presented as poster presentation at the 6th Systemic Sclerosis World congress (2020) and European Alliance of Associations for Rheumatology congress (2019)
ABSTRACT

Objectives: Systemic sclerosis (SSc) is a progressive fibro-inflammatory disease. Activation of interferon pathways may play a role in the early onset of the disease. We studied the role of advanced glycation endproducts (AGEs) and high mobility group box 1 (HMGB1) on interferon-regulated gene upregulation in the possible onset of skin fibrosis in SSc.

Methods: AGE and HMGB1 expression was investigated in 17 skin biopsies of SSc patients and 5 healthy controls (HCs). AGE accumulation was assessed as skin autofluorescence (SAF). Interleukin-6 (IL-6), HMGB1, soluble receptor for AGE (sRAGE), and interferon-γ-inducible protein 10 (IP-10) levels were determined in sera of 20 SSc patients and 20 HCs. In vitro, healthy human dermal fibroblasts were stimulated with AGE-modified albumin (AGE-BSA) and HMGB1. Inflammatory, profibrotic, and interferon-regulated genes were measured by RT-qPCR. Myofibroblast differentiation was assessed by α-smooth muscle actin (α-SMA) staining.

Results: AGE expression in skin of SSc patients was increased compared to HCs, which was also seen for SAF, and for HMGB1, sRAGE, and IP-10 levels in serum. In vitro, stimulation with AGE-BSA and HMGB1 resulted in a strong increase in IP-10 mRNA expression, and moderate IL-6 increase. Myofibroblast differentiation was seen after dermal fibroblast stimulation with AGE-BSA and HMGB1.

Conclusion: AGEs accumulate in skin of SSc patients. In vitro, AGEs and HMGB1 lead to IL-6 mRNA induction, prominent induction of IP-10, and to a lesser extent other interferon-regulated genes in fibroblasts, partly in a RAGE-dependent manner, and promote myofibroblast formation. This potentially links the AGEs/HMGB1-RAGE axis to inflammation and interferon activation as a putative pathway in SSc pathogenesis.
INTRODUCTION

Systemic sclerosis (SSc) is a rare, progressive autoimmune disease associated with increased morbidity and mortality rates\textsuperscript{1,2}. It is characterised by vasculopathy, low-grade inflammation, and myofibroblast activation leading to abundant collagen deposition in the skin, vasculature, and internal organs\textsuperscript{3}. Unfortunately, the process initiating this disease remains elusive, and therapeutic options are limited. Thus, there is a need for the identification of early disease mechanisms contributing to SSc as possible targets for disease-modifying interventions. It has been suggested that the interferon signalling pathway is upregulated in patients with SSc and may play a role in fibrosis early in the disease course\textsuperscript{4}.

Fibroblast activation is a crucial process in the development of fibrosis in SSc. Activation of inflammatory and fibrotic pathways leads to myofibroblast formation and characteristic tissue damage\textsuperscript{5}. In SSc, it has been shown that the interferon pathway is upregulated upon activation of fibroblasts\textsuperscript{6}.

Advanced glycation endproducts (AGEs) are oxidative stress derived compounds that are formed during glycoxidative stress and/or ‘dicarbonyl stress’, and are considered to be damage associated molecular patterns (DAMPs). A recent study has demonstrated that the expression of the AGE N\textsuperscript{ɛ}-(carboxymethyl)lysine (CML) and the receptor for advanced glycation endproducts (RAGE) is increased in skin tissue of SSc patients\textsuperscript{7}. Moreover, it was shown that levels of soluble RAGE (sRAGE), and of the RAGE-ligand high mobility group box 1 (HMGB1) are elevated in sera and skin tissue of SSc patients\textsuperscript{8}. HMGB1 is a DNA-binding nuclear protein which can be released by activated, apoptotic, or necrotic cells, and is also considered a DAMP\textsuperscript{9}. Recently the involvement of DAMPs, toll-like receptor (TLR)-signalling, and type 1 interferon signature in SSc was reviewed by Frasca et al.\textsuperscript{10}.

Although DAMPs may activate fibroblasts by interaction with TLRs, several other pattern recognition receptors may also play an important role. This prompted us to investigate whether AGEs and HMGB1 play a role in fibroblast activation through RAGE, and may induce the activation of proinflammatory, profibrotic, and interferon-inducible pathways related to the pathogenesis of SSc in a translational study. In addition to looking at the expression of AGEs and HMGB1 in skin and their serum levels in patients with SSc compared to matched healthy controls (HCs), we investigated whether in vitro stimulation of healthy human dermal fibroblasts by
AGE-bovine serum albumin (AGE-BSA) and HMGB1 leads to enhanced expression of proinflammatory, profibrotic, and interferon-inducible genes.

MATERIALS AND METHODS

Patient population
We studied the role of AGEs and HMGB1 in inflammation and fibrosis in SSc at three different levels. With immunohistochemistry protein expression was investigated in skin biopsies of 17 SSc patients and 5 HCs (study I). With ELISA protein expression was investigated in sera of 20 SSc patients and 20 age- and sex-matched HCs (study II). Finally, in an in vitro study healthy human dermal fibroblasts were stimulated with AGE-BSA and HMGB1 (study III). SSc patients fulfilled the 2013 ACR/EULAR clinical criteria. The studies were conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent and the local ethics committee of the University Medical Centre Groningen (METc study I 2018/373; study II 2014/337), The Netherlands, approved the studies.

Immunohistochemistry
Distribution of CML (ab30917, Abcam, Cambridge, UK), pentosidine (orb27502, Biorbyt Ltd., Cambridge, UK), Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine ([MG-H1], STA-011, cell biolabs, inc., NL), and HMGB1 (ab18256, Abcam) was assessed. Also, staining of myxovirus resistance protein A ([MxA], R&D, AF7946) as a marker of interferon type 1 expression was performed. Subsequently, slides were incubated with either anti-mouse (DakoCytomation, P0260), goat (DakoCytomation, P0449), or rabbit (DakoCytomation, P0448) horseradish peroxidase (HRP)-labeled secondary antibodies. Slides were stained with diaminobenzidine (DAB) colorant (Dako, K4006) and counterstained with haematoxylin. The intensity was semi-quantitatively assessed by two independent investigators (IMA and BDvdM) on endothelium, fibroblasts, extracellular matrix including collagen and elastin, and infiltrate. These were graded as follows: 0 (no staining), 0.5 (weak), 1 (moderate), 2 (intermediate), and 3 (strong) intensity.

Serum markers
Levels of interleukin-6 (IL-6), HMGB1, sRAGE, and interferon-γ-inducible protein 10 (IP-10) were determined in serum of 20 SSc patients and 20 age- and sex-matched HCs by enzyme-linked immunosorbent assay (ELISA) kits (HMGB1 with TECAN; others
with Duosets from R&D systems), according to manufacturer's instruction. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels were determined routinely and obtained from patients' charts.

**Skin autofluorescence**

Skin autofluorescence (SAF), a non-invasive method to assess dermal AGE accumulation, was obtained with the AGE Reader mu (DiagnOptics Technologies, NL) which has been extensively validated in various diseases and HCs to AGEs measured in skin biopsies. SAF was assessed on a standardised location on the volar side of the forearm, approximately 10 cm below the elbow crease. Details of this method are published elsewhere.

**Cell culture and treatment**

Healthy human dermal fibroblasts obtained from tissue available from mamma resection, were cultured in Dulbecco's modified Eagle's medium ([DMEM] high glucose, L0104-500; Biowest), and supplemented with 10% FCS, 1% penicillin, and streptomycin. Cells were incubated at 37°C (5% CO₂ atmosphere) and routinely passaged upon reaching approximately 80-100% confluence, using 0.5 mmol ethylenediaminetetraacetic acid ([EDTA] Cat no. 1084180250; MERCK) and 0.05% trypsin (1689349; MP) in PBS.

Cells were incubated with 50 µg/ml AGE-BSA (121800-10MG; EMD Millipore), 2 µg/ml HMGB1 (Bovine HMGB1; 9050; Chondrex), or 10 ng/ml recombinant human tissue growth factor-β1 ([TGF-β1] 100-21-10ug; Peprotech). As a negative control, cell medium without stimulus was used. After 24 h, cells were collected in TRIzol for mRNA detection. Furthermore, to investigate the role of RAGE, a specific RAGE inhibitor FPS-ZM1 (553030-25MG; EMD Millipore) was added at a concentration of 10 µM 30 min before stimulation with TGF-β1, AGE-BSA, and HMGB1. After the experiment, cells were lysed in TRIzol and stored in -80°C until further analysis.

**RNA isolation and RT-qPCR**

Total mRNA was isolated from TRIzol according to standard procedures, and cDNA was synthesised from total RNA using M-MLV Reverse Transcriptase (Invitrogen, oligo(dT) 12-18). The mRNA levels of IL-6, collagen-1α (Col-1α), connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA), interferon genes (IFN α-induced44L [IFI44L], Myxovirus resistance protein 1 [Mx1], Lymphocyte antigen 6 complex, locus E [LY6E], and IP-10), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were
measured by an Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with specific Taqman assays (IL-6 [Hs00174131_m1], α-SMA [ACTA2, Hs00909449_m1], Col-1α [Hs00164004_m1], CTGF [Hs00170014_m1], IFI44L [Hs00915292_m1], and IP-10 [Hs00171042_m1], all by Thermo Fisher Scientific). The amount of target was normalised to an endogenous reference (GAPDH [Hs99999905_m1]), and expressed as relative expression (2^−ΔCT) or as fold induction compared to an unstimulated sample (2^−ΔΔCT).

**Immunohistochemical staining of fibroblasts**

Fibroblasts were seeded in DMEM + 10% FCS. Cells were treated with 1–10-50 ng/ml TGF-β1, 1-100 µg/ml AGE-BSA, and 1–10 µg/ml HMGB1. After 24 h of stimulation, the medium was removed and cells were washed with PBS. Cells were fixed with 1% paraformaldehyde (244747; Bufa B.V., Pharmaceutical products) at room temperature (RT) for 20 min. After fixation, cells were washed in PBS, permeabilised with 0,2% Triton X-100, and blocked with 2% BSA/PBS. First, cells were stained with human α-SMA mouse monoclonal antibody (ab7817; Abcam) for 1 h at RT and conjugated with polyclonal rabbit anti-mouse immunoglobulins/HRP 1:50 (p0260; Dako) for 30 min, as secondary antibody. These antibodies were diluted in PBS 1% BSA. Second, cells were washed in PBS, stained with DAB (K3468; Dako) for 15 min, and counterstained with haematoxylin for 5 sec after washing with PBS.

**Statistical analysis**

Statistics were performed using IBM SPSS Statistics version 23. Data are presented as median with interquartile range (IQR) or number (%). Statistical analysis was performed using the nonparametric Mann-Whitney U test and Wilcoxon matched-pairs signed rank test for determining comparison between groups, and the nonparametric Spearman’s rank correlation test for correlations. A P-value <0.05 was considered as statistically significant.

**RESULTS**

**Patient characteristics**

Characteristics of the study groups are shown in Table 1. The median age of patients with SSc in study I was 59 years (51-65), 14 of them were female. The median disease duration was 16 years (7-21). In study II the median age and gender distribution of patients with SSc was comparable to the HCs. The median disease duration was 2 years (1-8).
Table 1. Participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SSc patients</td>
<td>Healthy controls</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>14 (82.4)</td>
<td>14 (70.0)</td>
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<tr>
<td>Age in years, median (IQR)</td>
<td>59 (51-65)</td>
<td>52 (45-62)</td>
</tr>
<tr>
<td>Caucasian ethnicity, n (%)</td>
<td>17 (100.0)</td>
<td>19 (95.0)</td>
</tr>
<tr>
<td>Smoker (ever), n (%)</td>
<td>12 (70.6)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>RP duration in years, median (IQR)</td>
<td>20.0 (12.0-38.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Disease duration since first non-RP symptom in years, median (IQR)</td>
<td>16.0 (7.0-21.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Organ involvement, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>5 (29.4)</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>PAH</td>
<td>1 (5.9)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>ILD</td>
<td>5 (29.4)</td>
<td>9 (45.0)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>12 (70.6)</td>
<td>8 (40.0)</td>
</tr>
</tbody>
</table>

SSc: systemic sclerosis; RP: Raynaud’s phenomenon; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease; NA: not applicable.

Localisation of AGEs, HMGB1, and MxA in skin biopsies of SSc patients (study I)

Staining of MG-H1 and pentosidine showed an enhancement in skin of SSc patients compared to control skin (Figure 1). In extracellular matrix, CML staining was higher in SSc patients, while it was higher in HCs when endothelium, fibroblasts, and infiltrates were also counted. HMGB1 staining was seen in nuclei of cells as expected, and no visible extranuclear or extracellular HMGB1 expression was found in both skin of SSc patients and HCs (data not shown). Expression of MxA in skin of SSc patients was primarily seen in the vascular wall, this applied also for skin of HCs (Supplementary Figure).

Markers of inflammation in sera of SSc patients (study II)

Serum levels of CRP (p=0.04), sRAGE (p=0.02), HMGB1 (p=0.03), and IP-10 (p=0.009) were significantly increased in SSc patients, as compared to HCs (Figure 2). Also, ESR levels were significantly increased in patients with SSc (p=0.02). IL-6 was not increased in SSc patients (p=0.9). Moreover, SAF was significantly increased in the skin of SSc patients, as compared to HCs (SSc: median 2.2 AU [1.8-2.5]; HCs: median 1.8 AU [1.4-2.1]; p=0.02).
Figure 1. Expression and localisation of advanced glycation endproducts in representative skin sections of patients with systemic sclerosis.

The total score (sum of vascular wall, fibroblasts, ECM, and infiltrate) of MG-H1 (A), CML (E), and pentosidine (L) staining was investigated in the dermis of SSc patients and healthy controls. Also, the score per compartment was investigated for MG-H1 (B), CML (F), and pentosidine (J). Figure C, G, and K show images of negative controls. Figure D, H, and L show representative images of MG-H1 (D), CML (H), and pentosidine (L) staining on SSc skin. Scale bar, 100 µm. SSc: systemic sclerosis; ECM: extracellular matrix; MG-H1: Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; CML: NƐ(carboxymethyl)lysine.
Figure 2. Serum levels of inflammatory and oxidative stress markers in systemic sclerosis patients and healthy controls.

Levels of CRP (A), ESR (B), IL-6 (C), IP-10 (D), HMGB1 (E), and sRAGE (F) were measured in serum of SSc patients and healthy controls (HC).

SSc: systemic sclerosis; IL-6: interleukin-6; IP-10: interferon-γ-inducible protein 10; HMGB1: high mobility group box 1; sRAGE: soluble receptor for advanced glycation endproducts.
In vitro experiments: mRNA expression of inflammatory, profibrotic, and interferon genes in dermal fibroblasts (study III)

The relative expression of IL-6, Col-1α, CTGF, α-SMA, IFI44L, Mx1, IP-10, and Ly6E mRNA, and the effect with the RAGE inhibitor is shown in Figure 3A-F. The results revealed that IL-6, Col-1α, CTGF, IFI44L, and Mx1 mRNA expression increased significantly following TGF-β1 stimulation compared to control (unstimulated cells) (p<0.05). The mRNA expression of IL-6, α-SMA, IFI44L, Mx1, and especially IP-10 increased after stimulation with AGE-BSA and HMGB1, suggesting activation of both inflammatory and interferon pathways.

We investigated the role of the receptor RAGE in inducing these disease specific genes by using the specific RAGE inhibitor FPS-ZM1. In Figure 3B, D, and F the effect of RAGE inhibition on mRNA expression is shown on genes that were more than 1.5 fold increased after stimulation. The mRNA levels of IL-6, Col-1α, CTGF, and IFI44L induced by TGF-β1 were not inhibited by the RAGE inhibitor FPS-ZM1 (Figure 3B). The mRNA levels of AGE-BSA- and HMGB1-induced IL-6 were significantly inhibited by addition of FPS-ZM1 (p<0.05), and inhibition of IP-10 mRNA showed a trend (p=0.06) (Figure 3D and F). A trend was also seen in inhibition of Mx1 after stimulation with HMGB1 (p=0.06).

The expression of α-SMA in stimulated and unstimulated dermal fibroblasts

The expression of α-SMA in fibroblasts treated with TGF-β1, AGE-BSA, and HMGB1 is shown in Figure 4A-H. Administration of TGF-β1 resulted in formation of SMA fibers. Expression of α-SMA was shown in the presence of 10 µg/ml AGE-BSA and 100 µg/ml AGE-BSA (Figure 4E and F), and of 1 µg/ml HMGB1 (Figure 4G) and 10 µg/ml HMGB1 (Figure 4H).
Figure 3. The mRNA expression of inflammatory, fibrotic, and interferon genes, and the effects of RAGE blockade.

Dermal fibroblasts were stimulated with TGF-β1 (A), AGE-BSA (C), and HMGB1 (E), and the receptor RAGE was inhibited by addition of FPS-ZM1 after exposure to TGF-β1 (B), AGE-BSA (D), and HMGB1 (F). RAGE: receptor for advanced glycation endproducts; TGF-β1: tissue growth factor-β1; AGE-BSA: advanced glycation endproduct-bovine serum albumin; HMGB1: high mobility group box 1.
Figure 4. The expression of α-SMA in TGF-β1-, AGE-BSA-, and HMGB1-treated dermal fibroblasts.

The expression of α-SMA was investigated in unstimulated dermal fibroblasts (negative control) (A), and in stimulated fibroblasts with 1 ng/ml TGF-β1 (B), 10 ng/ml TGF-β1 (C), 50 ng/ml TGF-β1 (D), 10 µg/ml AGE-BSA (E), 100 µg/ml AGE-BSA (F), 1 µg/ml HMGB1 (G), and 10 µg/ml HMGB1 (H). Scale bar, 100 µm.

α-SMA: α-smooth muscle actin; TGF-β1: tissue growth factor-β1; AGE-BSA: advanced glycation endproduct-bovine serum albumin; HMGB1: high mobility group box 1.
DISCUSSION

In this study, we show increased expression of AGEs in skin of SSc patients. Moreover, we report that the RAGE-ligands AGEs and HMGB1 induce upregulation of inflammatory, profibrotic, and interferon-regulated genes in healthy human dermal fibroblasts, and induction of differentiation into myofibroblasts. This suggests a potential role of AGEs and HMGB1 in promoting disease specific pathways, and, especially, in the onset of fibrosis, potentially mediated by RAGE. Furthermore, the upregulation of interferon-regulated genes is most prominent, suggesting a potential role for interferon type 1 in the development of fibrosis, as shown by the formation of myofibroblasts. In Figure 5 a schematic representation is shown of the possible contribution of the AGEs/HMGB1-RAGE axis to disease progression in SSc.

**Figure 5. Schematic figure showing the possible contribution of the AGEs/HMGB1-RAGE axis to disease progression in systemic sclerosis.**

Activation of RAGE by ligands such as AGEs and HMGB1 (1) results in myofibroblast formation and activation (2). This ultimately leads to vasculopathy, fibrosis, and organ failure leading to disease progression in SSc (3). This figure was created with BioRender.com.

SSc: systemic sclerosis; RAGE: receptor for advanced glycation endproducts; AGEs: advanced glycation endproducts; HMGB1: high mobility group box 1; TLRs: toll-like receptors; IL-6: interleukin-6; Col-1α: collagen-1α; CTGF: connective tissue growth factor; α-SMA: α-smooth muscle actin; IFI44L: interferon α-induced 44-like; Mx1: myxovirus resistance protein 1; LY6E: lymphocyte antigen 6 complex, locus E; IP-10: interferon-γ-inducible protein 10.
Several research groups have pointed at the role of interferon type 1 in the pathogenesis of SSc. This has been shown by a prominent interferon signature in peripheral blood mononuclear cells, skin, and lungs of SSc patients. Additional evidence comes from a study by Assassi et al. showing that a subset of patients with SSc exhibits a ‘lupus like’ interferon signature, and fits within the spectrum of diseases mediated by interferon.

Considering the increased interferon-regulated gene expression by fibroblasts after exposure to AGEs and HMGB1 in our study, we performed inhibitory experiments to investigate involvement of RAGE. We showed that inhibition of RAGE resulted in downregulation of inflammatory and interferon-regulated genes. A recent study by Senatus et al. on the biology of RAGE revealed a new link between signalling of RAGE and interferon type 1. They showed that deletion of AGER (the gene encoding RAGE) in regressing atherosclerotic plaques reduced the interferon pathway in macrophages, especially reducing interferon regulatory factor 7 (IRF 7) expression. IRF 7 is a major mediator in interferon type 1 signalling. It leads to secretion of interferon type 1 after binding to its promoter region in the nucleus. Wu et al. demonstrated that IRF 7 may represent a link between the interferon type 1 signature and fibrosis in the pathogenesis of SSc. Next to upregulation of IRF 7 mRNA and protein levels in skin tissue and fibroblasts of patients with SSc compared to HCs, they showed a decrease in fibrosis parameters such as profibrotic genes in knockdown IRF 7 SSc fibroblasts, and, also, dermal thickness and hydroxyproline, an important collagen constituent, in IRF 7 knockout mice. These findings are in line with our results demonstrating upregulation of interferon-regulated genes and inhibition of interferon-regulated genes by the RAGE inhibitor FPS-ZM1 in dermal fibroblasts. Moreover, the findings by Wu et al. underline the possibility that the formation of myofibroblasts in our study is the consequence of interferon upregulation.

Previously, a study by Brkic et al. found an interferon type 1 signature before overt fibrosis of the skin in SSc patients, and suggested a potential role of interferon type 1 in the development of fibrosis in SSc. In our study, we showed expression of MxA in skin of SSc patients, indicating interferon type 1 activation. Moreover, total AGE staining and SAF, a non-invasive measurement to assess accumulation of AGES in dermal tissue, was increased in our SSc patients compared to HCs, and, fibroblast stimulation with AGE-BSA resulted in myofibroblast differentiation. Lohwasser et al. showed that interactions between CML-BSA and RAGE may contribute to a profibrotic pattern in human foreskin fibroblasts. This study and our results suggest
that RAGE stimulation with AGEs may induce fibroblast differentiation and formation of SMA fibers. A recent review included several studies which show also the possibility of AGE-RAGE interactions contributing to the development of fibrosis. One example is kidney fibrosis as a result of interactions between AGEs and RAGE, which lead to stimulation of several pathways such as TGF-β, CTGF, MAPK, and NF-κB.

There are some limitations that our study presents. Interactions with other receptors of AGE-BSA and HMGB1, such as TLRs, have not been ruled out. Nevertheless, in addition to our experiments in sera and skin of SSc patients, our results give new insights into possible additional contributors to the complex SSc pathophysiology.

Another limitation is the limited number of SSc patients used for measuring cytokines in sera, and, therefore, multivariate regression analysis has not been performed. Sample size calculation was not performed. Moreover, skin biopsies were taken in another SSc cohort than the SSc patients from whom we collected blood samples. Although our results underline the possible contribution of the AGEs/HMGB1-RAGE axis to the pathogenesis of SSc, our results should be confirmed in a larger prospective study.

In conclusion, the results from our translational study indicate that the AGEs/HMGB1-RAGE axis may play a role in enhancing inflammation, and, particularly, the onset of fibrosis in SSc through a proinflammatory and profibrotic environment, and increased interferon activation. Although validation in an independent cohort is mandatory, our results may give us valuable insight into the pathogenic mechanisms involved in SSc pathogenesis, potentially paving the way for new therapies directed at the AGEs/HMGB1-RAGE axis.
REFERENCES


Supplementary Figure. Expression and localisation of interferon type 1 in representative skin sections of patients with systemic sclerosis.

The total score (sum of vascular wall, fibroblasts, ECM, and infiltrate) of MxA in the dermis of SSc patients and healthy controls (A), the score per compartment (B), a negative control (C), and a representative image of MxA staining (D) on SSc skin are shown. Scale bar, 20 µm.

SSc: systemic sclerosis; ECM: extracellular matrix; MxA: myxovirus resistance protein A.