Scleroderma-specific autoantibodies embedded in immune complexes mediate endothelial damage: an early event in the pathogenesis of systemic sclerosis

CURRENT STATUS: UNDER REVIEW

Arthritis Research & Therapy  ▶ BMC

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DOI:
10.21203/rs.3.rs-18870/v1

SUBJECT AREAS
Neurobiology of Disease  Pathology

KEYWORDS
Systemic sclerosis, Autoantibodies, Immune complexes, Toll-like Receptors, Endothelial cells, Fibrosis, Inflammation
Abstract

Background: Consistently with their diagnostic and prognostic value, autoantibodies specific for systemic sclerosis (SSc) embedded in immune complexes (ICs) elicited a pro-inflammatory and pro-fibrotic cascade in healthy skin fibroblasts, engaging Toll-like Receptors (TLRs) via their nucleic acid components. The objective of this study was to investigate the pathogenicity of SSc-ICs in endothelial cells (ECs).

Methods: ICs were purified from sera of SSc patients bearing different autoantibody specificities (antibodies against DNA topoisomerase I, centromeric proteins, RNA polymerase and Th/To), patients with systemic lupus erythematosus (SLE), primary anti-phospholipid syndrome (PAPS) or healthy controls (NHS) using polyethylen glycol precipitation. Human umbilical vein ECs (HUVECs) were incubated with ICs, IL-1β, Poly I:C, LPS or ODN CpG. mRNA levels of endothelin-1 (ET-1), collagenα1 (collα1), interferon (IFN)-α and IFN-β were investigated by Real-Time PCR; ICAM-1 expression was evaluated by cell-ELISA; secretion of IL-6, IL-8 and tumour growth factor (TGF)-β1 in culture supernatants was measured by ELISA. Intracellular signalling pathways culminating with NFkB, p38MAPK, SAPK-JNK and Akt were assessed by Western Blotting. Healthy skin fibroblasts were stimulated with supernatants from HUVEC incubated with ICs, and tumour growth factor (TGF)-β1secretion, mRNA levels of collα1 and matrix metalloproteinase (mmp)-1 were evaluated.

Results: All SSc stimulated IL-6 secretion compared to NHS-ICs; ACA-ICs and anti-Th/To-ICs increased ICAM-1 expression; all SSc-ICs but anti-Th/To-ICs augmented IL-8 levels; all SSc-ICs but ACA and ARA-ICs up-regulated et-1 and all SSc-ICs but ARA-ICs affected TGF-β1 secretion. Collα1, IFN-α and IFN-β mRNA levels were not affected by any SSc-ICs. A differential modulation of tlr expression was observed: tlr2, tlr3 and tlr4 were upregulated by ATA-ICs and ACA-ICs, while anti-Th/To-ICs resulted in tlr9 up-regulation. All SSc-ICs activated p38MAPK and AKT, all SSc-ICs but ARA-ICs yielded the activation of NFkB; ATA-ICs and ACA-ICs increased the activation rate of both subunits of SAPK-JNK. When healthy skin fibroblasts were stimulated with supernatants from HUVECs incubated with SSc-ICs, TGF-β1 secretion and collα1 expression levels were significantly modulated.

Conclusions: These data provide the first demonstration of the pathogenicity of ICs from scleroderma
patients with different autoantibodies in ECs. Endothelial activation induced by SSc-ICs ultimately led to a pro-fibrotic phenotype in healthy skin fibroblasts.

**Full Text**

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

**Tables**

| Gene   | TaqMan® Gene Expression ID |
|--------|----------------------------|
| thr2   | Hs01872448_s1              |
| thr3   | Hs01551078_ml              |
| thr4   | Hs00152939_ml              |
| thr7   | Hs01933259_s1              |
| thr8   | Hs00152972_ml              |
| thr9   | Hs00370913_s1              |
| ifni-α | Hs00855471_g1              |
| ifni-β | Hs01077958_s1              |
| et-1   | Hs00174961_ml              |
| collα1 | Hs00164004_ml              |
| mmp-1  | Hs00899658_ml              |
| gapdh  | Hs99999905_ml              |

**Figures**
ICAM-1 expression and IL-6 and IL-8 secretion in HUVECs stimulated with SSc-ICs, PAPS-ICs, SLE-ICs or NHS-ICs. Endothelial cells were incubated with SSc-ICs, PAPS-ICs, SLE-ICs or NHS-ICs (1:2 dilution). IL-1β (50 U/ml) and LPS (1 μg/ml) were used as positive controls. *p<0.01; **p<0.001; ***p<0.0001 versus medium. (A): ICAM-1; (B): IL-6; (C): IL-8.

et-1 mRNA expression levels in HUVECs stimulated with SSc-ICs, PAPS-ICs, SLE-ICs and NHS-ICs; TGF-β1 secretion in HUVEC stimulated with SSc-ICs or NHS-ICs. Endothelial cells were incubated with SSc-ICs, PAPS-ICs, SLE-ICs or NHS-ICs (1:2 dilution). IL-1β (50 U/ml) and LPS (1 μg/ml) were used as positive controls. *p<0.01; **p<0.001; ***p<0.0001 versus medium. (A): et-1; (B): TGF-β1.
tlr mRNA expression levels in HUVECs stimulated with SSc-ICs, PAPS-ICs, SLE-ICs and NHS-ICs. Endothelial cells were incubated with SSc-ICs, PAPS-ICs, SLE-ICs or NHS-ICs (1:2 dilution). LPS (1 μg/ml), Poly I:C (1 μg/ml) and ODN CpG (5 μM) were used as positive controls. (A): tlr2; (B): tlr3; (C): tlr4; (D): tlr9. *p<0.01; **p<0.001; ***p<0.0001 versus medium.
Intra-cellular signaling pathways in HUVECs stimulated with SSc-ICs, PAPS-ICs, SLE-ICs or NHS-ICs. Endothelial cells were incubated with SSc-ICs, PAPS-ICs, SLE-ICs or NHSICs (1:2
IL-1β (50 U/ml) was used as positive control. (A): pNFkB/NFkB; (B): pp38/p38; (C): pp46SAPK-JNK/p46SAPK-JNK; (D): pp54SAPK-JNK/p54SAPK-JNK; (E): pAKT/AKT. Results are expressed as the ratio of phosphorylated to non-phosphorylated forms, evaluated using Image J software. Western Blotting images are representative of a single experiment.

pNFkB: phosphorylated NFkB; p38MAPK: phosphorylated p38MAPK; pp46SAPK-JNK: phosphorylated p46SAPK-JNK; pp54SAPK-JNK: phosphorylated p54SAPK-JNK; pAKT: phosphorylated AKT. *p<0.01; **p<0.001; ***p<0.0001 versus medium.

Figure 5

TGF-β1 secretion and collα1 and mmp-1 mRNA expression in fibroblasts stimulated with supernatants from HUVEC incubated with SSc-ICs or NHS-ICs. Fibroblasts were exposed to supernatants from HUVECs incubated with SSc-ICs or NHS-ICs (1:2 dilution). TGF-β1 (10 ng/ml) was used as positive control for collagen and mmp-1 synthesis. (A): TGF-β1; (B): collα1; (C): mmp-1. *p<0.01; ***p<0.0001 versus medium.