



The Proadhesive Phenotype of Systemic Sclerosis Skin Promotes Myeloid Cell Adhesion



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ABSTRACT

Purpose. Systemic sclerosis (SSc) is characterized by microvascular abnormalities and leukocyte infiltration. Previous studies have suggested a proadhesive phenotype in SSc skin. CD44, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), molecules that mediate leukocyte adhesion to endothelial cells and other cell types and extravasation, are overexpressed in SSc, but the functional consequences of this overexpression are not known. Additional adhesion molecules known to mediate leukocyte adhesion and migration include those present at intracellular junctions, such as junctional adhesion molecule-B (JAM-B), JAM-C, and CD99, however their expression and role in the pathogenesis of SSc is unknown. The aim of this study was to determine the expression levels of these adhesion molecules on SSc dermal fibroblasts and their role in facilitating myeloid cell adhesion to SSc skin.

Methods. The expression of JAM-B, JAM-C, CD44, CD99, and VCAM-1 on SSc fibroblasts was determined using cell surface ELISAs. Myeloid U937 cell-SSc dermal fibroblast adhesion assays or *in situ* adhesion assays to clinically less involved (proximal arm) or more involved (distal forearm) skin were performed.

Results. CD44, JAM-B and JAM-C were expressed on SSc (n=4) dermal fibroblasts, as was CD99, which was overexpressed (2.8 fold vs. normal (NL) dermal fibroblasts, p<0.05). Expression of VCAM-1 on SSc dermal fibroblasts was highly inducible by tumor necrosis factor- α (145 fold change versus non-stimulated, n=4, p<0.05). Neutralizing antibodies to JAM-B, JAM-C, CD44, or CD99 did not inhibit the binding of U937 cells to SSc dermal fibroblasts, but neutralizing anti-ICAM-1 antibody did (n=3, p<0.05). In addition, blocking ICAM-1 inhibited U937 cell adhesion to both proximal (n=5, 49% of maximal binding, p<0.05) and distal SSc skin (n=6, 39% of maximal binding, p<0.05). VCAM-1 also facilitated U937 binding to SSc skin, as its neutralization lead to 41% (n=4, p<0.05) reduction in binding to proximal SSc skin and 35% (n=6, p<0.05) reduction in binding to distal SSc skin. The combination of anti-ICAM-1 and anti-VCAM-1 resulted in a reduction of 61% for both proximal and distal SSc skin (n=5, n=6, both p<0.05).

Conclusions. These studies show that CD99 is overexpressed on SSc dermal fibroblasts vs. NL dermal fibroblasts, but does not mediate myeloid cell adhesion. However, we demonstrate an important role for ICAM-1 and VCAM-1 in the retention of myeloid cells in SSc skin, suggesting that targeting these molecules may be useful novel SSc therapies.

INTRODUCTION

Systemic sclerosis (Scleroderma, SSc) is a multisystem disorder characterized by Raynaud's phenomenon, vasculopathy, and fibrosis of the skin and internal organs. Patients with SSc have decreased numbers of capillaries, capillary hemorrhages, and clusters of enlarged, distorted, capillary loops which can be seen in the skinfold of the fingers, which correlate with sclerodermatous involvement of the skin and internal organs. ECs, monocytes, and fibroblasts seem to be the key players in the pathogenesis of SSc.

Previous studies have suggested a proadhesive phenotype in SSc. Serum levels of several adhesion molecules are increased in SSc compared to healthy controls. Serum levels of soluble E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) correlate with SSc disease severity. Moreover, the levels of soluble E-selectin and VCAM-1 in SSc serum correlate with a positive response to some therapies.

In addition to increased levels of soluble adhesion molecules in SSc serum, we and others have shown that several adhesion molecules are overexpressed in SSc skin and on SSc dermal fibroblasts including VCAM-1, ICAM-1, P-selectin, E-selectin, CD44, and integrins β 1, β 2, and α 6. Aberrant adhesion molecule expression may play multiple roles in SSc skin pathogenesis. The increased expression of select adhesion molecules may lead to increased accumulation of leukocytes in SSc skin. Abraham et al. demonstrated that SSc fibroblasts have a greater propensity for binding T lymphocytes compared to normal fibroblasts, in part by their overexpression of ICAM-1. Overexpression of select adhesion molecules resulting in the accumulation of specific, activated leukocytes may play a role in the induction of fibrosis via cytokine release leading to excess extracellular matrix synthesis. Alternatively, the accumulation of leukocytes due to increased adhesion molecule expression, and subsequent angiogenic/angiostatic cytokine release, may play a role in the disorganized angiogenesis in SSc skin.

We hypothesized that adhesion molecules that are overexpressed in SSc skin may play a role in SSc pathogenesis by promoting the adhesion of monocytes. We first sought to determine the expression of adhesion molecules in SSc skin. These molecules include junctional adhesion molecule-B (JAM-B), JAM-C, and CD99, which have previously been shown to play a role in leukocyte adhesion and trans-endothelial migration. We then sought to determine which adhesion molecules mediate myeloid cell adhesion to SSc skin. Our results demonstrate an important role for ICAM-1 and VCAM-1 in the retention of myeloid cells in SSc skin.

MATERIALS AND METHODS

Patient samples. Punch biopsy skin samples (4 mm) were obtained from SSc and normal volunteers. Two biopsies were taken from SSc patients, one from the proximal arm (less clinically involved) and the other from the distal forearm (more clinically involved). All SSc patients fulfilled the ACR criteria for diffuse SSc.

Immunohistochemistry. Frozen skin samples were subjected to immunohistochemistry using standard procedures. Cell surface ELISA. SSc and normal fibroblasts were seeded in 96-well plates, stimulated with or without TNF- α , IL-1 β , IFN- γ , IL-17, or IL-18 (25 ng/ml), and cell surface ELISAs were performed.

In vitro cell adhesion assays. Adhesion of U937 cells to SSc fibroblasts was tested in the presence or absence of anti-JAM-B, JAM-C, CD99, or ICAM-1 antibodies, or irrelevant IgG controls (25 ng/ml).

Stampier-Woodruff assays. *In situ* adhesion assays were performed using frozen SSc skin samples incubated with neutralizing antibodies against ICAM-1, VCAM-1, or a combination of the two antibodies, or IgG control.

Immunohistochemical analysis of JAM-B, JAM-C, and CD99 on SSc and normal skin ECs

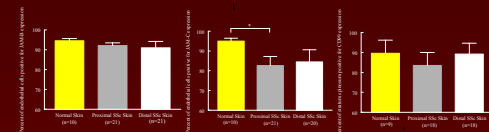


Figure 1. Frozen sections of proximal and distal SSc and NL skin were stained for JAM-B, JAM-C, and CD99 expression. Means are given with SEM. n = the number of patients. p<0.05 was considered significant.

Immunohistochemical analysis of CD99 on SSc and normal skin mononuclear cells

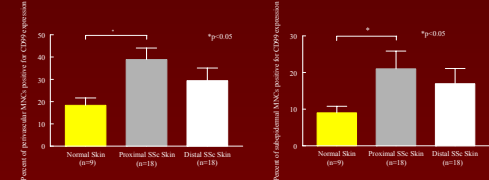


Figure 2. Frozen sections of proximal and distal SSc and NL skin were stained for CD99 expression. Means are given with the SEM. n = the number of patients. p<0.05 was considered significant.

JAM-B, JAM-C, CD44, and CD99 are expressed on SSc dermal fibroblasts

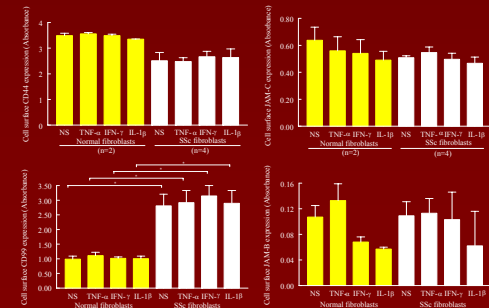


Figure 3. Cell surface ELISAs were performed to determine if JAM-B, JAM-C, CD44, and CD99 were expressed on the surface of normal and SSc dermal fibroblasts and if their surface expression was dependent on TNF- α , IFN- γ , or IL-1 β . Means \pm the SEM are shown. n = the number of patient derived fibroblast cell lines. p<0.05 was considered significant.

The expression of VCAM-1 and ICAM-1 on SSc dermal fibroblasts is highly inducible

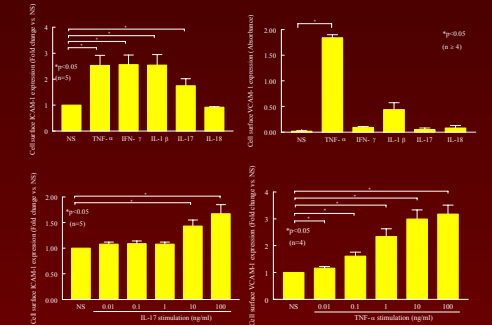


Figure 4. Cell surface ELISAs were performed to determine if VCAM-1 and ICAM-1 expression on the surface of SSc dermal fibroblasts was inducible by cytokine stimulation. Means \pm the SEM are shown. n = the number of patient derived fibroblast cell lines. p<0.05 was considered significant.

ICAM-1 mediates adhesion of U937 cells to SSc dermal fibroblasts

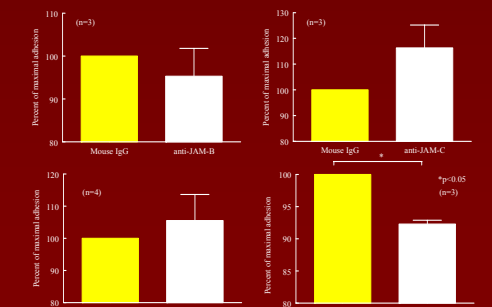


Figure 5. Adhesion assays were performed using U937 cells and SSc dermal fibroblasts. The percent of maximal binding was defined as the number of cells adhering to the fibroblasts in the presence of the test antibody divided by the number of adherent cells on the control treated fibroblasts. Means are shown \pm the SEM. n = the number of SSc patient derived fibroblast cell lines. p<0.05 was considered significant.

ICAM-1 and VCAM-1 mediate adhesion of U937 cells to proximal and distal SSc skin

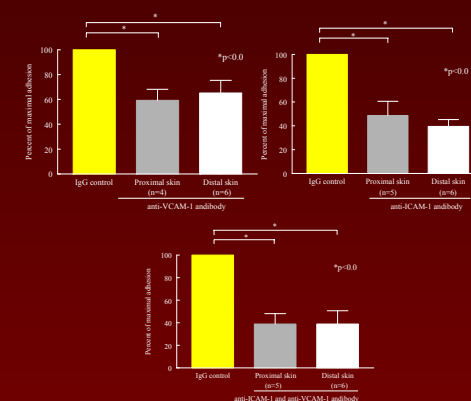


Figure 6. Stampier-Woodruff *in situ* assays were performed using frozen skin sections and fluorescent-labeled U937 cells. The percent of maximal binding was defined as the number of adherent cells on the test sections divided by the number of adherent cells on the control sections. Magnification was 400x. Means are given \pm SEM. n = the number of patients. p<0.05 was considered significant.

CONCLUSIONS

- JAM-B, JAM-C, and CD99 are aberrantly expressed in SSc skin.
- JAM-B, JAM-C, and CD99 do not mediate myeloid cell adhesion to SSc dermal fibroblasts.
- ICAM-1 and VCAM-1 mediate myeloid cell adhesion to SSc skin

We demonstrated that ICAM-1 and VCAM-1 functionally mediate myeloid cell adhesion to SSc skin, and thus potentially contribute to the binding and retention of leukocytes in SSc skin. Targeting ICAM-1 or VCAM-1 may be useful in SSc.

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