Junctional adhesion molecule-A is abnormally expressed in diffuse cutaneous systemic sclerosis skin and mediates myeloid cell adhesion

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ABSTRACT

Objective. While the pathogenesis of systemic sclerosis (SSc) is incompletely understood, vascular changes play a role. Junctional adhesion molecules (JAMs) are novel members of the immunoglobulin superfamily expressed by endothelial and other cells. JAM-A plays a prominent role in promoting angiogenesis. Here we determined the role of JAM-A in the pathogenesis of SSc.

Methods. Biopsies from proximal (less involved) and distal (involved) arm skin and serum were obtained from patients with SSc and normal (NL) volunteers. To determine the expression of JAM-A on SSc dermal fibroblasts and in SSc skin, cell surface ELISAs and immunohistology were performed. An ELISA was designed to determine the amount of soluble JAM-A (sJAM-A) in SSc and NL serum. Myeloid U937 cell-SSc dermal fibroblast or skin adhesion assays were performed.

Results. The stratum granulosum and dermal endothelial cells (ECs) from distal arm SSc skin exhibited significantly decreased expression of JAM-A compared to NL. However, sJAM-A was elevated in the serum of patients with SSc compared to NL. Conversely, JAM-A was increased on the surface of SSc compared to NL dermal fibroblasts. JAM-A accounted for a significant portion of U937 binding to SSc dermal fibroblasts. In addition, JAM-A contributed to U937 adhesion to both distal and proximal SSc skin.

Conclusions. Decreased endothelial JAM-A in SSc skin may reflect decreased angiogenesis with a compensatory increase in sJAM-A in serum. Increased SSc fibroblast JAM-A may serve to recruit and retain myeloid cells, which in turn secrete angiogenic factors. These results may help to understand disordered angiogenesis in SSc.

INTRODUCTION

Systemic sclerosis is a multi-system disorder characterized by Raynaud's phenomenon, proliferative vascular lesions and fibrosis of the skin and various internal organs. The pathogenesis of SSc is complex and remains incompletely understood, however fibroblasts, monocytes, and ECs seem to be key players . These cells facilitate excessive synthesis of extracellular matrix proteins and deposition of increased amounts of collagen, immune activation, and vascular damage, all of which are known to be important in the development of this illness.

Adhesion molecules play multiple roles in angiogenesis. Specific adhesion molecule expression can mediate angiogenesis indirectly by promoting the migration of monocytes. These monocytes are then capable of becoming tissue macrophages and secreting angiogenic factors. Cellular adhesion molecules may have a role in the immunopathogenesis of SSc.

JAMs belong to the immunoglobulin superfamily and to the cortical thymocyte marker for xenopus family of molecules. There are currently 5 known members of the family: JAM-A, JAM-B, JAM-C, JAM4, and JAML. JAM-A has been shown to be expressed on ECs and epithelial cells, as well as leukocytes including neutrophils, monocytes, B- and T-lymphocytes, and platelets. JAM-A has been implicated in a variety of physiologic and pathologic processes involving cellular adhesion, tight junction assembly, and leukocyte transmigration. Recently, JAM-A has been shown to play a role in angiogenesis in that it mediates basic fibroblast growth factor (bFGF) induced angiogenesis through its interaction with integrin αvβ3.

As SSc is characterized by both inflammatory cell infiltration and vasculopathy, we hypothesized that JAM-A may play a role in its pathogenesis. Here we demonstrate aberrant expression of JAM-A in SSc skin and sJAM-A in SSc serum. Moreover, we show a novel role for JAM-A in mediating myeloid cell adhesion to SSc skin.

MATERIALS AND METHODS

- Skin punch biopsies and peripheral blood samples were obtained from subjects with SSc (all with diffuse disease) and control subjects.
- Standard H&E staining and immunoperoxidase staining was used to assess the blood vessels and JAM-A in SSc and normal skin tissues.
- Cell surface ELISA was used to detect JAM-A expression in NL and SSc dermal fibroblast cell lines with/without stimulation by TNF-α, IL-1β or IFN-γ.
- A ELISA method was designed to test serum soluble JAM-A level.
- U937-fibroblast cell adhesion assays and Stamper-Woodruff adhesion assays were used to test JAM-A's role in mediating myeloid cell adhesion.

RESULTS

sJAM-A is elevated in SSc serum

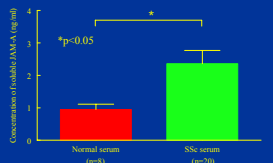

Figure 1. The concentration of sJAM-A in SSc serum (2.36±0.41 ng/ml) was significantly greater compared to NL control serum (0.95±0.16 ng/ml). n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

Distal SSc skin has fewer blood vessels than NL skin

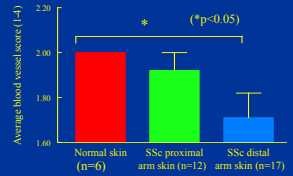

Figure 2. Skin sections from proximal and distal SSc skin and NL skin were hematoxylin and eosin stained, and were graded for the degree of vascularity by a pathologist unaware of the source of the samples. Blood vessels were scored using a scale of: 0=avascular; 1=slight decrease; 2=normal; 3=slight increase; 4=marked increase. n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

JAM-A mediates U937 cell binding to SSc dermal fibroblasts

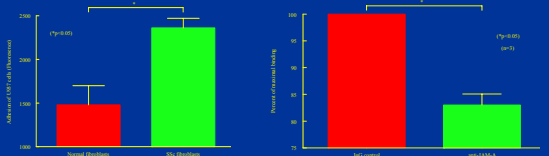

Figure 5. Adhesion assays were performed using PMA-stimulated U937 cells and SSc or NL dermal fibroblasts. n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

JAM-A expression in NL and SSc skin

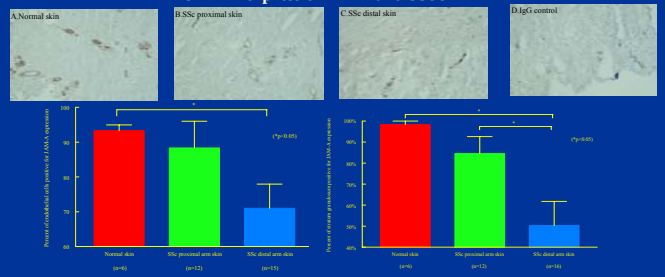

Figure 3. Representative photos of JAM-A endothelial cell staining in NL skin (A), proximal SSc skin (B), distal SSc skin (C), and of the isotype control (D) are shown, all at 200x. The percentage of positive cells was calculated semi-quantitatively as positively stained cells in proportion to all cells of a cell type by a pathologist unaware of the source of samples. (E) Dermal ECs from proximal and distal SSc skin exhibited decreased expression of JAM-A (71% in distal skin and 88% in proximal skin) compared to NL skin (93%). (F) JAM-A was less expressed in the stratum granulosum of SSc skin (50% in distal skin and 85% in proximal skin) compared to NL skin (98%). n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

JAM-A mediates U937 cell binding to SSc skin

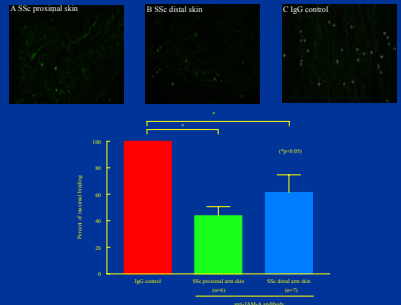

Figure 6. Stamper-Woodruff *in situ* assays were performed using frozen skin sections and fluorescent-labeled U937 cells. The inhibitory effect of the anti-JAM-A antibody treatment was given as the percentage of maximal binding, which was defined as the number of adherent cells in the control antibody treated sections. n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

JAM-A is overexpressed on SSc dermal fibroblasts

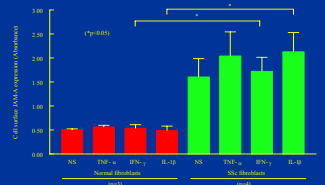

Figure 4. Cell surface ELISAs were performed to determine the expression of JAM-A on SSc and NL dermal fibroblasts. n = the number of patients. Means with SEM are shown. p<0.05 was considered significant.

CONCLUSIONS

- JAM-A expression is decreased on SSc dermal ECs vs. normal ECs.
- JAM-A expression is decreased in the stratum granulosum of SSc skin.
- JAM-A is over-expressed on SSc dermal macrophages and fibroblasts, indicating a possible role for JAM-A in leukocyte retention in SSc skin.
- sJAM-A is elevated in SSc serum.
- JAM-A mediates U937 cell binding to SSc dermal fibroblasts and adhesion to SSc skin.

Taken together, these results may help to understand disordered angiogenesis in SSc.