

Systemic sclerosis sera affect fibrillin-1 deposition and focal adhesion molecule expression by dermal blood microvascular endothelial cells: therapeutic implications of cyclophosphamide

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Systemic sclerosis (SSc, scleroderma) is characterized by fibrosis of the skin and internal organs, autoantibodies and diffuse microangiopathy. In SSc, fibrillin-1 microfibrils are disorganized and unstable. Mutations in *FBN1* gene and anti-fibrillin-1 autoantibodies have also been reported in SSc patients. Fibrillin-1 microfibrils sequester in the extracellular matrix the latent form of the potent pro-fibrotic cytokine transforming growth factor- β (TGF- β). We herein report the effects of SSc sera on the deposition of fibrillin-1 and microfibril-associated glycoprotein-1 (MAGP-1) and the expression of focal adhesion molecules by dermal blood (B-MVECs) and lymphatic (Ly-MVECs) microvascular endothelial cells. Cells were challenged with sera from SSc patients, naïve or under cyclophosphamide (CYC) treatment, and healthy controls. Fibrillin-1/MAGP-1 synthesis and deposition and the expression of $\alpha\beta3$ integrin/phosphorylated-FAK and vinculin/actin were evaluated by immunofluorescence and quantified by morphometric analysis. Fibrillin-1 and MAGP-1 co-localized in all experimental conditions, forming a honeycomb pattern in B-MVECs and a dense mesh of short segments in Ly-MVECs. In B-MVECs, fibrillin-1/MAGP-1 production and $\alpha\beta3$ integrin expression significantly decreased upon challenge with sera from naïve SSc patients compared with healthy controls. Vinculin was overexpressed in the cells exposed to the serum of naïve patients with the diffuse form of SSc. B-MVECs challenged with sera from CYC-treated SSc patients showed fibrillin-1, $\alpha\beta3$ integrin and vinculin levels comparable to those of controls. Ly-MVECs challenged with SSc sera did not differ from controls in the expression of any of the molecules assayed. Due to the critical role of fibrillin-1 in sequestering TGF- β in the extracellular matrix, its decreased deposition by B-MVECs challenged with SSc sera might contribute to dermal fibrosis. CYC treatment might limit fibrosis through the maintenance of physiologic fibrillin-1 synthesis and deposition by B-MVECs. CYC-induced normalization of $\alpha\beta3$ integrin expression may restore effective interaction of endothelial cells with fibrillin. Since vinculin is necessary for angiogenesis, its normalization upon CYC treatment may contribute to restore the impaired angiogenesis found in SSc.

Key words

Systemic sclerosis, endothelial cells, fibrillin-1, focal adhesion molecules, cyclophosphamide.