

Thrombospondin-1 up-regulates expression of cell adhesion molecules and promotes monocyte binding to endothelium

Natalya V. Narizhneva,* Olga V. Razorenova,* Eugene A. Podrez,[†] Juhua Chen,* Unni M. Chandrasekharan,[†] Paul E. DiCorleto,[†] Edward F. Plow,* Eric J. Topol,*[‡] and Tatiana V. Byzova*^{‡,1}

Departments of *Molecular Cardiology, [†]Cell Biology, and [‡]Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, Ohio, USA



To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.04-3310fje>; doi: 10.1096/fj.04-3310fje

SPECIFIC AIMS

Expression of cell adhesion molecules (CAM) by endothelium and subsequent monocyte adhesion and transmigration is an initial step in inflammatory response and atherosclerotic plaque formation. However, the mechanisms of their regulation are not well defined. The aim of this study was to identify a mechanism of regulation of CAM expression by endothelium that links platelet activation and atherogenesis. We considered a role for thrombospondin-1 (TSP-1), a matricellular protein released in abundance from activated platelets, as an inducer of CAM expression and monocyte adhesion to endothelium.

PRINCIPAL FINDINGS

1. TSP-1 induces expression of CAM on various types of endothelium

To analyze the effect of TSP-1 on CAM expression by endothelium, various concentrations of TSP-1 were added to endothelial cells (EC) of different origin, and CAM expression was assessed by FACS and Western blot analyses. Treatment of EC with platelet-derived as well as recombinant TSP-1 resulted in up-regulation of intracellular cell adhesion molecule (ICAM-1) in a concentration-dependent manner. This response was not restricted to certain types of endothelium, since similar results were observed using human aortic endothelial cells (HAEC), human microvascular endothelial cell line (HMVEC) (Fig. 1A), and the well-characterized human umbilical vein endothelial cells (HUVEC). TSP-1 also was able to stimulate expression of vascular cell adhesion molecule-1 (VCAM-1) (Fig. 1B) and E-selectin. For all 3 types of CAM monitored, maximal expression was observed at a concentration of 3 μ g/mL TSP-1. The stimulatory effect of TSP-1 on expression of CAM was lower than or similar to that induced by

TNF- α . In contrast to CAM involved in monocyte/leukocyte adhesion, TSP-1 treatment did not affect the expression of other cellular receptors on endothelium, including CD36 and integrins, demonstrating the specificity of its effect.

2. Stimulating effects of TSP-1 on expression of CAM are mediated by IAP binding peptide

To characterize the structure/functional aspects of TSP-1-stimulated up-regulation of CAM, we tested a series of peptides and fragments derived from TSP-1 known to interact with different TSP-1 receptors. CD36 binding peptide, as well as N-terminal fragment of TSP-1, were ineffective in stimulating ICAM-1 expression on HMVEC (Fig. 1A). In contrast, RFYVVMWK peptide, a binding site for IAP, but not control RFYGGMWK peptide, significantly up-regulated ICAM-1 (Fig. 1A). The IAP binding peptide was also a potent stimulator of VCAM-1 expression on HMVEC (Fig. 1B).

3. TSP-1 up-regulates monocyte adhesion to endothelium

TSP-1 stimulated monocyte adhesion to endothelium in a concentration-dependent manner; a saturation level was achieved at the concentration of 3 μ g/mL TSP-1 (Fig. 1C). Similar to results on CAM expression, IAP-specific peptide RFYVVMWK resulted in a dramatic increase of monocyte adhesion whereas the control RFYGGMWK peptide had no effect (Fig. 1D). Thus, TSP-1 appears to induce monocyte adhesion to EC via its interaction with IAP.

¹ Correspondence: Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Department of Molecular Cardiology, Cardiology, and Taussig Cancer Center, Cleveland Clinic Foundation, NB50, 9500 Euclid Ave., Cleveland, OH 44195, USA. E-mail: byzovata@ccf.org

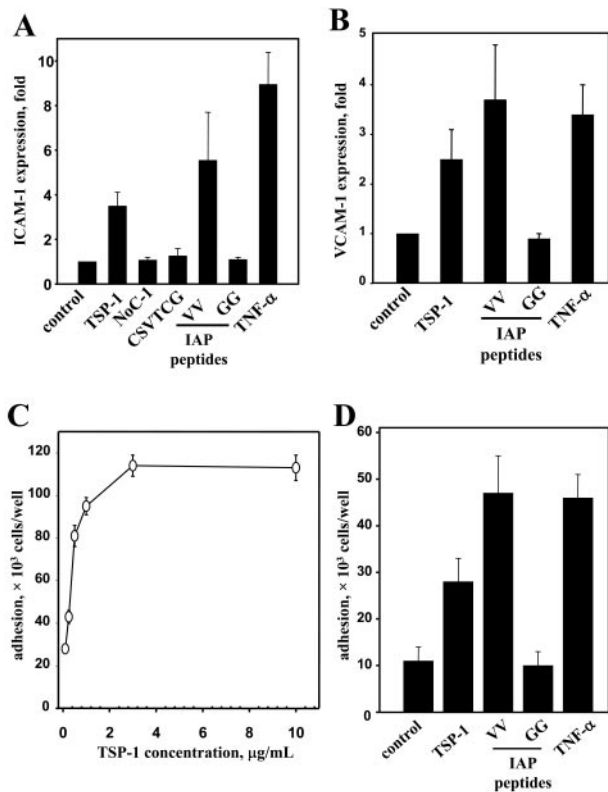


Figure 1. Expression of CAM and monocyte adhesion to HMVEC. *A, B*) Expression of ICAM-1 (*A*) and VCAM-1 (*B*) on HMVEC. Cells were incubated for 17 h in the presence of 10 ng/mL TNF- α , 3 μ g/mL TSP-1, 3 μ g/mL NoC-1 (amino acids 1-356 of TSP-1), or 50 μ M TSP-1-derived peptides: CD-36 binding peptide CSVTCCG, IAP binding peptide “VV” (RFYVVMWK), and control peptide “GG” (RFYGGMWK). CAM expression was analyzed by flow cytometry. The increases in means of fluorescence intensity over control (PBS) are shown ($P \leq 0.02$). Data are means \pm SD of 3–5 separate experiments. *C, D*) Adhesion of monocytes to endothelium. HMVEC grown in 24-well plates were treated with TSP-1 for 17 h or remained untreated. THP-1 cells were added to HMVEC at 3.75×10^5 cells/well. After 45 min incubation at 37°C, unbound monocytes were removed by repeated washing and bound cells were quantified by measuring radioactivity remaining in wells. Data are normalized on background level. *C*) Effect of various concentrations of TSP-1 on monocyte adhesion. Data are means \pm SD of 3 separate experiments. *D*) Effect of TSP-1 and TSP-1-derived peptides on adhesion of THP-1 cells. The concentration of TSP-1 as well as NoC-1 fragment of TSP-1 (amino acids 1-356) was 3 μ g/mL; IAP binding peptide “VV” (RFYVVMWK), control peptide “GG” (RFYGGMWK), and CD-36 binding peptide (CSVTCCG) was 75 μ M, and TNF- α was 10 ng/mL. Data are means \pm SD of 3 separate experiments.

4. TSP-1/IAP interaction is critical for up-regulation of CAM by TSP-1 and TNF- α

Knockdown of IAP using siRNA completely inhibited the stimulating effect of TSP-1 on CAM expression in HMVEC (Fig. 2A) and HUVEC (Fig. 2B), providing further support that IAP is a cellular receptor responsible for TSP-1-induced CAM expression. Blockade of TSP-1 binding to IAP by specific antibody to TSP-1 also

provided > 80% inhibition of TSP-1 induced CAM expression. Taken together, these results demonstrate a key role of IAP as a mediator of TSP-1-induced CAM expression.

HUVEC response to TNF- α was impaired as a result of IAP knockdown (Fig. 2C). Similar results were obtained using HMVEC (Fig. 2D). Likewise, the disruption of TSP-1 interaction with IAP using antibodies Ab-3 significantly diminished CAM expression stimulated by TNF- α (Fig. 2E). At 4 μ g/mL, Ab-3 was able to totally block CAM up-regulation by TNF- α at concentrations of 2 or 5 ng/mL. It appears that the TSP-1–IAP interaction plays an important role in regulation of CAM expression stimulated by TNF- α .

TNF- α increases expression of TSP-1 in a concentration-dependent manner as determined by Western blot and FACS analyses as well as by studies using TSP-1 promoter-luciferase construct. IAP expression was increased by TNF- α by > 2-fold. Thus, it appears that TNF- α up-regulates both IAP and TSP-1 and that these two proteins are involved in the TNF- α stimulated expression of CAM.

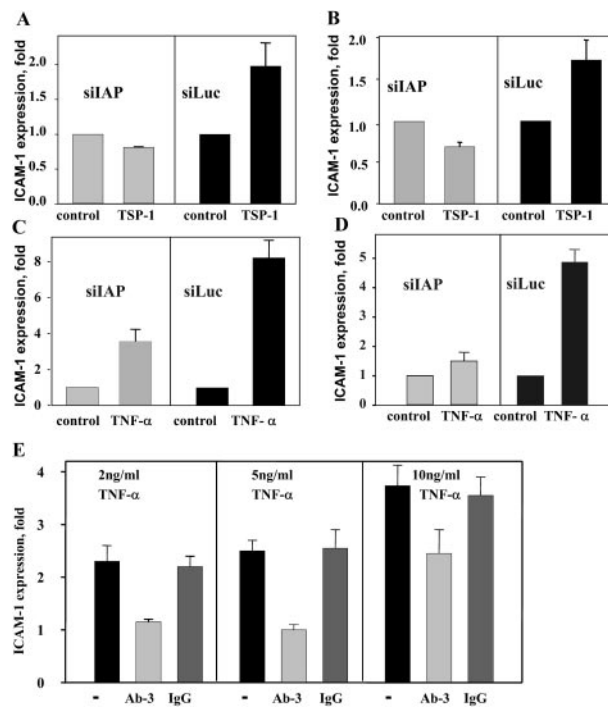


Figure 2. Expression of CAM on EC infected with siAP or siLuc. *A, B*) Expression of ICAM-1 on HMVEC (*A*) or HUVEC (*B*) in the presence of 3 μ g/mL TSP-1 (17 h treatment). *C, D*) Expression of VCAM-1 on HUVEC (*C*) and ICAM-1 on HMVEC (*D*) in the presence of 10 ng/mL TNF- α (17 h treatment). *E*) Effect of Ab-3 TSP-1-blocking antibodies or normal IgG on the expression of ICAM-1 on HUVEC in the presence of 2, 5, and 10 ng/mL of TNF- α . Antibodies concentration was 4 μ g/mL. Data were analyzed by flow cytometry; increases in means of fluorescence intensity over control (PBS) are shown ($P \leq 0.01$). Data are means \pm SD of 5 separate experiments.

CONCLUSIONS AND SIGNIFICANCE

We have shown that TSP-1, via its receptor IAP, targets endothelium and promotes expression of CAM and subsequent attachment of monocytes. This finding assigns a new role to TSP-1 and suggests that this matrix protein, which is abundant at the sites of vascular injury and platelet activation, may trigger the initial stages of inflammation and atherogenesis. The effect of TSP-1 on CAM expression and monocyte adhesion to endothelium provides a mechanism for the stimulatory effect of activated platelets on atherosclerosis development. Vascular expression of TSP-1 is increased as a result of injury simultaneously with IAP, supporting our conclusions and providing *in vivo* evidence for potential communication between IAP and TSP-1.

TNF- α up-regulates both TSP-1 and IAP expression on EC, and inhibition of either component of TSP-1/IAP complex decreases CAM expression stimulated by this cytokine. These data suggest a positive feedback mechanism in which low doses of TNF- α induce interaction between TSP-1 and IAP, which in turn results in

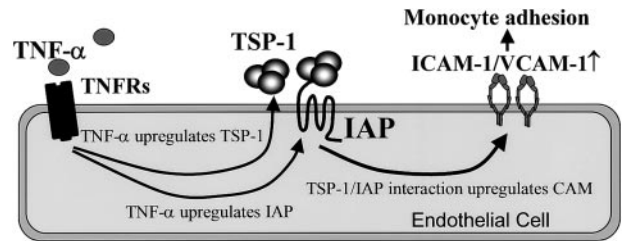


Figure 3. Role of TSP-1-IAP complex in regulation of CAM expression and monocyte adhesion to endothelium. TSP-1 interacts with IAP on EC, leading to up-regulation of CAM (ICAM-1 and VCAM-1). TNF- α simultaneously up-regulates TSP-1 and IAP expression; the TSP-1-IAP complex, in turn, mediates induction of CAM and subsequent monocyte adhesion to EC.

enhanced CAM expression (**Fig. 3**) and possibly increased resistance to infection.

Understanding a new role for TSP-1/IAP in the regulation of endothelial functions related to vascular injury, atherosclerosis, and inflammation may help to develop new therapeutic approaches. **FJ**