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Thromsbospondin-1 binds to the heavy chain of elastase activated coagulation factor V (FV_{aHNE}) and enhances thrombin generation on the surface of a promyelocytic cell line

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Abstract

Introduction: Thrombospondin 1 (TSP1) has the ability to bind to HL-60 cells and to reversibly inhibit human neutrophil elastase (HNE). Human factor V (FV) can be cleaved by HNE thereby providing FV with cofactor activity (FVa_{HNE}). Experiments were performed to evaluate the ability of HNE expressed on the surface of HL-60 cells to generate FVa_{HNE} to support thrombin generation, and to determine the effect of TSP1 on this reaction.
Results: Western blot analysis showed TSP1 forming a complex with FVa_{HNE} within a region corresponding to the heavy chain of FV. Enzymatic reactions were performed to determine the role of TSP1–HNE–FVa_{HNE} on the surface of HL-60 cells, namely the assembly of the prothrombinase complex. Thrombin generation was measured by the chromogenic substrate S2238. Exposure of factor V to HL-60 cells prior to the addition of prothrombin and activated factor X provided FV with cofactor activity. HL-60 cells were found capable of synthesizing factor V with cofactor activity, but HL-60 cells failed to synthesize and/or to provide factor X with enzymatic activity.

Abbreviations: TSP1, thrombospondin-1; HNE, human neutrophil elastase; HBSS, Hanks Balanced Salt Solution; RT-PCR, reverse trasnscriptase polymerase chain reaction; APL, acute promyelocytic leukemia; HBD, heparin binding site; TFPI, tissue factor pathway inhibitor; FXa, activated factor X; FV, factor V; TF, tissue factor.

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The ability of HL-60 cells to synthesize FV and TSP1 was demonstrated. The addition of exogenous TSP1 enhanced both the rate and amount of thrombin generated on the HL-60 cell surface.
Conclusion: Despite the ability of TSP1 to reversibly inhibit HNE in a purified system, TSP1 expression favors the reactions leading to thrombin generation on the HL-60 cell surface. These observations are relevant to clinical conditions where there is a prothrombotic state such as malignant tumors.
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Introduction

It has been appreciated for decades that activation of coagulation is invariably linked to immune-inflammatory responses in vivo and that systemic inflammation is a potent prothrombotic stimulus. Inflammatory mechanisms upregulate procoagulant factors, downregulate fibrinolytic activity as well as the naturally occurring inhibitors of coagulation [1,2]. It is known that deregulation of coagulation contributes to vascular injury and atherosclerosis, however, the molecular basis of the interface between coagulation and inflammation is not completely understood.

Neutrophils and monocytes represent the first line of defense during the inflammatory response and are involved in both the acute and chronic phases of multiple human disease conditions. Human neutrophil elastase (HNE) is an enzyme normally constrained within the primary azurophilic granules of neutrophils [3], and is released into the phagosome and the extracellular milieu upon neutrophil activation. HNE has been implicated in the pathogenesis of inflammatory disorders such as pulmonary emphysema [4], cystic fibrosis [5], glomerulonephritis [6] and rheumatoid arthritis [7]. In blood plasma, its catalytic activity is regulated primarily by α1-protease inhibitor and secondarily by α2-macroglobulin [8]. HNE can be reversibly inhibited by thrombospondin-1 (TSP1) [9,10]. TSP1 is a multifunctional extracellular matrix protein synthesized and secreted by normal and transformed cells in culture including fibroblasts [11], endothelial cells [12], smooth muscle cells [13], and platelets [14]. In preliminary studies we have shown the ability of TSP1 to bind human coagulation FV; other studies have indicated that TSP1 is capable of interacting with other ligands including plasminogen, fibrinogen and fibronectin [15]. TSP1 has the ability to interact with human neutrophils [16] and monocytes [17] promoting neutrophil chemotaxis and monocyte haptotaxis. In addition, TSP1 has the ability to interact with HL-60 cells [18], a promyelocytic cell line. Our laboratory has delineated a region within the TSP1 type-3 repeats that has the ability to interact with human neutrophils [19]. This region is contiguous to a region with the ability to inhibit human neutrophil elastase [10]. It has been shown that HNE cleaves FV to FV_{HNE}, providing FV with cofactor activity (FVa_{HNE}) thus promoting thrombin generation from the prothrombinase complex in the presence of phospholipid vesicles [20]. Prothrombinase is the complex of factors Xa (FX_a), Va, membrane surface and Ca^{++} ions. The functional role of prothromobinase is to catalyze the conversion of prothrombin (FII) to thrombin. The complex that activates FX is FIXa, FVIIIa, membrane surface and Ca^{++} ions (intrinsic pathway), or FVIIa, tissue factor (TF)/membrane surface and Ca^{++} ions (extrinsic pathway) [21]. The prothrombinase complex leads to the generation of activated FX (FXa) and thrombin, potentially leading to cell activation as well as cell proliferation [25]. Thrombin has a variety of activities on cells that result in augmentation of the inflammatory response [22]. The prothrombinase complex has been shown to be assembled on the surface of many cells including platelets and monocytes [23,24]; however its assembly on the surface of HL-60 cells has not yet been investigated. The formation of the prothrombinase complex on the HL-60 cell surface is of relevance in acute promyelocytic leukemia (APL), since it will lead to the generation of activated FX and thrombin, potentially leading to activation of endothelial cells and fibrin formation (procoagulant state). In addition, studies have shown that thrombin, FXa, and the FVIIa—tissue factor complex (FVIIa—TF) have a strong effect on the migration of cultured smooth muscle cells [22,25]. The circulating naturally occurring inhibitor of the FVIIa—TF complex and FXa is tissue factor pathway inhibitor (TFPI). There is evidence that TFPI binds to TSP1 [26], an event that has been associated in vitro with a decreased ability of TFPI to inhibit FXa thus causing impairment in thrombin regulation which results in an excess generation of thrombin [27,28].

The present study was conducted to evaluate the assembly of the prothrombinase complex within the

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