Thrombosis Research (2005) **116**, 409—419

ELSEVIER

THROMBOSIS RESEARCH

intl.elsevierhealth.com/journals/thre

Regular Aarticle

Detached endothelial cells and microparticles as sources of tissue factor activity☆

Rafail I. Kushak[a], Eirini Nestoridi[a,b], Jack Lambert[a], Martin K. Selig[c], Julie R. Ingelfinger[b], Eric F. Grabowski[a,*]

[a]*Pediatric Hematology/Oncology, MassGeneral Hospital for Children, Harvard Medical School, Boston, MA, United States*
[b]*Pediatric Nephrology, MassGeneral Hospital for Children, Harvard Medical School, Boston, MA, United States*
[c]*Department of Pathology Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States*

Received 22 September 2004; received in revised form 5 January 2005; accepted 10 January 2005
Available online 12 April 2005

KEYWORDS
Endothelial cells;
Tissue factor (TF);
Tissue factor pathway inhibitor (TFPI);
Cell detachment;
Microparticles

Abstract

Introduction: Cytokine activation of endothelial cell monolayers is associated with cell detachment, microparticle shedding from plasma membranes, and phosphatidylserine appearance in the plasma membrane outer leaflets. While tissue factor expression on activated endothelial cells and microparticles is well documented, the contribution of detached endothelial cells to tissue factor activity is less clear. We studied tissue factor expression and the role of tissue factor pathway inhibitor on adherent and detached endothelial cells and on microparticles following endothelial cell activation with TNF-α.
Materials and methods: Detached endothelial cells and microparticles were obtained from cultures of human umbilical vein endothelial cells by differential centrifugation of cell culture supernatant. For microparticle capture, an antibody directed against CD146 was used. Functional tissue factor activity was measured by chromogenic assay and tissue factor antigen by ELISA. Endothelial cell and microparticle morphology was examined by light and transmission electron microscopy.

☆ This work was presented in part at the 2004 Pediatric Academic Societies Annual Meeting, San Francisco, CA (Pediatr. Res. 2004;55:287A).

* Corresponding author. MassGeneral Hospital for Children, Cardiovascular Thrombosis Laboratory, 55 Fruit Street, Jackson 1318, Boston, MA 02114, United States. Tel.: +1 617 726 5641; fax: +1 617 724 2297.
E-mail address: GRABOWSKI.ERIC@MGH.HARVARD.EDU (E.F. Grabowski).

0049-3848/$ - see front matter
doi:10.1016/j.thromres.2005.01.013

Results: After cell activation for 22 h, functional tissue factor activity was distributed as follows: 60%, adherent endothelial cells; 35%, detached cells; and 5%, microparticles. Tissue factor protein followed a similar distribution. Cell detachment was 47%. Electron microscopy demonstrated shedding of microparticles with a diameter of 0.1—0.6 μm. Cy3—annexin V revealed increased phosphatidylserine on activated adherent endothelial cells and microparticles. Pre-incubation of adherent and detached endothelial cells and microparticles with anti-tissue factor antibody blocked factor Xa production. Pre-incubation with anti-tissue factor pathway inhibitor antibody increased tissue factor activity of adherent endothelial cells 2.8-fold, detached cells 1.4-fold, and microparticles 45-fold.
Conclusions: Detached endothelial cells as well as microparticles from activated endothelial cell monolayers express tissue factor activity, and this activity on microparticles is markedly inhibited by microparticle-associated tissue factor pathway inhibitor.

Introduction

Endothelial cell (EC) activation by cytokines is associated with membrane blebbing and the shedding of microparticles (MPs) into the extracellular space. MPs, lipoprotein structures about 0.1—1.5 μm in diameter, are rich in outer leaflet phosphatidylserine [1—3]. MPs produced by vesiculation of endothelial and circulating blood elements (platelets, monocytes, erythrocytes) have been extensively studied in vitro in cell culture as well as in vivo in blood samples in a variety of disease states. Significant increases in MP production in comparison to controls have been found in the blood of patients with sickle cell disease [3], endotoxemia [4], HIV [5], type 1 and 2 diabetes [6,7], thrombotic thrombocytopenic purpura (TTP) [8], the lupus anticoagulant [2], myocardial infarction [9], hypertension [10], cardiopulmonary bypass surgery [11], meningococcal sepsis [12], acute coronary syndromes [13], and multiple sclerosis [14]. Because of their high outer leaflet phosphatidylserine content, MPs are prothrombotic and may particularly support the tissue factor (TF) pathway of coagulation [15].

Vascular endothelium is usually thromboresistant but assumes a procoagulant phenotype after exposure to pro-inflammatory cytokines (e.g., TNF-α, IL-1) or components of infectious agents (e.g., LPS) [16,17]. Pro-inflammatory cytokines may also induce apoptosis, a process associated with loss of membrane asymmetry and appearance of inner-leaflet phospholipid phosphatidylserine in the outer leaflet of the cell membrane [15]. The appearance of phosphatidylserine in the outer leaflet promotes coagulation by supporting the activity of surface-active enzyme complexes, such as TF-factor VIIa. The TF-factor VIIa complex rapidly converts zymogen factors IX and X to their active forms, leading to thrombin generation and fibrin formation [18]. TF activity is down-regulated in part by tissue factor pathway inhibitor (TFPI), the chief component of which is located on lipid rafts in a GPI (glycosylphosphatidyl inositol)-anchored form. There is also a minor TFPI component in a loose association with cell membrane glycosaminoglycans [19]. TFPI inhibits the initiation of the coagulation cascade by binding in a quaternary complex with TF, factor Xa, and factor VIIa, and also in a binary complex with factor Xa on the cell surface [20]. Heparin infusion increases TFPI concentration in the circulation by causing the release of TFPI bound to cell surface glycosaminoglycans [21,22].

Pro-inflammatory cytokines also appear to induce EC detachment in cell culture. This process occurs in a dose-dependent manner and increases when cytokines (TNF-α, IL-1) are used in combination with cycloheximide or actinomycin D or in the absence of growth factors [23,24]. Detached ECs may be found not only in cell culture supernatants but also in vivo in the circulation. Increased circulating ECs have been detected in blood samples in many of the same clinical states cited above [25—27]. However, to our knowledge, the procoagulant activity of detached ECs in cell culture has not been studied. The quantitative relationship between procoagulant activity of detached ECs and remaining adherent ECs in an EC monolayer is also not known. The aim of the present study was to quantify the sources of TF in culture medium represented by residual adherent ECs, detached ECs, and MPs in order to define their contribution to the procoagulant state observed with ECs after their activation by TNF-α. A secondary aim was to demonstrate the role of TFPI with regard to each of these sources.