

Thrombus Formation Elevates Tissue Factor (TF) Expression in Atherosclerotic Rabbit Serum

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Abstract: Background: Tissue factor (TF), expressed in endothelial cells and enriched in human atherosclerotic lesions, acts as a critical initiator of blood coagulation in acute coronary syndrome. TF expressed on the surface of vascular wall acts as the major procoagulant for thrombus formation. To test the hypothesis that localized thrombosis is associated with systemic inflammation, we investigated whether atherosclerotic rabbits exhibited significantly high levels of TF at the site of thrombus. **Materials and Methods:** Rabbit atherosclerosis was induced by balloon deendothelialization and feeding a high cholesterol diet (1%) for 6 months, and thrombosis was triggered by Russell viper venom and histamine. Plasminogen activator inhibitor 1 (PAI-1) levels of sections from the non-thrombosis and thrombosis areas of rabbit thoracic aorta were detected by Immunohistochemical staining method. **Results:** Sites of thrombus formation have higher level of TF expression than that of non-thrombus area. There is marked increase in TF staining noted at the site of thrombosis. **Discussion:** Since this particular observation is also supported by increased serum C-reactive protein (CRP) levels, we propose that local event is defined by activation of CD40L connecting to macrophage CD40. This leads to local generation of tissue factor that acts as a nidus for thrombus formation. This might be a potential link between generalized systemic inflammation and localized thrombosis. [Report and Opinion. 2009;1(1):91-94]. (ISSN: 1553-9873).

Keywords: tissue factor; atherosclerosis; thrombosis; rabbit; serum

Abbreviations: CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor 1; TF, tissue factor

1. Introduction

Tissue factor (TF), expressed in endothelial cells and enriched in human atherosclerotic lesions, acts as a critical initiator of blood coagulation in acute coronary syndrome (Ott, 2003). Recent study have shown that activated platelets increase TF in human monocytes (Buffon, 1999). Since TF expression in monocytes and macrophage has been shown to be induced by cross-linking processes, it is plausible that CD40L expressing platelet interaction with CD40 expressing monocytes and macrophages cross link to induce TF production from the monocytes and macrophages. CD40 is a costimulatory protein found on antigen presenting cells. CD40 binds to CD154 (CD40L) on T cells to activate the antigen presenting cell and produce a variety of downstream effects. TF expressed on the surface of vascular wall acts as the major procoagulant for thrombus formation (Golino, 2003). To test the hypothesis that localized thrombosis is associated with systemic inflammation, we investigated whether atherosclerotic rabbits exhibited significantly high levels of TF at the site of thrombus.

All the life cells in the earth have a time to live and a time to die. There are two ways in which cells die: (1) Cells are killed by injury or disease. (2) Cells suicide. Programmed cell death is also called apoptosis, which is cell suicide. Apoptosis is a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage (Ma, Cherng, 2005). Inflammation is one of the common reasons that gives cells problem (disease) even die. Inflammation plays a pivotal role in atherosclerosis. In addition to being a risk marker for cardiovascular disease, much recent data suggest that CRP promotes atherogenesis via effects on monocytes and endothelial cells. The metabolic syndrome is associated with significantly elevated levels of CRP. PAI-1 is also elevated in the metabolic syndrome and in diabetes, and endothelial cells are the major source of PAI-1. According to the report by Devaraj in 2003, CRP induces PAI-1 expression and activity in human aortic endothelial cells and thus has implications for both the metabolic syndrome and atherothrombosis (Devaraj, 2003).

The rise in rabbit serum CRP and PAI-1 as early as 12 to 24 hr after thrombus-triggering may indicate a potential use as immediate to evaluate not only the long-term risk but also a more short-term risk markers of events for thrombosis if PAI-1 levels increase acutely. The time factor in CRP and PAI-1 rise could be helpful in clinical assessment of evolving cardiovascular events.

2. Materials and Methods

Rabbit atherosclerosis was induced by balloon deendothelialization and feeding a high cholesterol diet (1%) for 6 months, and thrombosis was triggered by Russell viper venom and histamine. TF levels of sections from the non-thrombosis and thrombosis areas of rabbit thoracic aorta were detected by immunohistochemical staining method.

For immunohistological analysis, rabbit aortas will be rapidly removed and frozen in liquid nitrogen followed by snap-froze in OCT compound (Tissue-Tek) after the rabbit are killed. Cryostat sections (7 μ m) of the aorta will be prepared, and air-dried 30 min at room temperature prior to washing with 0.1 M PBS followed staining with the respective antibody (Primary antibody: monoclonal antibody against rabbit tissue factor, Product #4510, American Diognostica Inc., Greenwich, CT; Secondary antibody: Rhodamine Red-X-AffiniPure F(ab')₂ Frag Mouse Anti-Rat IgG (H+L), Product #212-296-168, Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA, <http://www.jacksonimmuno.com>): As negative controls, isotype control IgG will be used. After incubation with the appropriate biotinylated, affinity-purified secondary antibodies, the sections will be incubated with alkaline phosphatase-labeled streptavidin solution and visualized using a fast red substrate kit. Quantitative analysis of atherosclerotic lesions was performed by a single observer blinded to the experiment protocol. All images will be captured by microscope equipped with camera and analyzed using Adobe Photoshop 6.0 and National Institute of Health Image 1.62 Software. For all samples, the average value for 5 locations for each animal was used for analysis. Satin dye: SlowFade® Light Antifade Kit, Product #S7461, Molecular Probes, Inc., Eugene, OR.

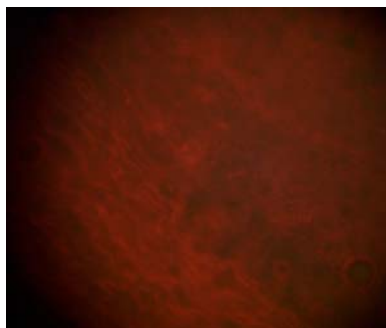
Plasminogen activator inhibitor 1 (PAI-1) levels of sections from the non-thrombosis and thrombosis areas of rabbit thoracic aorta were detected by Immunohistochemical staining method.

3. Results

It has been shown that activated platelets express CD40L and CD40L receptor, namely CD40, that are found on the surface of monocytes and macrophages interact with CD40L forming a CD40L/CD40 complex which then leads to down stream events. To test the hypothesis that localized thrombosis are associated with systemic inflammation, we investigated whether atherosclerotic rabbits which exhibited significantly high levels of serum TF at the site of thrombus. As shown in Figure 1, we have demonstrated that sites of thrombus formation have high level of TF expression. Since this particular observation is also supported by increased serum C-reactive protein (CRP) levels (Ma, 2004), we propose that local event is defined by activation of CD40L connecting to macrophage CD40. This leads to local generation of tissue factor which acts as a nidus for thrombus formation.

Sites of thrombus formation have higher level of TF expression than that of non-thrombus area. There is marked increase in TF staining (arrow) noted at the site of thrombosis (Figure 1).

A. Without Thrombus Formation



B. With Thrombus Formation



Figure 1. Immunohistochemical staining of section from thoracic aorta of an atherosclerotic rabbit demonstrates background staining in an area without thrombus (A) compared to an area of the aorta with thrombus (B). There is marked increase in TF staining (arrow) noted at the site of thrombosis.

PAI-1 result is described in another article.

4. Discussion

Since this particular observation is also supported by increased serum CRP levels, we propose that local event is defined by activation of CD40L connecting to macrophage CD40. This leads to local generation of tissue factor that acts as a nidus for thrombus formation. This might be a potential link between generalized systemic inflammation and localized thrombosis.

For the further study, it is important to demonstrate that systemic inflammation with rise in CRP levels stimulates CD40L activation in platelets, and enhances macrophage production of TF. Consequently, this might be a potential link between generalized systemic inflammation and localized thrombosis. Accordingly, future studies will be needed to investigate CD40L and sCD40L on platelets and TF expression in macrophages at the sites of thrombus formation by co-localization cell type specific markers and TF using immunohistochemical methods, and correlate these data to serum inflammatory markers to show the overall mechanisms underlying CRP mediated systemic inflammation developing into local thrombosis.

The Proposed mechanism of CRP mediated systemic inflammation developing into local thrombosis is shown in Figure 2.

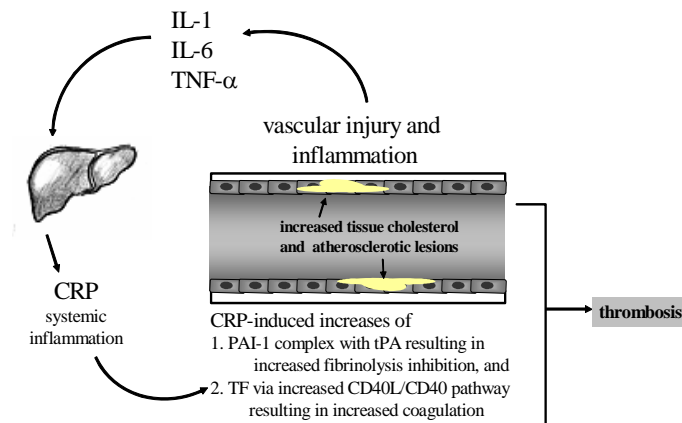


Figure 2. Proposed mechanism of CRP mediated systemic inflammation developing into local thrombosis. Administration of RVV and histamine in an atherosclerotic rabbit results in increased IL-6 (and PAI-1). IL-6 serves as a major stimulator of hepatic CRP secretion. Increased serum CRP stimulates endothelial cells (EC) synthesis and secretion of PAI-1. CRP-induced tissue factor (TF) occurs by increased CD40L-CD40 mediated activation leading to increased coagulation. Localized TF expression by macrophages acts as a nidus for formation of thrombus at a specific site. Together with increased coagulation and fibrinolysis inhibition results in thrombosis.

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