Role of Thromboxane Receptor in C-Reactive Protein–Induced Thrombosis

Etty Grad, Rachel M. Pachino, Garret A. FitzGerald, Haim D. Danenberg

Objective—Thromboxane A₂ and prostacyclin are thromboregulatory prostaglandins. The inflammatory C-reactive protein (CRP) promotes thrombosis after vascular injury, presumably via potentiation of thromboxane activity. Using a genetic approach, we investigated the role of thromboxane receptor (TP) pathway in CRP-induced thrombosis.

Methods and Results—Four genetically engineered mice strains were used: C57BL/6 wild-type, human CRP transgenic (CRPtg), thromboxane receptor–deficient (Tp−/−), and CRPtgTp−/− mice. CRP and TP expression were correlated, and suppression of CRP expression using small interfering RNA/CRP led to reduction in TP expression. Platelet–endothelial adherence was increased in CRPtg and suppressed in CRPtgTP−/− and CRPtg cells that were suppressed with TP small interfering RNA. TP deficiency in both platelets and endothelial cells was synergistic in affecting platelet–endothelial interactions.

Conclusion—TP pathway is of major importance in CRP-induced thrombosis. The expression of TP is increased in CRPtg endothelial cells, and its blockade significantly suppresses the prothrombotic effect of CRP. (Arterioscler Thromb Vasc Biol. 2012;32:2468-2474.)

Key Words: C-reactive protein ■ thromboxane receptor ■ platelets ■ endothelium ■ thrombosis

Elevation of baseline blood C-reactive protein (CRP), an acute-phase reactant, is a strong and independent predictor of cardiovascular disease.¹⁻³ CRP interacts with endothelial cells through a variety of mechanisms, provoking endothelial dysfunction. CRP binding to endothelium impairs endothelium-dependent relaxation and deregulates cell signaling, leading to alteration of prothrombotic/antithrombotic pathways and increased cell permeability.⁴⁻⁵

Increasing evidence suggests that CRP may mediate, and its blood levels may predict, syndromes of vascular occlusion.⁵ Prostaglandins such as thromboxane A₂ and prostacyclin are bioactive lipid mediators that contribute to the complex process of thromboregulation.⁶ Accordingly, thromboxane receptors (TPs) play a pivotal role in cardiovascular diseases; their stimulation elicits not only platelet aggregation and smooth muscle contraction but also expression of adhesion molecules and adhesion and infiltration of monocytes/macrophages.⁷

Recently, we showed that CRP modulates the cyclooxygenase-prostanoid pathway in a manner that disrupts thromboregulation.⁸ CRP suppresses prostacyclin synthase expression and thereby reduces the homeostatic increase in prostacyclin biosynthesis that follows vascular injury, while potentially augmenting thromboxane bioactivity by increasing its receptor expression. The efficacy of low-dose aspirin in cardioprotection can be attributed to suppression of platelet cyclooxygenase-1–derived thromboxane A₂,⁹ and we showed that treatment with aspirin before vascular injury attenuated the prothrombotic phenotype seen in CRP transgenic mice.⁸ The regimen we used in that study suppressed thromboxane A₂ formation dramatically and prostacyclin to a lesser extent, a pattern reminiscent of what is achieved in humans during low-dose aspirin therapy.¹⁰

In this study, a TP knockout (TPKO) mouse was crossed with a human CRP transgenic mouse to elucidate whether TP expression is crucial for CRP prothrombotic activity.

Methods

Mice

Four mouse strains were used: wild-type (WT), human CRP transgenic (CRPtg), TP-deficient mice (Tp−/−), and CRPtgTp−/− mice, all with a C57BL/6 background. All mice used in this study were males aged 10 to 14 weeks. Animal use was approved by the Institutional Animal Care and Use Committee. WT mice were purchased from Harlan Laboratories (Israel). CRPtg were derived from mice originally described by Ciliberto et al.¹¹ Tp−/− were kindly provided by Prof Thomas M. Coffman from the Department of Medicine at the University of North Carolina and were generated as previously described.¹² CRPtg were crossed with Tp−/−, and offspring transgenic for human CRP and carrying the mutant allele for TP (CRPtgTP−/−)
were intercrossed to obtain animals that were homozygous for the targeted TP mutation and the human CRP transgene.

**Genotyping and Phenotyping**

Presence of human CRP and mutant TP gene was determined using polymerase chain reaction of genomic DNA isolated from mouse tails. The primer set for human CRP is as follows: forward 5′-TTT ACA GTG GGT GGG TCT GAA ATA-3′ and reverse 5′-GGG CCA CAG CTG GGG TTT GGT GAA-3′. The primer set for TPKO is as follows: forward 5′-TGG GGG TAG CRA TGG TGT TC-3′, reverse 5′-GGG AGA AGG GCC GTG TGA T-3′, and ApoE NEO 5′-CTT CCT CGT GCT TTA CGG TA 3′.

The expression of TP in WT and TP<sup>−/−</sup> mice was measured by Western blot analysis. Heart tissues were lysed, homogenized under ultrasound, and boiled for 5 minutes. Transferred proteins were blotted overnight for TP (1:500 dilution; Cayman). Proteins from 4 WT and 4 TP<sup>−/−</sup> mice were analyzed.

Serum human CRP levels were measured using ELISA kit according to the manufacturer’s instructions (Bender MedSystems, Vienna, Austria).

**Human CRP and TP Flow Cytometry in Human Umbilical Vein Endothelial Cells After siRNA/CRP Transfection**

CRP and TP expression were measured in human umbilical vein endothelial cells after 24 hours of incubation with negative control or small interfering RNA (siRNA)/CRP. Cells were grown in culture medium without antibiotics (MEM 199; Biological Industries, Israel), supplemented with 20% FCS, 2 mmol/L l-glutamine (purchased from Biological Industries, Israel), and endothelial cell growth supplement (Biomedical Technologies, Stoughton, MA) and heparin) until they reached 60% to 70% confluence. The Stealth siRNA against human CRP and Stealth RNAi-negative control duplexes were designed by Invitrogen (USA). siRNA/CRP sequence 5′-3′ sense: GGU CUA AGG AUA AGG AUA CAG UUU; antisense: AAA CUG UAU CCC UAU AUC CUU AGA CC. The negative control was designed by Invitrogen to be of the same chemical structure as the target RNAi molecules with no homology to any known vertebrate gene. siRNA/CRP or negative was transfected using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer’s instructions.

Cells were detached using nonenzymatic cell dissociation solution (Sigma, USA) and stained for 30 minutes with human fluorescein isothiocyanate-conjugated CRP antibody (BETHYL, USA) and rabbit anti-tissue factor (Cayman, USA) after 30 minutes of staining with goat and donkey anti-rabbit Cy5 (Jackson ImmunoResearch, PA) and other matching controls (for CRP, Goat IgG Control, R&D Systems, USA, and for TP, anti-rabbit Cy5). Positive cells were measured using FACS DIVA flow cytometer (BD).

**Isolation and Culture of Murine Heart Endothelial Cells (MHECs)**

The method for isolation and purification of endothelial cells was modified from a previously published protocol. In brief, sheep anti-rat Dynal beads (Invitrogen, USA) were coated with either rat anti-platelet endothelial cell adhesion molecule-1 or rat anti-intercellular adhesion molecule-2 (Becton Dickinson Labware, Bedford, MA) monoclonal antibody (1.5 µg antibody for 1×10<sup>7</sup> beads) according to the manufacturer’s instructions. Three hearts from 8- to 12-week-old mice were aseptically harvested and placed in 4°C cold digestion medium (DMEM supplemented with 10% FBS, 12.5 µU/ml nystatin, 100 µg/ml streptomycin, 100 U/ml penicillin [all purchased from Biological Industries, Israel]). Hearts were minced finely and digested in 10 mL of type I collagenase (180–200 U/mL in PBS; Worthington) at 37°C for 45 minutes. The digested tissue was mechanically dissociated by triturating with 14G 6″ needle connected to a 10-mL syringe, filtered through a 100-µm disposable cell strainer (Becton Dickinson Labware, Bedford, MA) and centrifuged (1000g, 10 minutes, 4°C). Cell pellets were resuspended in cold 0.1% BSA in PBS and incubated with 50 µL platelet endothelial cell adhesion molecule-1–coated beads (10 minutes, 4°C). Beads were recovered magnetically, cells were plated in fibronectin-coated (Biological industries, Israel) flasks, and suspended in culture medium (DMEM, 20% FBS, 1% l-glutamine, 12.5 µU/mL nystatin, 100 µg/mL streptomycin, 100 µU/mL penicillin [all from Biological Industries, Israel]), 1% amino acids (Sigma), 100 µg/mL heparin (20 U/mL; Sigma), and 100 µg/mL endothelial cells growth supplement (BD). After reaching 70% to 80% confluence (≤5–9 days), cells were detached using warm trypsin, resuspended in 0.1% BSA/PBS, and incubated with intercellular adhesion molecule-2–coated beads (10 minutes, 4°C). Bead-bound cells were washed, plated in culture medium, and further passed at 1:3 ratio. Confluent endothelial monolayers of multiple preparations were used at passages 2 to 3 for this study.

**Expression of TP in CRPtg and WT MHECs**

Expression of TP was measured by Western blot. Total protein was extracted from ~1×10<sup>7</sup> cells using 100 µL of cell lysis solution (Sigma, Israel), supplemented with 1 µL protease inhibitor cocktail (Sigma, Israel). Twenty-microgram extracts were loaded onto 5% to 15% gradient SDS-PAGE. Transferred proteins were blotted overnight, and expression of TP (Cayman, USA) was normalized to actin (1:4000; MP Biomedical).

**In Vivo Effect of TP Deficiency on Thrombogenesis**

To test whether and to what extent TP deficiency affects CRP-induced thrombosis activity, WT, CRPtg, TP<sup>−/−</sup>, and CRP<sup>−/−</sup> mice (10–15 mice/group) underwent arterial injury by photochemical reaction as described previously. The time required to form an occlusive thrombus, defined as absence of blood flow for ≥5 minutes, was recorded. The measurement was stopped at 150 minutes if no occlusive thrombus was recorded.

**Isolation and Labeling of Human Platelets**

This study conformed to the principles of Helsinki declaration. Whole fresh venous blood was collected from healthy, young volunteers (22- to 35-year-old, n=10) into vacutainer tubes with sodium citrate. After resting for 30 minutes, platelet-rich plasma was prepared by centrifugation at 165g for 12 minutes. Platelets were labeled with calcein-AM (30-minute room temperature; Molecular Probes, Eugene, OR) and reconstituted with autologous packed cells immediately before platelet function testing in a cone and platelet analyzer assay. In murine platelet experiments, we used murine blood collected directly from the hearts of WT mice and TP<sup>−/−</sup> mice. The preparation of labeled murine platelets was identical to that of human platelets.

**siRNA/TP Transfection**

Cells were harvested, transferred onto a 4-well plate, and grown in culture medium (described above) without antibiotics until they reached 60% to 70% confluence. The stealth siRNA against TP and stealth RNAi-negative control duplexes were designed by Invitrogen (USA). siRNA-annealed oligonucleotide duplexes for TP sequence 5′-3′ sense: UCG CUC UGC UGU GCG GCC UUG UUC U; antisense: AGA ACA AGG CCG CAC AGC AGA GCC A. In the preliminary experiment, we have shown that the siRNA/TP reduced TP mRNA in CRPtg-derived MHECs by 60%, relative to nontreated cells (control), after 24-hour incubation. The negative control was designed by Invitrogen to the same chemical structure as the target RNAi molecules with no homology to any known vertebrate gene. Negative control RNAi did not change the mRNA expression of any known vertebrate gene. Negative control RNAi did not change the mRNA expression of any known vertebrate gene. Negative control RNAi did not change the mRNA expression of any known vertebrate gene. siRNA or negative was transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions, 24 hours before adhesion assay, Western blot, or real-time polymerase chain reaction experiments.
Platelet Adhesion Assay

Platelet adhesion to MHECs was studied using cone and platelet analyzer assay, as described previously.\(^4\) Forty thousand MHECs at passages 2 to 3 were plated on 4-well plates. Adhesion assays were performed 2 to 3 days later on confluent cells. Twenty-four hours before adhesion assay, medium was replaced with fresh culture medium containing no additives (control), siRNA/TP, or negative siRNA control. Immediately before exposure to shear stress in the cone and platelet analyzer (300 rpm for 2 minutes), the medium was removed and replaced with 180 µL of calcine-labeled platelets containing reconstituted whole blood. After cone and platelet analyzer assay, the platelets were washed 3× with PBS (Biological Industries, Israel). Subsequently, 10 arbitrary chosen high-power fields (x100) were viewed and photographed using fluorescent microscopy. While performing the adhesion assays, 2 pictures were taken from each microscopic field: one of phase contrast for endothelial morphology and the other with fluorescein isothiocyanate labeling for platelets. Using this technique, we explored the phase contrast field for nonendothelial cells. Fields that were contaminated by smooth muscle cells were excluded from fluorescein isothiocyanate labeling analysis for platelet adhesion. Each experiment was repeated up to 8×, and the number of adherent platelets in each field was calculated.

Statistics

Values are means±SD. The Student t test was used to assess differences between 2 groups. For >2 groups, we used the nonparametric Kruskal-Wallis test and the Mann-Whitney nonparametric test for multiple pairwise comparisons with the Bonferroni correction for the significance level. Differences were considered significant at \(P<0.05\).

Mice Characteristics

Mice expressing human CRP were successfully crossbred with TPKO mice. As shown in Figure 1A, we used 4 genetically distinct mice strains: WT mice not containing the human CRP transgene (right) and are TP\(^{-/-}\) (left); CRPtg mice containing human CRP transgene (right) and are TP\(^{+/-}\) (left); TP\(^{-/-}\) not containing the human CRP transgene (right) and not expressing TP (left); and CRPtgTP\(^{-/-}\) containing human CRP transgene (right) and are TP\(^{-/-}\) (left). Western Blot analysis confirmed that heart tissue of TPKO mice does not express TP unlike hearts of WT mice (Figure 1B). The expression of human CRP on the other hand, was similar in CRPtg mice and CRPtgTP\(^{-/-}\) mice. CRP serum levels at baseline in CRPtg were 3.6±3 mg/L, and in CRPtgTP\(^{-/-}\) mice were 2±0.3 mg/L (n=5, no significant difference).

**Figures**

**Figure 1.** Mice characteristics and thromboxane receptor (TP) expression. A, Mice genotype was studied using polymerase chain reaction (PCR) amplification technique with specific primer sets. Representative photographs of genotyping (TP [left] and CRP [right]) of all 4 mice congenes: (1) Wild-type (WT) mice not containing the human CRP transgene and are TP\(^{-/-}\); (2) CRP transgenic (CRPtg) mice containing human CRP transgene and are TP\(^{-/-}\); (3) TP-deficient mice (TP\(^{-/-}\)) not containing the human CRP transgene and not expressing TP; and (4) CRPtgTP\(^{-/-}\) containing human CRP transgene and are TP\(^{-/-}\). B, TP protein expression was completely blocked in TP knockout mice (Western blot analysis).

**Figure 2.** C-reactive protein (CRP) small interfering RNA (siRNA) reduces CRP and thromboxane receptor (TP) expression. A, Western blot showing TP protein expression is augmented in CRP transgenic murine heart endothelial cells. B, Representative flow cytometry analysis of human umbilical vein endothelial cells after 24 hours of incubation with siRNA against human CRP or negative control demonstrated a reduction in cells expressing CRP (B and C, respectively) and cells expressing TP (D and E, respectively). The expression was measured relative to negative control. FITC indicates fluorescein isothiocyanate; FCS, forward scatter; siCRP, siRNA against human CRP.
polymerase chain reaction analysis in MHECs from CRPtg and WT, was not different (data not shown).

To further elucidate CRP–TP interaction, we analyzed with flow cytometry the expression of human CRP and TP in endothelial cells (human umbilical vein endothelial cells) treated with siRNA against human CRP. As shown in Figure 2B–2E, siRNA against human CRP reduced CRP expression relative to negative treatment, with reduction in CRP-expressing cells from 62.72% to 49.93% (Figure 2B and 2C, respectively). Furthermore, this treatment reduced TP-expressing cells from 66.25% to 50.94% (Figure 2D and 2E, respectively). The experiment was repeated 4x with similar results (30.8±12.8% reduction in CRP expression and 43.4±17.6% reduction in TP expression after siRNA against human CRP treatment relative to negative).

**TP Deficiency Prevents Arterial Occlusion After Photochemical Injury**

Thrombotic occlusion of the carotid artery after photochemical injury was shorter in CRPtg mice than in WT mice (35±3.4 versus 67±8.9 minutes, respectively; *P*<0.01, *n*=10–15 per group; Figure 3) as we previously reported. TPKO significantly prolonged the time till thrombus formation in both WT and CRPtg mice; after 150 minutes, in most cases, thrombus was not observed and flow was not hampered (146±3.8 minutes in TP−/− and 136±13.8 minutes in CRPtgTP−/− mice; *P*<0.01 relative to WT and CRPtg mice, *n*=10–15). As a result, time till total occlusion in TPKO and CRPtgTPKO mice was similar, whereas time till total occlusion in TPKO and CRPtgTPKO was statistically different compared with all other tested groups.

**TP Deficiency in CRPtg Mice Reduced Platelet Adhesion to Endothelial Cells**

To further elucidate the mechanisms underlying CRP/TP modulation of thrombosis, we measured the adhesion of platelets to endothelial cells ex vivo. Platelet adhesion under normal arterial shear flow conditions to MHECs derived from all 4 congenic mice was studied using the cone and plate(let) apparatus. The number of adherent human platelets to MHECs derived from human CRPtg was significantly higher relative to WT-derived cells (*P*<0.01; Figure 4A and 4B; 223±63% versus 100±24.6%, respectively). In cells derived from mice lacking the TP (TP−/−), the adhesion of platelets (103±19%) was not different from that recorded in WT cells. Crossbreeding of CRPtg with the TP-deficient mice (CRPtgTP−/−) markedly reduced platelet adherence in comparison with cells obtained from CRPtg cells (147±38%; *P*<0.05 versus CRPtg cells).
siRNA Against TP in CRPtg MHECs Reduced Human CRP Proadhesiveness

To further delineate whether increased TP expression in CRPtg MHECs modulates endothelial–platelet adhesion, its expression was investigated in endothelial cells derived from CRPtg applying siRNA as an alternative mode of TP suppression. Treatments with siRNA/TP decreased platelet adhesion to CRPtg-derived endothelial cells by 60% compared with control negative siRNA (Figure 5).

Platelets Derived From TPKO Mice Are Less Adhesive to MHECs

To settle the differences between the results obtained in vivo in TP−/− mice which demonstrated that the thrombus formation time is prolonged and the in vitro findings of no difference between the number of platelets adhered to TP−/− MHECs and WT MHECs, we conducted another experiment using murine platelets instead of human platelets. As shown in Figure 6, TPKO in platelets and endothelial cells mimics the in vivo situation in TP−/− mice, with marked reduction in platelets adherence to MHECs (from 100±30% in WT-derived platelets and MHECs to 36.4±32% in TP−/− derived platelets and MHECs, P<0.01, n=3). TPKO only in platelets (WT endothelium) or TPKO only in endothelium (WT platelets) leads to a smaller reduction in platelet adhesion to MHEC relative to WT platelets and WT MHECs (70±31% and 67±32%, respectively; P<0.01, n=3).

Discussion

Inflammation is pivotal in vascular injury and repair and thus fundamental in the pathogenesis of cardiovascular disease. The inflammatory marker CRP is a powerful predictor for the occurrence and prognosis of cardiovascular events. Long considered a mere marker, a direct proinflammatory effect of CRP was recorded on cardiovascular cells. Furthermore, in vivo studies demonstrated that CRP is prothrombotic and augments myocardial infarction severity. A possible pathway for CRP prothrombotic activity is the prostanoid pathway. Previous studies have shown that CRP upregulates the expression of TP both in vitro and in vivo. This phenomenon was observed in human umbilical vein endothelial cells after incubation with recombinant CRP and in the arteries and cardiac tissue of human CRPtg mice after vascular injury. The present study aimed at elucidating whether TP is indeed crucial in modulating the prothrombotic effect of CRP.

Using a genetic approach, we confirmed that TP pathway indeed modulates CRP-induced platelet endothelial adhesion and intravascular thrombosis. Abrogation of TP expression by specific siRNA or through its genetic knockout as in the CRPtgTP−/− mice significantly reduced the prothrombotic effect of CRP.

HMG CoA reductase inhibitors (statins) and inhibitors of the renin–angiotensin system, especially angiotensin-converting enzyme inhibitors, appear to convey protection against cardiovascular disease, an effect that exceeds their original cholesterol-lowering effect and antihypertensive effect, respectively. However, because neither of these 2 classes of drugs has major antithrombotic effects, in large numbers of patients at risk...
for cardiovascular disease, aspirin is administered to prevent thrombotic and embolic complications of atherosclerosis. Studies in animal models and cells have shown that the effects of TP antagonists are also, like statins and angiotensin-converting enzyme inhibitors, far behind their original antithrombotic effects. TP antagonists also reduce eicosanoid production that accompanies the widespread vascular and organ inflammation and oxidant stress associated with vascular disease.

CRP increases reactive oxygen species production in vascular smooth muscle cells, activates nicotinamide adenine dinucleotide phosphate-oxidase, and upregulates reactive oxygen species generation through FcγRs in human aortic endothelial cells and in other inflammatory cells that are present in the atherosclerotic plaque area such as macrophages. Reactive oxygen species enhance the stability and increase the density of functional TPs at the cell membrane, which might explain the increased TP expression observed in CRPtg MHECs (Figure 2A) and CRPtg tissues after vascular activation. The activation of TPs in endothelial cells, in turn, inhibits NO production and increases TP expression and stabilization in a positive feedback manner. We have previously shown that NO production in endothelial cells is reduced in CRPtg mice. Thus, increased reactive oxygen species production that is induced by CRP is a putative link among the generation of deleterious eicosanoids, the posttranscriptional stabilization of TPs, and the decreased production of NO that leads to an imbalance in thromboregulation and subsequent thrombosis after vascular injury as observed in CRPtg mice (Figure 7). Taken in conjunction, this experimental evidence indicates that TPs are very likely to play a pivotal role in CRP-induced thrombosis.

Platelets are a major player in the initiation of atherothrombosis. In murine models, platelet–endothelial cell adhesion occurs simultaneously with vascular inflammatory gene expression before leukocyte recruitment and plaque formation. The adhesion of platelets to the vessel wall precedes monocyte infiltration at the site of lesion formation. The results of our studies support the multiactivity of TP. Although in vivo intravascular thrombosis studies (the photochemical injury model; Figure 3) provide us with the combined thrombotic activity, in vitro platelet adhesion studies focus on 1 component of this complex process (Figure 4). In vitro we isolated the effect of TP derived from endothelial cells on endothelium–platelet interaction by using endothelial cells that are TP deficient and normalplatelets derived from human volunteers. Lack of TP on endothelial cells does not affect the platelet–endothelial interactions (platelet adhesion to MHECs derived from TP−/− versus MHECs derived from WT mice; Figure 4). In contrast, the effect of TP−/− in vivo is vaster and involves not only endothelial cells but also platelets and possibly other cells. Replacing human platelets with murine platelets derived from TP−/− or WT mice has shown that the effect of TP on platelet adhesion to endothelial cells is synergistic, causing a larger reduction in platelet adhesion when both the platelets and the MHECs are derived from TP−/− and therefore TP deficient (Figure 6). However, when only platelets or only MHECs are derived from TP−/−, the number of platelets that adhere to endothelial cells is reduced relative to WT platelets and WT MHECs but significantly higher compared with combined TP−/− platelet and TP−/− MHEC interaction. The intravascular thrombosis studies demonstrated the reversal of CRP-induced thrombosis in TP-deficient mice (CRPtg/TP−/−). However, intravascular thrombosis was extremely slow in TP−/− mice as well. These findings further support the multipathobiological activity of TP and its crucial role in vascular thrombosis.

TP is present in atherosclerotic lesions that are prone to plaque rupture and thrombosis and as seen is activated by CRP. TP promotes platelet aggregation and vasoconstriction and stimulates the expression of endothelial adhesion molecules and leukocyte adherence, induces apoptosis, and accelerates progression of atherosclerotic lesions. Viles-Gonzalez et al have shown a regression of already established atherosclerotic lesions after 6 months of treatment with a selective TP inhibitor in a atherosclerotic rabbit model fed with atherogenic diet. Thus, we believe that this important modulator of vascular disease interacts with CRP in facilitating acute thrombotic vascular occlusion.

In conclusion, using crossbreeding techniques and blocking of TP expression, it appears that CRP-induced thrombosis is modulated, at least in part, by an upregulation of TP expression. Suppression of TP pathway inhibits CRP-induced thrombosis and thus may have a therapeutic role in reducing cardiovascular morbidity that is promoted by inflammation and CRP.

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**Disclosures**

None.

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