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ORIGINAL ARTICLE

Human C-reactive protein enhances thrombus formation after neointimal balloon injury in transgenic rabbits

S. MATSUDA,* A. YAMASHITA,* Y. SATO,* S. KITAJIMA,† T. KOIKE,‡ C. SUGITA,* S. MORIGUCHI-GOTO,* K. HATAKEYAMA,* M. TAKAHASHI,* C. KOSHIMOTO,§ Y. MATSUURA,* T. IWAKIRI,* Y. E. CHEN,¶ J. FAN‡[1] and Y. ASADA*[1]

**Department of Pathology, Faculty of Medicine, University of Miyazaki, Miyazaki; †Analytical Research Center for Experimental Sciences, Saga University, Saga; ‡Department of Molecular Pathology, Interdisciplinary Graduate School of Engineering and Medicine, University of Yamanashi, Yamanashi; §Department of Bio-resources, Division of Biotechnology, Frontier Science Research Center, University of Miyazaki, Miyazaki, Japan; and ¶Cardiovascular Center, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*

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Summary. *Background:* High plasma levels of C-reactive protein (CRP) constitute a powerful predictive marker of cardiovascular events. Several lines of evidence suggest that CRP has prothrombogenic effects. However, whether CRP directly participates in the pathogenesis of thrombosis in vivo has not been fully clarified. *Objective:* To test whether human CRP (hCRP) affects arterial thrombus formation after balloon injury of smooth muscle cell (SMC)-rich or macrophage-rich neointima. *Methods:* We compared the susceptibility of transgenic (Tg) rabbits expressing hCRP (46.21 ± 13.85 mg L^{-1}, $n = 22$) and non-Tg rabbits to arterial thrombus formation after balloon injury of SMC-rich or macrophage-rich neointima. *Results:* Thrombus size on SMC-rich or macrophage-rich neointima was significantly increased, and was accompanied by an increase in fibrin content in hCRP-Tg rabbits, as compared with non-Tg rabbits. Thrombus size did not significantly differ between SMC-rich and macrophage-rich neointima in hCRP-Tg rabbits. Tissue factor (TF) mRNA expression and activity in these neointimal lesions were significantly increased in hCRP-Tg rabbits as compared with non-Tg rabbits. The degree of CRP deposition correlated with the elevated TF expression and thrombus size on injured neointima. In addition, hCRP isolated from hCRP-Tg rabbit plasma induced TF mRNA expression and activity in rabbit cultured vascular SMCs. *Conclusions:* These results suggest that elevated plasma hCRP levels promote thrombus formation on injured SMC-rich neointima by enhancing TF expression, but have no additive effects in macrophage-rich neointima.

Keywords: cardiovascular diseases, prognosis, smooth muscle cells, thrombosis, tissue factor.

Correspondence: Yujiro Asada, Department of Pathology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan.
Tel.: +81 985 85 2810; fax: +81 985 85 7614.
E-mail: yasada@fc.miyazaki-u.ac.jp

[1]These authors are joint senior authors.

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Introduction

C-reactive protein (CRP) is an inflammatory acute-phase reactant that has emerged as a powerful predictor of cardiovascular diseases. High levels of plasma CRP are associated with future cardiovascular events in apparently healthy individuals and with a worse prognosis in patients with acute coronary events [1].

On the other hand, CRP has also been implicated in the pathogenesis of cardiovascular diseases such as atherothrombosis. This notion was initially suggested by the demonstration that: (i) CRP is expressed in atherosclerotic lesions [2,3], where its concentration is associated with plaque instability [4]; and (ii) CRP induces proinflammatory changes in cultured vascular cells [5,6]. However, recent studies with transgenic (Tg) rabbits [7] and Tg mice [8–11] from various laboratories have indicated that CRP does not directly participate in the progression of atherosclerosis. These findings are also supported by human genetic studies [12,13], suggesting that CRP triggers cardiovascular events through other mechanisms, such as the promotion of thrombosis. This notion is supported by the following findings. Tg mice expressing CRP have impaired endothelial functions [14], and CRP reduces the expression of tissue-type plasminogen activator (t-PA) [15] and increases that of tissue factor (TF) [16] in vascular cells. In addition, injection of purified CRP activates the blood coagulation system [17]. Danenberg *et al.* [18] demonstrated that human CRP (hCRP)-Tg mice have a higher rate of thrombotic occlusion after arterial injury than non-Tg mice, whereas others have found

that hCRP does not affect the incidence of thrombus in apolipoprotein E knockout mice [19,20]. Regardless of these persistent controversies, mouse models are not apparently appropriate for examining hCRP physiologic functions, because plasma levels of CRP, even in the presence of inflammatory stimuli, are extremely low in mice as compared with humans and rabbits [21].

Recently, we established Tg rabbits expressing hCRP in the liver, and showed that the hCRP-Tg rabbit model might be useful for investigating the relationship between CRP and cardiovascular disease [7]. As in humans, but not in mice, plasma CRP in the rabbit functions as an acute reactant protein during inflammation, and hCRP can activate the rabbit complement system [7]. We previously established a rabbit model of arterial intimal injury and thrombosis [22,23], and examined whether hCRP promotes neointimal proliferation and thrombus formation in this model *in vivo*.

Materials and methods

Transgenic rabbits expressing hCRP under the control of a liver-specific promoter were generated in our laboratory as previously described [7]. Here, we studied male hCRP-Tg rabbits (2.5–3.0 kg) expressing a plasma hCRP concentration of 46.21 ± 13.85 mg L^{-1} (n = 22) and non-Tg littermates as controls (n = 25). All animal research protocols were approved by the Animal Care Committee of Miyazaki University (No. 2006-069-4), and the animals received humane care according to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the US National Institutes of Health.

Femoral artery injury models

Non-Tg rabbits and hCRP-Tg rabbits were fed with a conventional diet (non-Tg, n = 15; hCRP-Tg, n = 12) or a 0.5% cholesterol diet (non-Tg, n = 10; hCRP-Tg, n = 10) for 1 week before and 3 weeks after balloon injury to determine the effect of hCRP on the development of thrombus formation.

Neointimal lesions in the femoral artery were induced by the balloon-injury method, as previously described [22,23]. The rabbits were anesthetized with intravenous pentobarbital (25 mg kg^{-1}), and an angioplasty balloon catheter (diameter, 2.5 mm; length, 9 mm; Quantum, Boston Scientific, Galway, Ireland) was then inserted into the femoral artery under fluoroscopic guidance. The catheter was inflated to 1.5 atm and retracted by 50 mm three times to denude the endothelium. The neointimal lesions were collected for analysis 5 days and 3 weeks later (see below).

To induce thrombus formation on the neointimal surface, balloon injury was induced once again 3 weeks after the first balloon injury. A 2F balloon catheter (Baxter Healthcare, Irvine, CA, USA) was inserted via the anterior tibial artery into the femoral artery, inflated to 1.4 atm, and retracted by 30 mm three times. The rabbits were injected with intravenous heparin (500 U kg^{-1}) 15 min later, and killed with a pentobarbital overdose. Rabbits were also killed 5 days and 3 weeks after the first balloon injury, for assessment of cell proliferation and apoptosis (see Data S1). The rabbits were perfused with 50 mL of 0.01 mol L^{-1} phosphate-buffered saline (PBS) (pH 7.4) and then perfusion-fixed with 4% paraformaldehyde for histologic and immunohistochemical staining.

Blood samples were collected from the medial auditory artery into 3.8% sodium citrate (9 : 1, v/v) for evaluation of blood parameters, platelet aggregation, whole-blood coagulation and platelet adhesion under flow (see Data S1).

Histologic examinations and immunohistochemistry

The femoral arteries were fixed in 4% paraformaldehyde for 24 h at 4 °C, cut into five sections at 4-mm intervals, and embedded in paraffin. Serial sections (3-μm thick) were stained with hematoxylin and eosin (HE). Areas of neointimal lesions and thrombus size were quantified on HE-stained specimens with an image analysis system (Axio Vision 4.0.5; Carl Zeiss, Munchen, Germany) and light microscopy.

Serial sections (3-μm thick) were also immunohistochemically stained with antibodies (Abs) against hCRP, α-smooth muscle actin, rabbit macrophages, TF, rabbit fibrin, glycoprotein IIb–IIIa, and Ki-67 (Table S1). The sections were rinsed with PBS, and incubated with peroxidase-conjugated secondary Abs at room temperature for 60 min; staining was then visualized after the 3,3′-diaminobenzidine tetrahydrochloride reaction followed by counterstaining with Meyer's hematoxylin. The primary Abs were replaced with mouse non-specific IgG or sheep or guinea pig serum in control sections. Immunostained areas in whole cross-sectional areas of neointima and thrombus were quantified with a color imaging morphometry system (WinRoof, Mitani, Fukui, Japan). All quantitative analyses were performed in a blinded manner by two investigators.

Analysis of TF expression and activity

The neointima of the femoral arteries produced 3 weeks after the first balloon injury was carefully separated from the media and adventitia under a stereomicroscope. Total RNA was extracted with Trizol, and TF mRNA and β-actin expression was determined by quantitative real-time RT-PCR with SYBR Premix Ex Taq kits (Takara Bio, Shiga, Japan), according to the manufacturer's instructions. The panel of the primers is shown in Table S2.

Rabbit plasma clotting time initiated by the neointimal homogenate was measured with a coagulation timer (Thrombotrack, AXIS-SHIELD; PoC AS, Oslo, Norway) [23,24] for evaluation of neointimal TF activity. The neointima was homogenized in Tris-buffered saline (pH 7.4) containing 5 mmol L^{-1} $CaCl_2$ and 0.1% Triton X (Nakalai Tesque, Kyoto, Japan), with a Polytron PT3000 (Kinematica, Littau, Switzerland). After centrifugation at 2500 × g for 10 min, the supernatant (vessel sample; 100 μL, containing 100 μg of

protein) was incubated for 1 min with rabbit plasma (100 μL) with or without anti-rabbit TF antibody (10 pg mL^{-1}, 4511; American Diagnostica, Stamford, CT, USA) or recombinant human TF pathway inhibitor (250 ng mL^{-1}; Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan); clotting was then initiated by adding 20 mmol L^{-1} $CaCl_2$ (100 μL). The protein concentrations were determined with the BCA protein assay kit (Pierce, Rockford, IL, USA). A standard curve was obtained from serial dilutions of recombinant TF (American Diagnostica), and TF activity is expressed as arbitrary units.

Expression and activity of TF mRNA in cultured vascular smooth muscle cells (SMCs)

Aortic SMCs isolated from the femoral arteries of non-Tg rabbits by the explant technique were cultured in smooth muscle growth medium (SmGM-2 bullet kit; Lonza, Basel, Switzerland) [24] for five passages. Trypsin-EDTA (Sigma, St Louis, MO, USA) was briefly added to 80–90% confluent SMCs, which were then suspended (1×10^5 cells in 100 μL of serum-free medium) in cuvettes and incubated with hCRP (20, 50 and 100 μg mL^{-1}) that was affinity-purified from hCRP-Tg rabbit plasma [7]. Total RNA was extracted, and the mRNA expression of TF and β-actin was determined as described above. The TF activity of SMCs was assessed from plasma clotting times. Briefly, rabbit plasma (100 μL) was added to viable SMCs (1×10^5 cells in 100 μL of serum-free medium) in cuvettes for analysis with a coagulation timer (AXIS-SHIELD), and clotting assays were performed as described above.

Statistical analysis

All data are presented as means ± standard deviations. Differences for individual groups were tested with Student's *t*-test and ANOVA (GraphPad Prism version 4.03; GraphPad Software, San Diego, CA, USA). Three groups of TF mRNAs and activities were analyzed by one-way ANOVA followed by Bonferroni's multiple-comparison test in the cell culture study. Platelet adhesion was analyzed in the flow chamber system by two-way ANOVA. Microscopic assessments of femoral arteries, TF mRNA and activity of neointima, blood parameters, platelet aggregation and thromboelastogram assay findings between two groups were compared with Student's *t*-test. Relationships between factors were evaluated with linear regression analysis. Statistical significance was established at $P < 0.05$.

Results

Effects of hCRP on the neointimal lesions

We initially investigated the effects of hCRP on the neointimal lesions of the femoral arteries induced by balloon injury. Figure 1 shows that neointimal lesions at 3 weeks after injury contained mainly SMCs with a few macrophages in rabbits fed with a conventional diet, but neointimal size and cellular components (both SMCs and macrophages) did not significantly differ between Tg and non-Tg rabbits. Neither cell proliferation nor apoptosis differed significantly in the neointima of Tg and non-Tg rabbits at 5 days and at 3 weeks after balloon injury (Fig. S1). Immunohistochemical staining frequently revealed hCRP-immunoreactive proteins in the neointimal lesions (but not in the normal intima; data not shown) of hCRP-Tg rabbits (Fig. 2). RT-PCR analysis did not detect any hCRP mRNA transcripts in the neointimal lesions (Fig. S2), suggesting that hCRP-immunoreactive proteins were derived from the circulation.

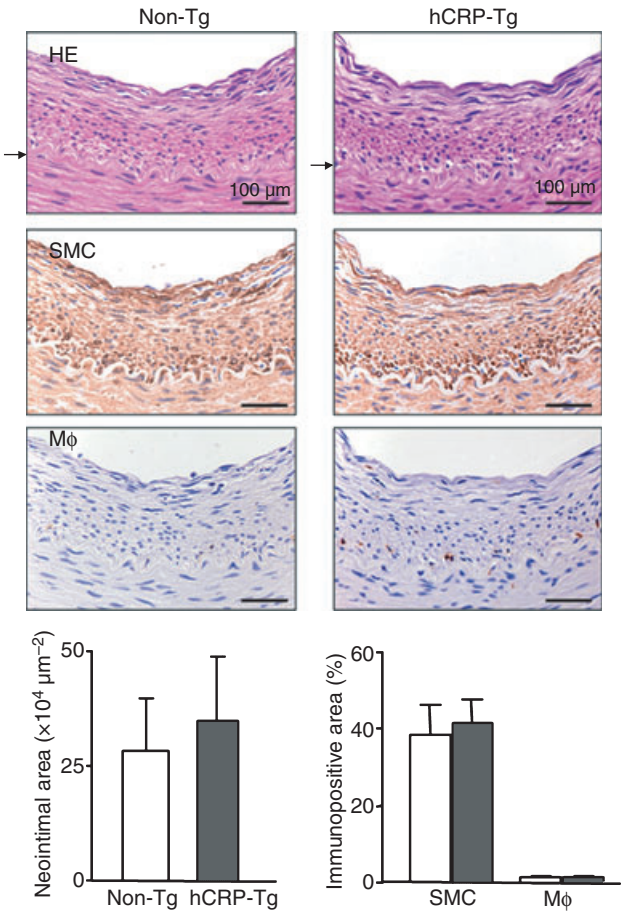

Fig. 1. Neointimal formation 3 weeks after balloon injury. Neointimal lesions mainly contain smooth muscle cells (SMCs) with a few macrophages (Mφ). Neointimal area and cellular components (SMCs and macrophages) do not significantly differ between transgenic (Tg) and non-Tg rabbits ($n = 6$ per group). Arrowheads indicate internal elastic lamina. hCRP, human C-reactive protein; HE, hematoxylin and eosin.

We immunohistochemically stained the neointima to determine whether hCRP affects TF expression in neointimal SMCs. We found that the level of immunoreactive TF proteins was increased three-fold in hCRP-Tg rabbits as compared with non-Tg rabbits (Fig. 2). Furthermore, TF mRNA expression and activities were increased 1.8-fold and 4.5-fold, respectively, in the neointimal lesions of Tg rabbits as compared with non-Tg rabbits (Fig. 3). Taken together, these data indicated that

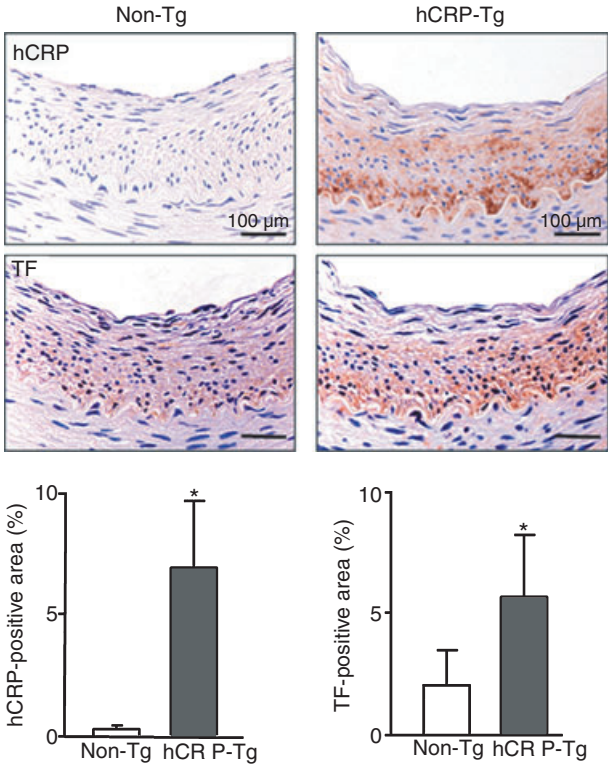

Fig. 2. Immunohistochemical demonstration of C-reactive protein (CRP) and tissue factor (TF) in neointima. Human CRP (hCRP)-immunoreactive protein is frequently present in neointima of hCRP-transgenic (Tg) rabbits, whereas TF-immunoreactive protein is present in that of both Tg and non-Tg rabbits. The positive area was quantified with an image analysis system, as described in Materials and methods ($n = 6$ per group). *$P < 0.01$ vs. non-Tg rabbits.

hCRP in neointimal lesions is associated with, or induces, TF expression but does not affect the SMC proliferation induced by balloon injury in hCRP-Tg rabbits.

Expression/activity of TF is induced by hCRP in cultured vascular SMCs

To determine whether hCRP can stimulate the TF expression by SMCs shown in hCRP-Tg rabbits, we investigated the effects of hCRP isolated from Tg rabbit plasma on the expression of TF in cultured rabbit SMCs. The expression of TF mRNA was significantly increased after incubation with hCRP (50 and 100 mg L^{-1}) for 1 h, but this effect disappeared after 6 h (Fig. 4, left). In contrast, TF protein activity induced by hCRP incubation was significantly increased by 6 h (Fig. 4, right).

Increased platelet–fibrin thrombus formation

We postulated that the pathologic procoagulant state of the neointima of hCRP-Tg rabbits would lead to enhanced thrombogenesis, and therefore compared thrombus formation induced by a second balloon injury on the neointima of the femoral arteries. Thrombi appeared on the injured neointima at 15 min after the injury, and homogeneously covered the damaged neointimal surface (Fig. 5A). Immunohistochemical staining showed that the neointimal thrombi consisted of aggregated platelets and fibrin in both non-Tg and Tg rabbits. Quantitative analysis revealed that thrombus areas and fibrin concentrations were increased 2.5-fold and 1.5-fold, respectively, in Tg rabbits as compared with non-Tg rabbits (Fig. 5C). The areas of CRP immunopositivity significantly correlated with areas of TF positivity and thrombus. The TF-positive area significantly correlated with thrombus and fibrin areas (Fig. 6A–D). To exclude the possibility that hCRP affects platelet aggregation, adhesion and whole-blood hemostatic parameters that could also lead to increased thrombus formation in Tg rabbits, we measured these parameters along with blood counts and prothrombin time (PT)/activated partial thromboplastin time (APTT) in non-Tg and Tg rabbits. However, they did not significantly differ (Fig. S3 and Table S3).

Neointima and thrombus formation in hyperlipidemic rabbits

Neointima and thrombus were induced in hCRP-Tg and non-Tg rabbits fed with a 0.5% cholesterol diet, to determine whether hCRP can stimulate TF expression in macrophage-rich neointima and increase thrombus formation. The macrophage content was significantly increased in the neointima of both types of rabbit fed with a cholesterol diet as compared with a conventional diet. However, neointimal size and cellular components did not significantly differ between non-Tg and hCRP-Tg rabbits (neointimal area, non-Tg 35.9×10^4 μm^2, hCRP-Tg 37.2×10^4 μm^2, $n = 5$ each; SMCs, non-Tg 40.2%, hCRP-Tg 45.8%; macrophages, non-Tg 13.0%, hCRP-Tg 14.4%). Immunohistochemical staining revealed hCRP-immunoreactive proteins in the macrophage-rich neointimal lesions. TF mRNA expression and activities in the neointima and thrombus 15 min after the second neointimal balloon injury were significantly increased in Tg rabbits as compared with non-Tg rabbits (Fig. S4A,Band Fig. 5B). However, thrombus size did not significantly differ between SMC-rich and macrophage-rich neointima in hCRP-Tg rabbits (Fig. 5C). Whole-blood cell counts, platelet function and blood coagulation parameters did not significantly differ between non-Tg and Tg rabbits (Table S4, Fig. S4C–E).

Discussion

We investigated the effect of hCRP on neointimal proliferation and thrombus formation on injured neointima after balloon injury in hCRP-Tg rabbits. The average plasma level of hCRP in Tg rabbits was 46 mg L^{-1}, which was in agreement with a study of interactions between CRP and vascular pathophysiology in humans with high CRP levels. A recent large-scale clinical study showed that the crude relative risk of cardiovascular diseases increases eight-fold among individuals with levels of CRP > 20 mg L^{-1} [25].

In the neointimal lesions of Tg rabbits, hCRP-immunoreactive proteins were frequently detected around SMCs and

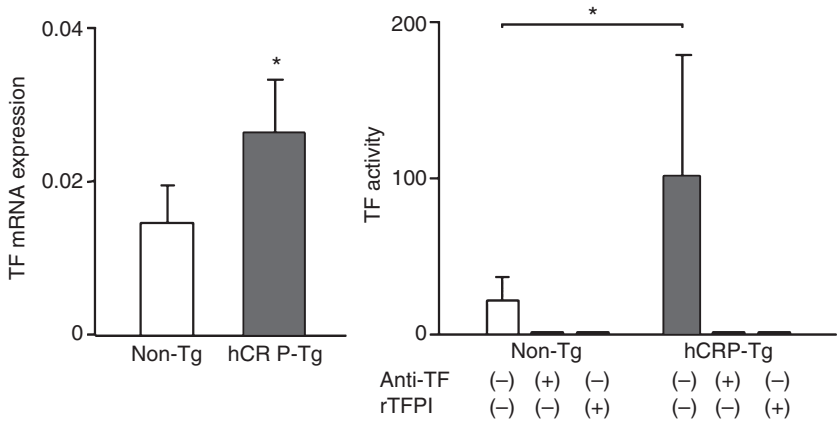

Fig. 3. Tissue factor (TF) mRNA expression and activity in neointima of femoral arteries. TF mRNA expression was analyzed by real-time RT-PCR, and is expressed as TF/β-actin ratios. The TF activity of neointima is 1.8-fold and 4.5-fold higher in terms of mRNA expression and activity, respectively, in transgenic (Tg) rabbits than in non-Tg rabbits. Anti-rabbit TF antibody (10 pg mL^{-1}, < 0.5 arbitrary units) or recombinant TF pathway inhibitor (rTFPI) (250 ng mL^{-1}, < 0.6 arbitrary units) completely blocked increased TF activity in Tg rabbits ($n = 6$ each; $*P < 0.01$ vs. non-Tg rabbits). hCRP, human C-reactive protein.

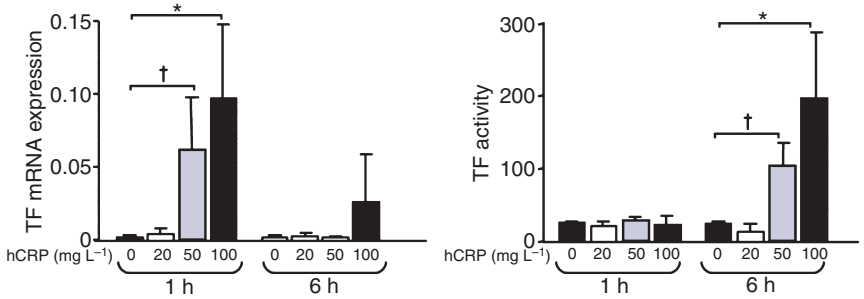

Fig. 4. Tissue factor (TF) mRNA expression and activity in cultured vascular smooth muscle cells (SMCs). TF mRNA expression and activity are expressed as TF/β-actin ratios and arbitrary units, respectively. Vascular SMCs isolated from normal rabbit femoral arteries were incubated with human C-reactive protein (hCRP) (20, 50 and 100 mg L^{-1}) purified from hCRP-transgenic (Tg) rabbit plasma for 1 or 6 h. TF mRNA expression was significantly increased after incubation with 50 and 100 mg L^{-1} hCRP for 1 h, but this effect disappeared after 6 h, whereas TF protein activity was significantly increased at 6 h. Anti-rabbit TF antibody (10 pg mL^{-1}) or recombinant TF pathway inhibitor (250 ng mL^{-1}) (data not shown) ($n = 6$ each) blocked this increase in activity caused by 100 mg L^{-1} hCRP. $*P < 0.01$, $\dagger P < 0.05$ vs. control).

macrophages in the femoral arteries, which is similar to CRP deposition in the atherosclerotic lesions of aortas and coronary arteries in Tg rabbits fed with a cholesterol-rich diet [7]. Despite such obvious CRP deposition in the lesions, we did not find any differences in vascular cell proliferation and apoptosis between the two groups, suggesting that CRP is not involved in the enhanced SMC proliferation and macrophage infiltration in vascular lesions. Although these findings contradict those of previous studies [26,27], they support the notion that CRP in the lesions is not atherogenic [7–11].

Because the primary sources of TF in the non-diseased vascular wall are SMCs, and TF expression was increased remarkably after injury [28,29], we compared TF expression in neointimal lesions of Tg and non-Tg rabbits. We found that both TF-immunoreactive proteins and mRNA expression activity were significantly increased, along with TF clotting, in the neointima of Tg rabbits as compared with non-Tg rabbits, suggesting that hCRP upregulates TF expression in neointimal SMCs. These findings are further supported by the observation that incubation of cultured SMCs with hCRP induces TF expression in vitro, which is consistent with other findings [30]. The induction of TF expression in SMCs by CRP is mediated by FcγRIII (CD16), a cell surface IgG Fc receptor, the p44/42 MAPK pathway, and the generation of reactive oxygen species [26]. It should be noted that TF activities in the uninjured aortas of both hCRP-Tg and non-Tg rabbits did not differ significantly (data not shown), indicating that local CRP deposition in the neointimal lesions is necessary for TF upregulation. This finding contrasts with the study by Wu *et al.* [16], who found that SMCs isolated from the normal aortas of CRP-Tg mice express more TF and TF activities than non-Tg mice. The discrepancy might be attributable to species differences in the biological effects of hCRP on vascular SMCs.

Because TF is a critical factor in the initiation of blood clotting, increased TF expression in the arterial wall might trigger thrombosis after injury such as plaque rupture. Our study showed that the size of thrombi formed on the neointimal surface of hCRP-Tg rabbits was significantly increased as

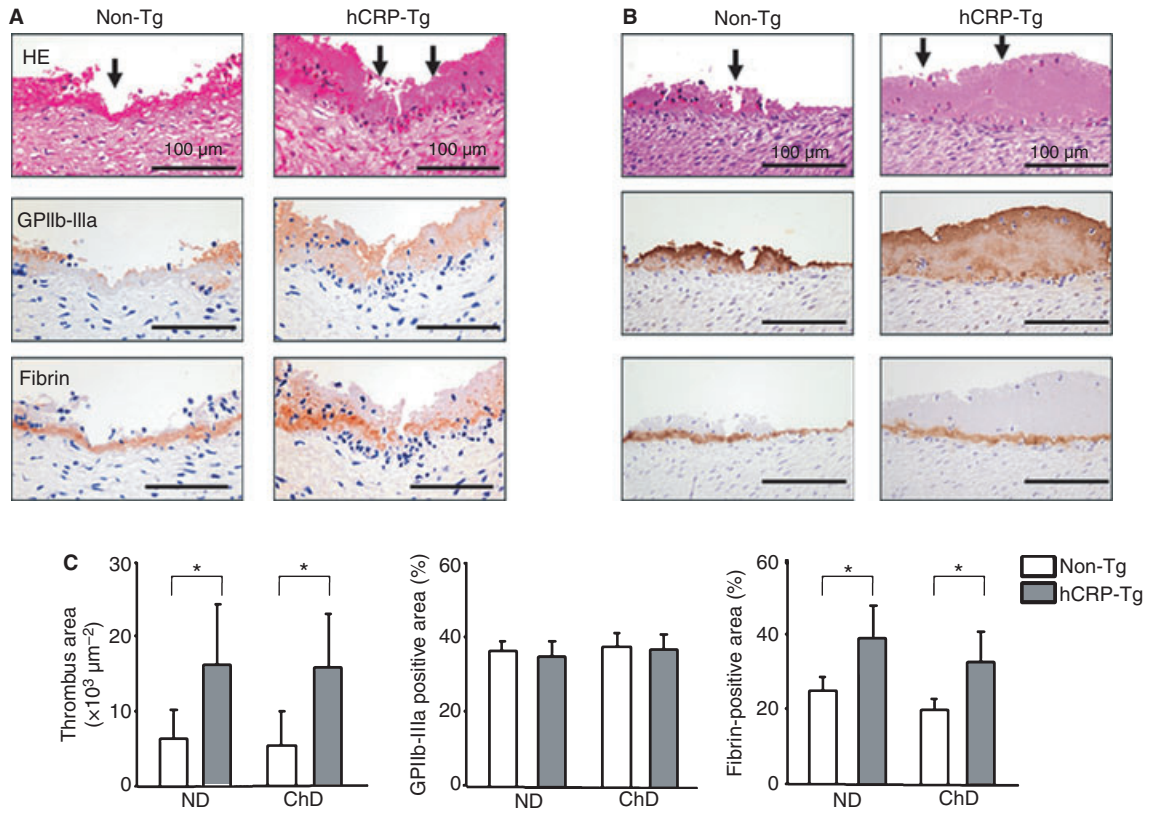

Fig. 5. Thrombus formation on neointima induced by balloon injury in femoral arteries. Thrombus size (arrows) and immunoreactive proteins of glycoprotein (GP)IIb–IIIa and fibrin on neointimas were quantified by image analysis, as described in Materials and methods (A) Thrombus formation on smooth muscle cell-rich neointima, $n = 6$ each. (B) Thrombus formation on macrophage-rich neointima, $n = 5$ each. (C) Thrombus area, GPIIB–IIIa-immunopositive-area and fibrin-immunopositive area. *$P < 0.01$ vs. non-transgenic (Tg) rabbits. ChD, cholesterol diet; hCRP, human C-reactive protein; HE, hematoxylin and eosin; ND, normal diet.

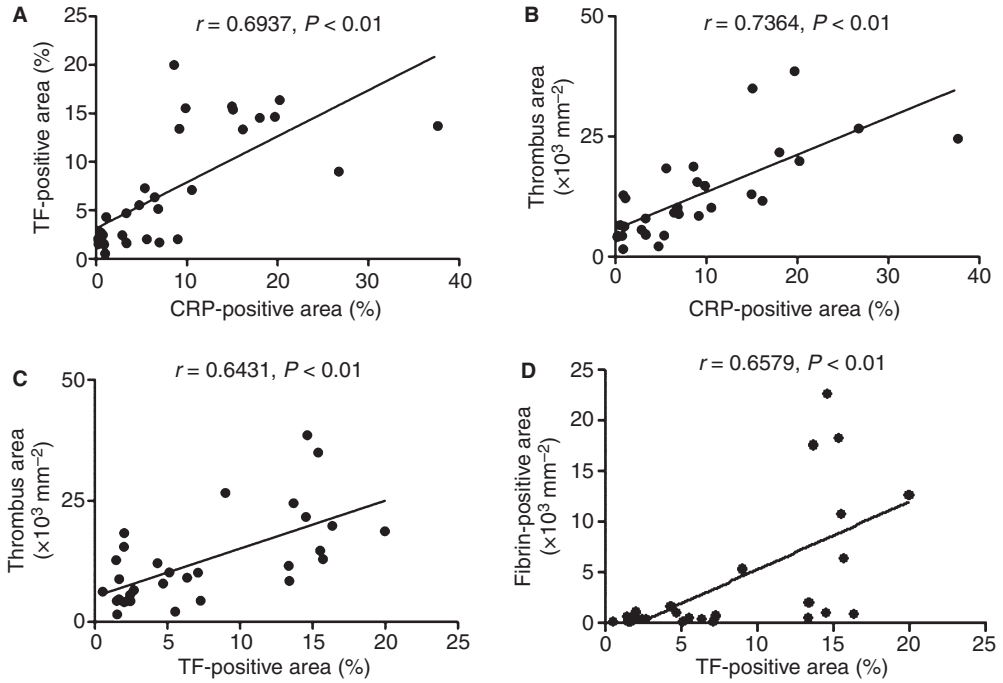

Fig. 6. Linear regression analyses of associations between C-reactive protein (CRP)-positive areas and areas of tissue factor (TF) positivity and thrombus, and between TF-positive areas and areas of fibrin positivity and thrombus ($n = 6$ each).

compared with that of non-Tg rabbits. Several mechanisms might be involved in the increased thrombogenesis in hCRP-Tg rabbits. First, elevated TF expression in neointimal SMCs induced by high levels of CRP in the lesions might directly promote thrombosis. Second, high plasma levels of CRP and lesional CRP might result in a prothrombogenic milieu in the arterial wall through reducing the level of t-PA, upregulating plasminogen activator inhibitor-1, and impairing endothelial functions [5,6]. Whether CRP itself is directly involved in the thrombosis remains unknown. Nevertheless, it is unlikely that CRP affects thrombosis by mediating platelet functions and the blood coagulation system, as we did not find any abnormalities in PT, APTT, or whole-blood hemostatic parameters. Therefore, our data do not support the notion that CRP modulates platelet activation and aggregation [6].

Thrombus size did not significantly differ between SMC-rich and macrophage-rich neointima in hCRP-Tg rabbits. Devaraj *et al.* [31] reported that CRP enhances oxidative stress and TF activity in rat peritoneal macrophages, and implied that CRP in atherosclerotic lesions affects thrombus formation after plaque disruption. Our results did not support this notion, and suggested a difference between vascular and peritoneal macrophages. Although macrophage-rich neointima develops under hyperlipidemic conditions, hCRP deposition did not enhance TF expression and thrombus formation in the lesions as compared with normolipidemic Tg rabbits. The present results could be partly explained by the effect of CRP on complement activation by enzymatically modified LDL (E-LDL). Bhakdi *et al.* [32] reported that CRP deposited in early atherosclerotic lesions is bound to E-LDL, and CRP bound to E-LDL can activate complement but inhibit the complement sequence at the stage of C3b/C5 [33]. E-LDL might reduce the biological effect of CRP in atherosclerotic lesions. The absence of additional enhancement of thrombus formation on macrophage-rich neointima suggests that CRP in macrophage-rich lesions is not thrombogenic.

The present study found higher plasma hCRP levels in hCRP-Tg rabbits than the risk levels generally proposed for humans. We could not assess which levels of CRP affect TF expression in vascular cells and thrombogenesis in vivo, and whether CRP enhances the expression of procoagulant molecules other than TF in hCRP-Tg rabbits. Future studies are required to address these issues, to confirm the roles of CRP in the development of atherothrombosis.

In conclusion, high plasma hCRP levels enhance thrombus formation in SMC-rich neointima via an increase in TF expression, but macrophage infiltration with CRP deposition in the lesions does not have additional effects. This indicates that CRP in macrophage-rich lesions might not be thrombogenic.

Addendum

S. Matsuda, A. Yamashita, S. Kitajima, T. Koike, Y. E. Chen, J. Fan and Y. Asada: contributed to the concept and design; S. Matsuda, A. Yamashita, Y. Sato, C. Sugita, S. Moriguchi-Goto, K. Hatakeyama, M. Takahashi, C. Koshimoto, Y. Matsuura, T. Iwakiri and Y. Asada: contributed to analysis and/or interpretation of data, and critical writing of the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.
Fig. S1. Assessment of cell proliferation and apoptosis.
Fig. S2. Expression of human C-reactive protein (hCRP) and rabbit CRP (rbCRP) mRNA in liver, normal femoral arteries and femoral arteries 3 weeks after balloon injury.
Fig. S3. Platelet functions and whole-blood coagulation in human C-reactive protein (hCRP)-transgenic (Tg) and non-Tg rabbits fed with a conventional diet.
Fig. S4. Localization of C-reactive protein (CRP), tissue factor (TF) expression, thrombus formation and blood parameters in human CRP (hCRP)-transgenic (Tg) and non-Tg rabbits fed with a 0.5% cholesterol diet.
Table S1. Antibodies used for immunohistochemical staining.
Table S2. Primers used for RT-PCR analysis.
Table S3. Blood parameters in human C-reactive protein (hCRP)-transgenic (Tg) and non-Tg rabbits fed with a conventional diet.
Table S4. Blood parameters in human C-reactive protein (hCRP)-transgenic (Tg) and non-Tg rabbits fed with a 0.5% cholesterol diet.

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