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Local Release of C-Reactive Protein From Vulnerable Plaque or Coronary Arterial Wall Injured by Stenting

Teruo Inoue, MD, FACC,* Toru Kato, MD,* Toshihiko Uchida, MD,† Masashi Sakuma, MD,† Atsuko Nakajima, BSc,‡ Mitsuei Shibazaki, BSc,‡ Yoshitaka Imoto, BSc,§ Masahiko Saito, MD,‖ Shigemasa Hashimoto, MD,* Yutaka Hikichi, MD,* Koichi Node, MD*

Saga, Koshigaya, Tokyo, and Ageo, Japan

OBJECTIVES The purpose of this study was to assess local release of C-reactive protein (CRP) from atherosclerotic plaques or the vessel wall injured by stenting.

BACKGROUND Recent research has focused on the local production of CRP, especially in inflammatory atherosclerotic plaques.

METHODS The study consisted of two separate protocols. In protocol 1, we measured serum high-sensitivity-CRP (hs-CRP) levels in coronary arterial blood sampled just distal and proximal to the culprit lesions in 36 patients with stable angina and 13 patients with unstable angina. In protocol 2, we measured serial serum hs-CRP levels and activated Mac-1 on the surface of neutrophils in both coronary sinus and peripheral blood in 20 patients undergoing coronary stenting.

RESULTS In protocol 1, CRP was higher in distal blood than proximal blood in both stable ($p < 0.05$) and unstable angina ($p < 0.01$). The translesional CRP gradient (distal CRP minus proximal CRP, $p < 0.05$) as well as the proximal CRP ($p < 0.05$) and distal CRP ($p < 0.05$) was higher in unstable angina than in stable angina. In protocol 2, the transcardiac CRP gradient (coronary sinus minus peripheral blood) and activated Mac-1 increased gradually after stenting, reaching a maximum at 48 h ($p < 0.001$ vs. baseline for both). There was a positive correlation between the transcardiac CRP gradient and activated Mac-1 at 48 h ($r = 0.45$, $p < 0.01$).

CONCLUSIONS C-reactive protein is an excellent marker for plaque instability or poststent inflammatory status, and its source might be the inflammation site of the plaque or the coronary arterial wall injured by stenting. (J Am Coll Cardiol 2005;46:239–45) © 2005 by the American College of Cardiology Foundation

Human C-reactive protein (CRP) is an acute-phase reactant and has long been considered merely an innocent bystander in the inflammatory process. High-sensitivity CRP (hs-CRP) is now recognized as a powerful predictor of cardiovascular events, including stroke, coronary heart disease, peripheral vascular disease, and sudden cardiac death (1).

Inflammation plays an essential role in the initiation and progression of coronary atherosclerosis, as well as atherosclerotic plaque rupture that culminates in acute coronary syndromes (ACS) (2). Elevated circulating CRP commonly accompanies ACS, reflecting primary inflammation of vulnerable plaques (3). In addition, percutaneous coronary intervention (PCI) produces a significant inflammatory reaction in the injured vessel wall that leads to the development of neointimal thickening and restenosis (4,5). C-reactive protein increases after PCI in a time-dependent manner, with the maximum response at 48 h (6,7), and predicts the occurrence of restenosis (8). In the process of inflammation at the site of the vessel wall injured by PCI, leukocyte integrin Mac-1 (CD11b/CD18) plays a central role. Percutaneous coronary intervention induces activation and up-regulation of Mac-1 on the surface of neutrophils, with the maximum response at 48 h after PCI, and this is also associated with restenosis (9–14).

Not only is CRP a powerful inflammatory marker, but recent evidence suggests that CRP may also directly participate in the inflammatory process of atherogenesis. Although it is thought that CRP is produced mainly in liver, reflecting a systemic inflammatory reaction (15), recent CRP research has focused on its localization in various tissues such as atherosclerotic plaques (16). However, it is not yet known if CRP is produced at the site of the vulnerable plaque or the vessel wall injured by PCI and whether locally released CRP plays any role in Mac-1 activation and restenosis.

In this study, we clinically assessed local CRP production, first at the vulnerable plaque and then at the site of vessel wall injured by PCI. We also assessed the relationship between local CRP production and activation of Mac-1 on the surface of neutrophils leading to restenosis.

METHODS

Study protocol PROTOCOL 1. The subjects included 49 patients with atherosclerotic coronary artery disease who

From the *Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga, Japan; †Department of Cardiology, Koshigaya Hospital, Dokkyo University School of Medicine, Koshigaya, Japan; ‡Department of Laboratory Medicine, Dokkyo University School of Medicine, Koshigaya, Japan; §Yufu Itonaga Co. Ltd., Tokyo, Japan; and ‖Department of Cardiology, Cardiovascular Center, Ageo Central General Hospital, Ageo, Japan. This study was supported in part by a grant from the Vehicle Racing Commemorative Foundation, Tokyo, Japan.

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Abbreviations and Acronyms

ACS = acute coronary symptoms
CRP = C-reactive protein
EDTA = ethylenediaminetetraacetic acid
hs-CRP = high-sensitivity C-reactive protein
MLD = minimal lumen diameter
PCI = percutaneous coronary intervention

underwent PCI for an isolated culprit lesion. Of these 49 patients, there were 36 patients with stable angina and 13 patients with unstable angina (4 patients in class I B, 7 in class II B, and 2 in class III B according to Braunwald's classification) but without angiographically detectable thrombus in the culprit lesion. The PCI procedure was performed using the over-the-wire technique. Before the PCI procedure, a 2-ml blood sample was taken via the balloon catheter when the catheter was advanced to a site just proximal to the target lesion. Next, a blood sample was also taken at a site just distal to the lesion after the catheter crossed through the lesion (Fig. 1). The PCI procedure was completed after collecting the two blood samples. Whole blood was immediately collected into tubes containing ethylene diaminetetraacetate (EDTA).

PROTOCOL 2. The subjects included 20 patients with stable angina and isolated atherosclerotic coronary artery disease of the proximal left anterior descending artery who underwent initial elective coronary stent implantation (April 1999 to March 2001) and follow-up coronary angiography. All patients exhibited clinically stable class I or II effort angina without previous myocardial infarction according to the Canadian Cardiovascular Society. None of these patients were included in protocol 1, but they were included among the subjects in our previous study (14). The target lesions were all type A or type B lesions, as described by the American College of Cardiology/American Heart Association Task Force. Patients were excluded who showed

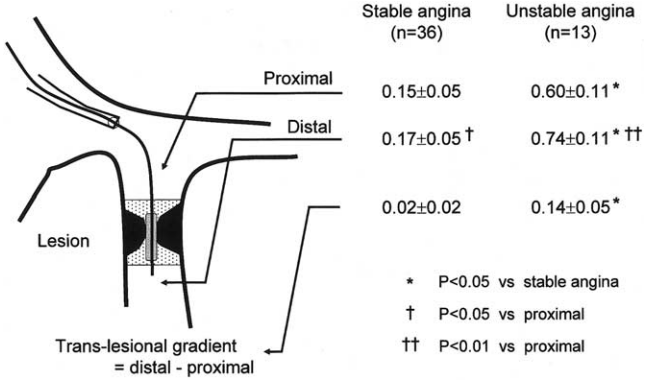

Figure 1. Translesional measurement of C-reactive protein (CRP). The distal CRP was higher than the proximal CRP in the groups with stable and unstable angina. Thus, a translesional CRP gradient was present. Both the proximal and distal CRP levels in unstable angina were higher than in stable angina. The translesional CRP in unstable angina was also greater than in stable angina.

Table 1. Patient Characteristics in Protocol 2

Age (yrs)	46–78 (63 ± 12)
Men/women	16/4
SVD/MVD	11/9
Diabetes	5 (25%)
Hypertension	7 (35%)
Total cholesterol (mg/dl)	158–279 (210 ± 44)
Triglycerides (mg/dl)	76–523 (188 ± 133)
HDL cholesterol (mg/dl)	34–58 (45 ± 11)
LDL cholesterol (mg/dl)	74–172 (128 ± 38)
Statin	4 (20%)
ACEI	4 (20%)
ARB	3 (15%)

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVD = multivessel coronary artery disease; SVD = single-vessel coronary artery disease.

no-reflow or slow-flow phenomena or post-PCI myocardial damage demonstrated by elevation of creatine kinase-MB isozyme level over twice the upper limit of the normal range. The characteristics of the patients are shown in Table 1. Percutaneous coronary intervention was performed using the standard technique with a femoral approach. Coronary lesions were assessed by quantitative coronary angiographic measurements. Reference diameter, lesion length, and minimal lumen diameter (MLD) were measured, and acute gain (MLD after PCI minus MLD before PCI) was determined. Late lumen loss (MLD after PCI minus MLD at 6 months of follow-up) was calculated as an index of neointimal thickening. Before PCI, a catheter was positioned in the coronary sinus and left for 48 h after the procedure for coronary sinus blood sampling. Coronary sinus blood as well as peripheral blood was collected before PCI and at 15 min, 24 h, and 48 h after coronary stenting. Whole blood was immediately collected into tubes containing acid citrate dextrose or EDTA.

In both studies, patients with acute or recent myocardial infarction and patients with a high CRP value (>1.5 mg/dl) were excluded. All of the patients received standard daily oral medications for angina, including 81 mg of aspirin, and none of the medications was discontinued or exchanged during PCI or the follow-up period. Intravenous heparin was administered to maintain an adequate activated clotted time during PCI and for 48 h after the procedure. In protocol 2, patients received 200 mg of oral ticlopidine 2 days before PCI and for one month after PCI (this is the current standard poststent antiplatelet regimen in Japan). Both protocols were approved by the local ethics committee, and written informed consent was obtained from each patient.

Laboratory measurements. The EDTA blood was centrifuged at 1,500 G for 15 min at room temperature for measurement of hs-CRP. The plasma was frozen and stored at −80°C until analysis. The hs-CRP was measured by particle-enhanced technology on the Behring BN II nephelometer (Dade Behring Inc., Newark, Delaware) (17). This assay used monoclonal anti-CRP antibodies and a calibrator that was traceable to World Health Organization reference

material. Using this method, the run-to-run coefficient of variation at hs-CRP concentrations of 0.047, 1.05, and 5.49 mg/dl, was 6.4%, 3.7%, and 2.9%, respectively, and the detection limit was 0.001 mg/dl (18).

The acid citrate dextrose whole blood was used for flow cytometric analysis to measure the expression of the activation-dependent neoepitope of Mac-1 on the surface of neutrophils. We used a purified monoclonal antibody, 8B2 (provided by Dr. Thomas Edgington, Department of Immunology, The Scripps Research Institute, La Jolla, California) with a high sensitivity and specificity for the recognition of the activation-dependent neoepitope of Mac-1 (14,19). Purified mouse immunoglobulin G1 was also used as an isotype-negative control. The fluorescein-conjugated second-step reagents used for indirect immunofluorescence were fluorescein isothiocyanate-conjugated F(ab′)2 fragment of antimouse immunoglobulin G goat immunoglobulins (Dako, Glostrup, Denmark). Indirect immunofluorescence labeling was performed on whole blood incubated with 8B2 (100 μg/ml). The flow cytometric analysis was performed using an EPICS XL flow cytometer (Coultronics, Sunnyvale, California). Cell surface antigen expression was quantified using QIFIKIT (Biocytex, Marseille, France). The antibody binding capacity was measured as an index of the 8B2 binding (i.e., expression of the activation-dependent neoepitope of Mac-1) on the surface of neutrophils (14).

Statistical analysis. Values were expressed as the mean ± SD. In both protocols, intergroup comparisons were performed using Student unpaired *t* test for continuous variables and the chi-square test for categorical variables. Intragroup comparisons were performed using Student paired *t* test. Serial changes in the variables were evaluated by repeated measures analysis of variance for intra- and intergroup comparisons. Correlations between two parameters were assessed using simple linear regression. Multiple regression analysis was performed for predicting late lumen loss in protocol 2. A p value of <0.05 was considered significant.

RESULTS

Protocol 1. There were no significant differences in age, gender, coronary risk factors, or medications that might affect inflammatory status between the group with stable angina and the group with unstable angina (Table 2). The distal CRP was higher than the proximal CRP in both groups of patients. Thus, a translesional CRP gradient (distal CRP minus proximal CRP) was present in stable angina as well as unstable angina. Both the proximal and distal CRP levels in the unstable angina group were higher than in the stable angina group. Translesional CRP in the unstable angina group was also greater than in the stable angina group (Fig. 1). In all patients, proximal CRP correlated with translesional CRP ($r = 0.62$, $p < 0.001$) (Fig. 2).

Table 2. Patient Characteristics in Protocol 1

	Stable Angina (n = 36)	Unstable Angina (n = 13)	p Value
Age (yrs)	69 ± 14	64 ± 11	NS
Men/women	28/8	9/4	NS
Single-vessel CAD/ multivessel CAD	16/20	7/6	NS
Target lesion (LAD/LCX/RCA)	18/6/12	7/2/4	NS
Smoking	28 (78%)	10 (77%)	NS
Diabetes mellitus	13 (36%)	3 (23%)	NS
Hypertension	20 (56%)	6 (46%)	NS
Total cholesterol (mg/dl)	198 ± 42	204 ± 34	NS
Triglycerides (mg/dl)	142 ± 86	146 ± 80	NS
HDL cholesterol (mg/dl)	49 ± 16	47 ± 14	NS
Statins	11 (31%)	4 (31%)	NS
Angiotensin receptor blockers	12 (33%)	3 (23%)	NS

CAD = coronary artery disease; LCX = left circumflex artery; HDL = high-density lipoprotein; LAD = left anterior descending artery; RCA = right coronary artery.

Protocol 2. The plasma CRP level increased after coronary stenting from baseline in a time-dependent manner, reaching a maximum at 48 h in both coronary sinus blood (0.18 ± 0.11 mg/dl to 1.20 ± 0.16 mg/dl, $p < 0.001$) and peripheral blood (0.12 ± 0.01 mg/dl to 0.79 ± 0.15 mg/dl, $p < 0.001$). The changes in CRP levels were more striking in the coronary sinus blood than peripheral blood ($p < 0.01$). The transcardiac gradient of CRP increased after PCI, reaching a maximum at 48 h (0.06 ± 0.18 mg/dl to 0.40 ± 0.20 mg/dl, $p < 0.001$) (Fig. 3). The antibody binding capacity for 8B2 (i.e., expression of Mac-1 activation neoepitope on the surface of neutrophils) began to increase 15 min after coronary stenting and reached a maximum at 48 h in both coronary sinus blood (5,080 ± 80 sites/cell to 7,360 ± 520 sites/cell, $p < 0.001$) and peripheral blood (5,040 ± 90 sites/cell to 6,320 ± 320 sites/cell, $p < 0.01$). The changes were also more striking in coronary sinus blood than peripheral blood ($p < 0.01$). The transcardiac gradient (coronary sinus blood minus peripheral blood) of 8B2 binding also increased after PCI and reached a maximum at 48 h (40 ± 140 sites/cell to 1,280 ± 720

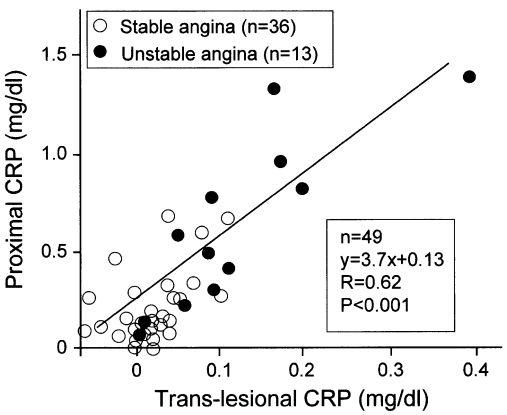

Figure 2. Correlation between proximal C-reactive protein (CRP) and translesional CRP. In all patients, there was a significant correlation between the two measurements.

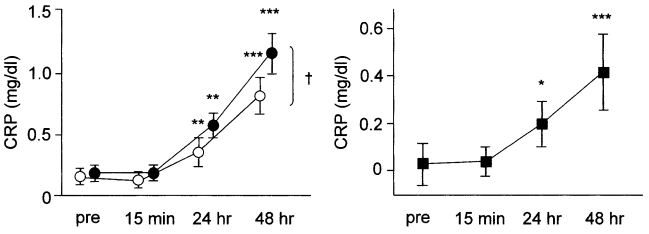

Figure 3. Serial changes in plasma C-reactive protein (CRP) levels after percutaneous coronary intervention (PCI). C-reactive protein increased after coronary stenting in a time-dependent manner, reaching a maximum at 48 h in both coronary sinus and peripheral blood. These changes were also more striking in coronary sinus blood **(left)**. The transcardiac gradient of CRP increased after PCI reaching a maximum at 48 h **(right)**. **Closed circles** = coronary sinus blood (CS); **open circles** = peripheral blood (P); **squares** = transcardiac gradient. *$p < 0.05$ vs. pre; **$p < 0.01$ vs. pre; ***$p < 0.001$ vs. pre; †$p < 0.01$ CS vs. P.

sites/cell, $p < 0.001$) (Fig. 4). The correlation between the transcardiac gradients of CRP and of 8B2 binding at each time point is shown in Figure 5. There was no significant correlation before PCI. However, positive correlations were seen at 15 min ($r = 0.31$, $p = 0.06$) and at 24 h ($r = 0.41$, $p = 0.05$) that reached borderline significance. There was a significant positive correlation at 48 h ($r = 0.45$, $p = 0.03$). Angiographic late lumen loss was predicted by the transcardiac gradient of 8B2 ($r = 0.44$, $p = 0.04$) and by the transcardiac gradient of CRP ($r = 0.48$, $p = 0.03$) at 48 h in a simple linear regression analysis (Fig. 6). A multiple regression analysis that included all of the variables shown in Table 3 demonstrated that both 8B2 ($r = 0.43$, $p = 0.03$) and CRP ($r = 0.44$, $p = 0.02$) were powerful independent predictors of late lumen loss.

DISCUSSION

In the present study, we demonstrated in protocol 1 that CRP was higher in blood sampled just distal to the site of an atherosclerotic coronary artery lesion compared with blood sampled at a site just proximal to the lesion. The presence of this translesional CRP gradient might have been due to CRP produced in the atherosclerotic plaque. Both

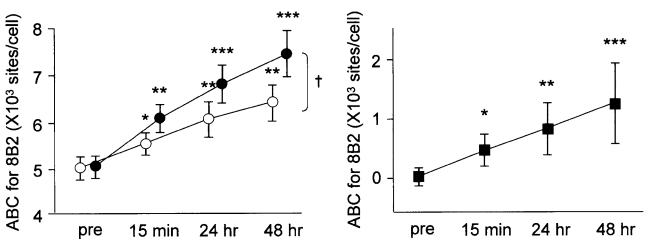

Figure 4. Serial changes in the expression of Mac-1 activation neo-epitope (8B2 binding) on the surface of neutrophils after PCI. Antibody binding capacity (ABC) of 8B2 began to increase immediately after coronary stenting reaching a maximum at 48 h in both coronary sinus and peripheral blood. These changes were more striking in coronary sinus blood **(left)**. The transcardiac gradient (coronary sinus blood minus peripheral blood) of 8B2 binding increased after PCI, reaching a maximum at 48 h **(right)**. **Closed circles** = coronary sinus blood (CS); **open circles** = peripheral blood (P); **squares** = transcardiac gradient. *$p < 0.05$ vs. pre; **$p < 0.01$ vs. pre; ***$p < 0.001$ vs. pre; †$p < 0.01$ CS vs. P. PCI = percutaneous coronary intervention.

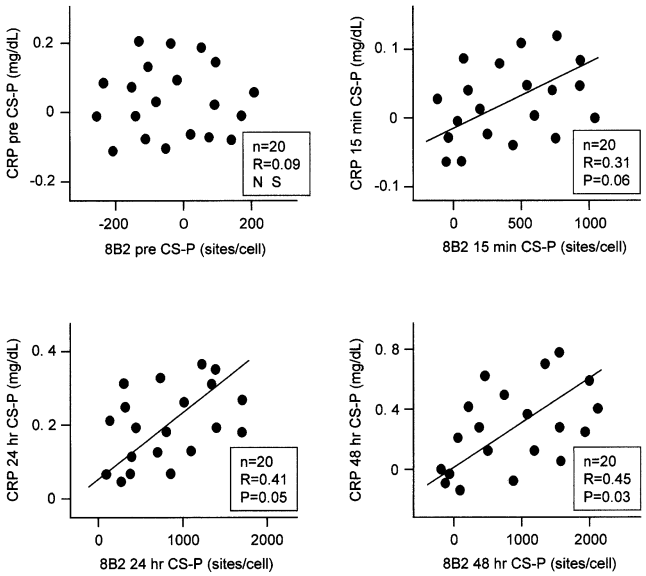

Figure 5. Correlation between the transcardiac gradients (CS-P) of CRP and 8B2 binding at each time point. There was no significant correlation before PCI **(upper left)**. However, positive correlations at 15 min **(upper right)** and at 24 h **(lower left)** reached borderline significance. There was a significant positive correlation at 48 h **(lower right)**. CS = coronary sinus blood; P = peripheral blood; PCI = percutaneous coronary intervention.

the proximal and distal CRP levels were higher and the translesional CRP gradient was greater in unstable angina than in stable angina. These results suggest that CRP production increases in the plaque, reflecting inflammation in the process of plaque instability. We further demonstrated in protocol 2 that PCI produced an inflammatory reaction, as demonstrated by increased CRP and Mac-1 activation on the surface of neutrophils, and that the PCI-induced maximum inflammatory reaction occurred 48 h after PCI. The PCI-induced CRP elevation and Mac-1 activation were greater in coronary sinus blood than in peripheral blood. These transcardiac measurements showed that CRP was produced and Mac-1 was activated across the vascular bed at the PCI site, indicating active local inflammation in the vessel wall injured by PCI.

Local CRP production in the atherosclerotic plaque or vessel wall. Although CRP, like other acute-phase proteins, is synthesized by the liver in response to systemic

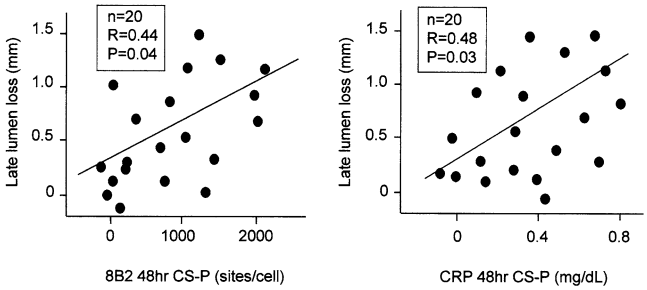

Figure 6. Relationship between angiographic late lumen loss and 8B2 binding or CRP. Late lumen loss correlated with the transcardiac gradient of 8B2 binding at 48 h **(left)** and that of CRP **(right)**. CRP = C-reactive protein.

Table 3. Multiple Regression Analysis for Predicting Angiographic Late Lumen Loss

	Regression Coefficient	p Value
Age (yrs)	0.086	0.522
Men/women	0.135	0.422
Diabetes (on/off)	0.364	0.052
Hypertension (on/off)	0.193	0.108
HDL cholesterol (mg/dl)	−0.274	0.072
LDL cholesterol (mg/dl)	0.225	0.086
Statin (on/off)	−0.264	0.075
ACEI (on/off)	−0.143	0.149
ARB (on/off)	−0.098	0.554
Reference diameter (mm)	0.064	0.587
Lesion length (mm)	0.216	0.097
MLD before PCI (mm)	0.128	0.186
Acute gain (mm)	0.148	0.138
Transcardiac 8B2 (sites/cell)	0.426	0.028
Transcardiac hs-CRP (mg/dl)	0.442	0.024

hs-CRP = high-sensitivity C-reactive protein; MLD = minimal lumen diameter; PCI = percutaneous coronary intervention; other abbreviations as in Table 1.

inflammation (15), a variety of cell types participate in CRP production, and CRP has recently been found in human atherosclerotic plaque (16). Torzewski et al. (20) observed that CRP colocalized with the terminal complement complex in atherosclerotic plaques. However, in that report the authors suggested that CRP was deposited from circulating CRP produced by the liver instead of local synthesis. In contrast, Yasojima et al. (21) suggested that cells in the arterial wall synthesized CRP. Using in-situ hybridization techniques, the authors showed that elongated muscle-like cells inside the atherosclerotic plaque were positive for CRP. In the findings by Ishikawa et al. (16,22), immunoreaction of CRP was localized and CRP messenger ribonucleic acid was expressed in vulnerable plaques, suggesting that CRP was synthesized locally in the plaques. Calabró et al. (23) demonstrated in their in vitro study that human coronary artery smooth muscle cells could produce CRP in response to inflammatory cytokines. We believe that the translesional gradient of CRP in our study provides strong and direct evidence for endogenous CRP production in coronary atherosclerotic plaques. Moreover, the magnitude of the translesional CRP gradient may be the most powerful indicator of a vulnerable plaque. In addition, our results showing an increased transcardiac gradient of CRP after PCI suggest that the vessel wall injured by PCI is partially responsible for CRP production.

Role of Mac-1 for inflammation in PCI-injured vessel wall. Percutaneous coronary intervention produces local mechanical vascular injury that promotes leukocyte recruitment and vascular inflammation (4,5). Accumulating neutrophils and monocytes release a variety of inflammatory mediators, including cytokines, chemokines, growth factors, and reactive oxygen intermediates. These mediators potentiate injury and promote smooth muscle cell proliferation, migration, and extracellular matrix deposition (9,24). Mac-1 orchestrates the recruitment of leukocytes by promoting firm adhesion to, and transmigration across, fibrinogen and platelet ligands, such as GP Ib alpha (25) and possibly intercellular adhesion molecule-2 (26), present at sites of vessel wall injury. Prior experimental and clinical studies have implicated Mac-1 in restenosis. Monoclonal antibody blockade (27) and the absence of Mac-1 (28) reduce neointimal thickening after experimental angioplasty and stenting. We demonstrated that PCI up-regulated Mac-1 expression on the surface of neutrophils that was associated with the subsequent development of restenosis (10–14). Maximum up-regulation of Mac-1 was observed 48 h after PCI in our serial sample analysis up to 144 h after PCI (10). We further demonstrated that PCI promoted an activation-dependent conformational change of existing Mac-1 on the surface of neutrophils before up-regulation of Mac-1 expression. Maximal Mac-1 activation was also observed 48 h after PCI and was similar to maximal Mac-1 up-regulation (14). Although the present study showed the same results as our previous work, an association between Mac-1 activation and CRP production at the site of PCI is new information. Both simple linear regression and multiple regression analysis showed that CRP levels as well as Mac-1 activation predicted angiographic late lumen loss (i.e., neointimal thickening or restenosis).

Measurement of hs-CRP. Laboratory, clinical, and epidemiologic evidence demonstrate a role for inflammation in atherogenesis. Prospective data from a population of apparently healthy men (29) and women (30) indicate that baseline levels of CRP predict the risk of cardiovascular events. The current methods of CRP measurement are capable of measuring CRP with a detection limit of 0.3 to 0.5 mg/dl. Although this detection limit is adequate for the traditional clinical use of CRP in monitoring infection, more sensitive measurement methods are required for assessing and predicting cardiovascular risk in healthy populations. In the present study, we used the BN II nepherometer to perform an ultrasensitive latex-enhanced immunoassay for hs-CRP with a detection limit of 0.001 mg/dl (18). During inflammation in the vulnerable plaques or post-PCI inflammatory reaction, an augmentation of inflammatory activity takes place, and there is an upward shift in the distribution of CRP values. Therefore, a highly sensitive measurement method may not be needed. However, in this study we had to evaluate small differences in CRP values between the proximal and distal sampling sites and between the peripheral and coronary sinus blood in order to establish the respective translesional and transcardiac gradients. Thus, a highly sensitive assay, such as latex nepherometry, was required for this type of analysis.

CRP as a functional protein in the post-PCI inflammatory process. In addition to CRP's serving as a powerful inflammatory marker, recent evidence suggests that it may directly participate in the inflammatory process of atherogenesis. C-reactive protein is thought to inhibit endothelial nitric oxide synthase and NO generation, to activate nuclear factor kappa B, and to up-regulate adhesion molecules such as intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 and angiotensin receptors (31). Thus, CRP

may be an important functional protein in the process of atherogenesis. We believe that our CRP data show not only a local production of CRP, but also suggest that CRP produced locally might act as a functional protein in the inflammatory process, leading to plaque instability or post-PCI restenosis.

Potential limitations. This study has several potential limitations. Because CRP activates the complement system (32,33) and promotes thrombosis in transgenic mice (34), there is a possibility that the translesional gradient of CRP was caused by CRP-enriched thrombus. Although our patients with unstable angina in protocol 1 had no angiographically detectable thrombus, the possible participation of thrombus-derived CRP cannot be excluded. Recent studies suggested that there are different types of vulnerable plaques besides culprit plaques in ACS patients. Ishikawa et al. (22) reported that a transcardiac gradient of CRP was present not only in patients with unstable angina and stable angina but also in control subjects, indicating the possibility of the vulnerable plaques besides the culprit lesion in stable angina patients and even in the control subjects. Although the comparison between translesional CRP and transcardiac CRP could not be performed in protocol 1, the presence of a baseline transcardiac gradient (0.06 ± 0.18 mg/dl) before PCI in protocol 2 raises the possibility of the presence of nonculprit vulnerable plaques. The reports from larger clinical trials showed that lesion length or stent length was a powerful predictor of angiographic late lumen loss. However, multiple regression analysis in protocol 2 showed that lesion length was of only borderline significance in predicting late lumen loss. This may have been due to small sample size or the fact that only type A or B lesions were included. In both protocols, we could not precisely assess whether medical treatments such as statins influenced CRP or Mac-1 because only a small number of patents received such medications. This possibility should be explored in future trials with a larger number of patients.

Conclusions: clinical implications. The presence of translesional or transcardiac gradients of CRP in our two study protocols suggests that at least some amount of the CRP in the serum was produced at the site of the lesion. This locally produced CRP may act as a promoter of the inflammatory process in vulnerable plaques or the vessel wall injured by PCI. Thus, evaluation of the local CRP is very important to recognize plaque instability or the pathophysiology of PCI-induced injury and repair. However, both translesional and transcardiac CRP measurements require a complex technique such as intracoronary or coronary sinus sampling and cannot be performed as routine clinical laboratory tests. On the other hand, our observation of a correlation between translesional CRP and proximal CRP in protocol 1 indicates that even systemic CRP may be a good surrogate of locally produced CRP, although proximal CRP was not always equal to systemic CRP. In addition, the measurement of Mac-1 activation on the surface of neutrophils requires an expensive, complex technique such as flow cytometry, whereas the measurement of systemic CRP is very simple. The correlation between transcardiac gradients of CRP and that of Mac-1 activation at 48 h post-PCI that we observed in protocol 2 indicates that CRP may be an appropriate surrogate for Mac-1 activation at the time of the peak inflammatory response after PCI. The measurement of CRP is very useful to understand the pathophysiology of ACS or the post-PCI inflammatory process. Because CRP is a functional protein that may promote the inflammatory process, CRP might also be a potential therapeutic target for plaque stabilization in ACS or for the prevention of restenosis after PCI.

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Reprint requests and correspondence: Dr. Teruo Inoue, Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, 5-1-1 Nabeshima, Saga 849-8501, Japan. E-mail: inouete@med.saga-u.ac.jp.

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