Increased C-reactive protein expression exacerbates left ventricular dysfunction and remodeling after myocardial infarction

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Submitted 4 January 2010; accepted in final form 15 September 2010

Increased C-reactive protein expression exacerbates left ventricular dysfunction and remodeling after myocardial infarction, Am J Physiol Heart Circ Physiol 299: H1795–H1804, 2010. First published September 17, 2010; doi:10.1152/ajpheart.00001.2010.—We previously reported serum C-reactive protein (CRP) elevation after acute myocardial infarction (MI) to be associated with adverse outcomes including cardiac rupture, left ventricular (LV) remodeling, and cardiac death. Experimental studies have indicated that CRP per se has various biological actions including proinflammatory and proapoptotic effects, suggesting a pathogenic role of CRP in the post-MI remodeling process. We tested the hypothesis that increased CRP expression would exacerbate adverse LV remodeling after MI via deleterious effects of CRP. Transgenic mice with human CRP expression (CRP-Tg) and their transgene-negative littermates (control) underwent left coronary artery ligation. There was no apparent difference in phenotypic features between CRP-Tg and control mice before MI. Although mortality and infarct size were similar in the two groups, CRP-Tg mice showed more LV dilation and worse LV function with more prominent cardiomyocyte hypertrophy and fibrosis in the noninfarcted regions after MI than controls. Histological evaluation conducted 1 wk post-MI revealed a higher rate of apoptosis and more macrophage infiltration in the border zones of infarcted hearts from CRP-Tg mice in relation to increased monocyte chemoattractant protein (MCP)-1 expression and matrix metalloproteinase (MMP)-9 activity. Increased CRP expression exacerbates LV dysfunction and promotes adverse LV remodeling after MI in mice. The deleterious effect of CRP on post-MI LV remodeling may be associated with increased apoptotic rates, macrophage infiltration, MCP-1 expression, and MMP-9 activity in the border zone.

Macrophage; apoptosis

EARLY REVASCULARIZATION AND pharmacological therapy have been used to improve clinical outcomes in patients with acute myocardial infarction (MI). However, in some instances, particularly in those in which myocardial ischemic damage is too severe and extensive to allow salvage, adverse left ventricular (LV) remodeling develops following MI. This deleterious remodeling is characterized by progressive LV dilation and depressed LV function. In the long-term, the development of LV remodeling leads to detrimental outcomes such as congestive heart failure and lethal arrhythmias. Although the precise mechanisms underlying adverse LV remodeling remain to be determined, the inflammatory response to myocardial tissue damage plays a pivotal role in the pathophysiology of the remodeling process (8).

C-reactive protein (CRP) is a major acute-phase inflammatory reactant produced predominantly in the liver. We previously reported that marked serum CRP elevation after acute MI is associated with adverse outcomes such as cardiac rupture, LV remodeling, LV mural thrombosis, and cardiac death (2, 3, 29). In addition, the CRP level in the acute phase of MI is a powerful independent marker of heart failure and long-term mortality (28). Although the CRP level is reportedly a significant risk factor for cardiovascular disease (23, 24), CRP has been regarded as simply a prognostic marker in clinical settings. However, recent experimental studies have indicated that CRP per se has various biological actions, including proinflammatory, thrombogenic, atherogenic, and proapoptotic effects (6, 7, 9, 10, 22, 26, 32, 33), suggesting a pathogenic role of CRP in the inflammatory response and myocardial tissue damage associated with LV remodeling after MI. Therefore, we hypothesized that increased CRP expression would have deleterious effects on adverse LV remodeling after MI.

To examine the direct in vivo involvement of CRP in the adverse remodeling process following MI, we generated transgenic mice with human CRP expression (CRP-Tg). Large anterior wall MIs were then induced in CRP-Tg mice and their transgene-negative littermates. We assessed survival, infarct size, and LV size and function after MI. We then sought to determine the mechanisms by which increased CRP expression might accelerate adverse LV remodeling after MI.

METHODS

Generation and characterization of CRP-Tg mice. The study protocol was approved by the Institutional Animal Care and Use Committee at Keio University School of Medicine (No. 050015), and animal use and care were in accordance with Institutional and National Institutes of Health (NIH) guidelines. To generate transgenic mice with ubiquitous expression of human CRP, human CRP cDNA was inserted into the unique EcoRI site between the CAG (modified chicken β-actin promoter with CMV-IE enhancer) promoter and 3′-flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector 4797 (Fig. 1A). The pronuclei of fertilized eggs from hyperovulated C57BL/6 mice were microinjected with this DNA construct. Founder animals were identified and bred with wild-type mice of the same strain. Gene presence was confirmed using genomic DNA purified from clipped tail tips (Fig. 1B); gene expression in the heart was confirmed by real-time RT-PCR with primers for human CRP (forward: GCTGGTTATTTGTGCTGTCTC; reverse: CAGTTCAAGACATTAGGACTGAA). Protein expression of human CRP in the heart was also confirmed by Western blotting of LV homogenates with antibodies recognizing human CRP (Sigma-Aldrich, St. Louis, MO; Fig. 1C). Human CRP expression in various organ tissues, including heart, lung, and liver, was determined by
immunohistochemistry (Fig. 1D). Blood sampling was taken, and the serum was stored at −80°C when animals were euthanized. Serum CRP levels were measured by latex agglutination immunoassay using the Nanopan CRP kit (Sekisui Medical, Tokyo, Japan). The detection limit of this assay was 0.1 ml/dL. Blood pressures were measured in awake animals by the tail-cuff method using the BP-98A system (Softtron; Tokyo, Japan). Mice were housed with free access to food and water and exposed to 12-h:12-h light-dark cycles.

MI. Male CRP-Tg mice (3 to 4 mo old; n = 59) and their age-matched, male transgene-negative littermates (control; n = 63) were used for the study. MI was induced by permanent ligation of the left coronary artery as described previously (13, 30). Briefly, mice were anesthetized with ketamine and xylazine, intubated, and connected to a rodent ventilator. The chest cavity was opened through the fourth intercostal space to expose the heart. Suture (7-0 silk) was tied around the proximal left coronary artery. Complete occlusion of the vessel was confirmed by the presence of myocardial blanching in the perfusion bed, and subsequently by histological assessment.

Survival study. MIs were induced in the CRP-Tg and control mice in a randomized, blinded fashion. This study was designed to determine the 5-wk survival of mice following MI; therefore, mice that did not survive the surgical procedure were not included in the analysis.

Hemodynamics. LV samples were fixed in Formalin, embedded in paraffin, and cut into 5-μm-thick cross sections. The sections from apex, mid-LV, and base were stained with hematoxylin and eosin and Masson’s trichrome. Infarct size was determined as the mean percent of infarct lengths divided by total LV circumferences in the three sections. Myocyte hypertrophy in the noninfarcted septum was assessed by measuring the mean myocyte cross-sectional area. The percent area of fibrosis within the remote and MI regions of the LV was also computed. In a subset of mice euthanized at 1 and 5 wk after MI, the atria and right ventricle were removed from the heart and the LV was opened by a long-axis incision. The LV was then laid flat on the plate. The endocardial and epicardial surfaces were photographed by a digital camera. Infarct size was measured as the ratio of infarct area to total LV area by planimetry as previously described (4). Infarct size was also assessed in a subgroup of animals euthanized at 24 h after MI to avoid differences in hypertrophy of the noninfarcted wall that might result from differences in human CRP expression. Hearts were excised and immersed in 1% agarose and sectioned perpendicular to the long axis into 1-mm-thick slices and stained with 1.0% 2,3,5-triphenyltetrazolium chloride (TTC) for 10 min at 37°C to delineate the infarct area from the noninfarcted viable area. Each slice was weighed and photographed, and the LV area and the area of infarction for each slice were determined by planimetry as described previously (30).

Echocardiography. Echocardiography was performed with a 12-MHz probe (EnVisor; Philips Medical Systems, Andover, MA) in mice before MI and 1 or 5 wk after MI. Animals were anesthetized with an intraperitoneal injection of tribromoethanol (125 μg/g). A parasternal short-axis view was obtained as a guide for LV M-mode imaging at the papillary muscle level. LV dimensions, including end-diastolic diameter, end-systolic diameter, and wall thickness, were measured using the leading-edge method on three consecutive cardiac cycles. The LV ejection fraction (EF) was calculated using the area-length method as described previously (30).

TUNEL staining. To evaluate the extent of apoptosis in infarcted hearts, TUNEL assays were performed on sectioned LV samples using the CardioTACS In Situ Apoptosis Detection Kit (R&D Systems, Minneapolis, MN) as previously described (30). Images of six to eight contiguous sections across the LV wall were obtained at the midventricle level to measure the number of TUNEL-positive cardiac myocyte nuclei in the noninfarcted remote, peri-infarct border, and infarct zones. Digital images were evaluated using NIH Image to count TUNEL-positive stained nuclei and the total number of nuclei in a nuclease pretreated section from the same region. The area of each section was planimetered to calculate the average density of nuclei (nuclei per squared micrometers), the TUNEL-positive stained nuclei (per squared micrometers), and the rate of TUNEL-positive nuclei (per 105 nuclei).

Quantitative real-time PCR. Total RNA was isolated by acid-phenol extraction in the presence of chaotropic salts (TRIzol; Invitrogen, Carlsbad, CA) and subsequent isopropanol-ethanol precipitation as described previously (18, 27). Real-time RT-PCR of each sample was carried out with a TaqMan RNA PCR kit and ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol. Expression of the housekeeping gene GAPDH was used for normalization. Bcl-2, Bcl-xL, Bax, and Bad assays were purchased as preoptimized kits from Applied Biosystems.

Immunohistochemical studies. Immunohistochemical studies were performed employing immunoperoxidase methods (18, 20). Hearts were fixed in Formalin, embedded in paraffin, and cut into 5-μm-thick cross sections. The sections were stained with antibodies against F4/80 (Novus Biological, Littleton, CO; dilution 1:50) for monocyte-derived macrophages, monocyte chemotactic protein (MCP)-1 (Hy-cult Biotech; Uden, The Netherlands; dilution 1:50), matrix metalloproteinase (MMP)-9 (Abcam, Cambridge, MA; dilution 1:100), and human CRP (Epitomics; Burlingame, CA; dilution 1:100). Because antibodies against neutrophils (Abcam; dilution 1:100) crossreacted with phagocytic macrophages, neutrophils were counted in the sections stained with hematoxylin and eosin by morphological assessment. For quantitative analysis, the number of positive cells was counted per field for five random fields and expressed as the number per squared millimeters.

Western blot analysis. LV samples were homogenized with lysis buffer containing 1% Triton X-100 and protease inhibitors. After centrifugation at 16,000 g for 30 min at 4°C, the supernatants were collected. Western blot analysis was performed as previously described (30). Equal quantities of proteins from LV samples were loaded on gels. Antibodies to MCP-1 (Abcam) and uncleaved and cleaved caspase-3 (Cell Signaling Technology, Danvers, MA) were used. After membranes were probed with primary antibodies, they were stripped of bound immunoglobulins and reprobed with antibodies against neutrophils (Abcam; dilution 1:100) crossreacted with phagocytic macrophages, neutrophils were counted in the sections stained with hematoxylin and eosin by morphological assessment. For quantitative analysis, the number of positive cells was counted per field for five random fields and expressed as the number per squared millimeters.

Serum MCP-1 measurement. Serum MCP-1 levels were measured by quantitative sandwich enzyme immunoassay technique using the Quantikine kit (R&D Systems) according to the manufacturer’s instruction.
**RESULTS**

**Characterization of CRP-Tg.** There were no differences in phenotypic features including general appearance, body weight, and organ weights between control and CRP-Tg mice in the non-MI setting (Table 1). Systolic blood pressures measured by the tail-cuff method were similar in control and

Table 1. *Morphometric data of control and CRP-Tg mice non-MI and post-MI*

<table>
<thead>
<tr>
<th></th>
<th>Non-MI</th>
<th>CRP-Tg</th>
<th>Post-MI</th>
<th>CRP-Tg</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CRP-Tg</td>
<td>Control</td>
<td>CRP-Tg</td>
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</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>14</td>
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</tr>
<tr>
<td>BW, g</td>
<td>28.8 ± 2.3</td>
<td>28.0 ± 2.1</td>
<td>30.2 ± 3.1</td>
<td>29.4 ± 3.8</td>
<td>ns</td>
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<td>LV + RV, mg</td>
<td>118 ± 13</td>
<td>112 ± 8</td>
<td>158 ± 15*</td>
<td>182 ± 14**</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>(LV + RV)/BW, mg/g</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>5.3 ± 0.7*</td>
<td>6.3 ± 0.9**</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Lung, mg</td>
<td>160 ± 21</td>
<td>161 ± 12</td>
<td>174 ± 16</td>
<td>203 ± 31†</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>Lung/BW, mg/g</td>
<td>5.6 ± 0.7</td>
<td>5.8 ± 0.5</td>
<td>5.8 ± 0.8</td>
<td>7.0 ± 1.4**</td>
<td>P = 0.003</td>
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<td>Liver, mg</td>
<td>1,285 ± 171</td>
<td>1,244 ± 153</td>
<td>1,199 ± 91</td>
<td>1,176 ± 153</td>
<td>ns</td>
</tr>
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<td>Liver/BW, mg/g</td>
<td>44.8 ± 5.6</td>
<td>44.5 ± 4.3</td>
<td>40.0 ± 3.8</td>
<td>40.2 ± 4.6</td>
<td>P = 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of animals studied. CRP-Tg, transgenic mice with human C-reactive protein expression; MI, myocardial infarction; BW, body weight; LV, left ventricle; RV, right ventricle; ns, not significant. *P < 0.05, non-MI vs. post-MI; †P < 0.05, post-MI CRP-Tg vs. post-MI control (Bonferroni’s test).

Statistical analysis. Results are presented as counts or means ± SD. Group comparisons were made using the Student’s t-test (two tailed), the Mann-Whitney U test or the one-way ANOVA with Bonferroni’s post hoc test for continuous data. Survival curves were computed using the Kaplan-Meier method and compared using the log rank test. The null hypothesis was rejected if P < 0.05. Analyses were performed using the GraphPad Prism (GraphPad software; San Diego, CA).

![Fig. 1. Generation and characterization of transgenic mice with human C-reactive protein (CRP) expression (CRP-Tg) mice. A: human CRP cDNA was inserted into the unique EcoRI site between the CAG (modified chicken β-actin promoter with CMV-IE enhancer) promoter and 3' flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector. B: gene expression was confirmed by RT-PCR using genomic DNA extracted from clipped tails. Lane 1 is the positive control. Lanes 2-5 are samples negative for the CRP transgene, and lanes 6-9 from positive samples. C: Western blotting of left ventricular (LV) homogenates showed marked protein expression of human CRP in LV samples from CRP-Tg mice but not in those from control mice. D–F: immunohistochemical studies confirmed local expression of human CRP (stained in brown) in sections from heart (D, 200×), liver (E, 400×), and lung (F, 400×).](http://ajpheart.physiology.org/)

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CRP-Tg mice before MI (control, 106 ± 6 mmHg; CRP-Tg, 105 ± 9 mmHg, n = 10 for each group; P = 0.77). Heart rates were also similar in control and CRP-Tg mice (control, 574 ± 84 beats/min; CRP-Tg, 575 ± 56 beats/min, n = 10 for each group; P = 0.96). Serum CRP levels were 32 ± 7 mg/l in non-MI CRP-Tg mice (n = 8), whereas CRP was undetectable in serum from control mice. Histological examination in the hearts from CRP-Tg mice identified cardiac myocytes and endothelial cells as a main source of human CRP (Fig. 1D). No pathological abnormalities such as inflammation, hypertrophy, and fibrosis were seen in the hearts from non-MI CRP-Tg mice, which were indistinguishable from those from control mice. The count of neutrophils in the hearts was not different between control and CRP-Tg mice; CRP-Tg mice, 16 ± 6 cells/mm², n = 6 for each group; P = 0.81. The count of macrophages in the hearts was also comparable between the two groups (control, 4 ± 4 cells/mm²; CRP-Tg, 5 ± 3 cells/mm², n = 6 for each group; P = 0.68).

Mortality and infarct size do not differ between control and CRP-Tg mice after MI. A total of 86 mice (45 controls and 41 CRP-Tg) underwent surgery for the survival study. Eleven mice (5 controls and 6 CRP-Tg) died of surgical complications: five mice (3 controls and 2 CRP-Tg) died before coronary ligation, three mice (2 controls and 1 CRP-Tg) died after coronary ligation but before extubation, and three mice (0 controls and 3 CRP-Tg) died immediately after extubation. The remaining 75 mice, consisting of 40 controls and 35 CRP-Tg mice, were included in the survival study. Kaplan-Meier analysis revealed that survival 5 wk after MI did not differ between the two groups (Fig. 2A). All animals had anterior wall MI at necropsy. LV rupture was found in six control mice and five CRP-Tg mice. Serum CRP levels were 43 ± 17 mg/l in the CRP-Tg mice that were euthanized 5 wk post-MI (n = 14).

We measured infarct sizes at 1 and 5 wk following MI using two approaches: 1) the ratio of infarct area to total LV area in the LV samples that were cut and opened and 2) the ratio of infarct lengths to total LV circumferences in the LV cross sections. Infarct size as assessed by either approach was comparable between the two groups (Fig. 2, D and E). Serum CRP levels were 51 ± 20 mg/l in the CRP-Tg mice that were euthanized 1 wk post-MI (n = 10). In addition, a separate group of animals were euthanized 24 h after MI to assess infarct size by TTC staining. Histological examination showed infarct size to be similar in the two groups (P = 0.85; Fig. 2, C and F). Infarct size was consistent with our previous data obtained using the same procedure (30). These results indicate that infarct size is not affected by human CRP expression in mice.

CRP-Tg mice have more prominent cardiac hypertrophy and fibrosis in the noninfarcted regions after MI than controls. As shown in Table 1, there was no group difference in body weight 5 wk after MI. The left and right ventricle weight and the left and right ventricle-to-body weight ratio were increased after MI, and these parameters were significantly higher in post-MI CRP-Tg mice than in post-MI controls. The lung weight and the lung-to-body weight ratio were higher in post-MI CRP-Tg mice than in post-MI controls. These data suggest that cardiac hypertrophy and lung congestion associated with MI are more pronounced in CRP-Tg mice.

Histological evaluation revealed the cross-sectional area of cardiac myocytes in the noninfarcted intraventricular septum was increased 5 wk after MI, and the increase in cardiomyo-

![Fig. 2](http://ajpheart.physiology.org/)

Fig. 2: A: seventy-five mice (40 controls and 35 CRP-Tg mice) that recovered from surgery were subjected to the survival study. Kaplan-Meier curve analysis demonstrated that mortality at 5 wk did not differ between the 2 groups (log rank, P = 0.28). B: transverse sections of LV at the midventricular level from a control mouse and a CRP-Tg mouse 1 wk after myocardial infarction (MI); Masson’s trichrome. C: representative pictures of sliced LV cross sections obtained from a control mouse and a CRP-Tg mouse 24 h after MI. Necrotic tissues (white) are distinct from viable tissues (red); 1% triphenyltetrazolium chloride (TTC). D: there was no group difference in infarct size 1 and 5 wk after MI as assessed by the ratio of infarct area to total LV area in LV samples. E: no group difference in infarct size was found as assessed by the ratio of infarct lengths to total LV circumferences in LV cross sections. F: histological examination by TTC staining showed infarct size to be similar in the 2 groups 24 h after MI.
cyte cross-sectional area was greater in CRP-Tg mice than in controls (non-MI control, 270 ± 12 µm²; non-MI CRP-Tg, 268 ± 16 µm²; MI control, 389 ± 25 µm²; MI CRP-Tg, 474 ± 62 µm², n = 6 for each group; P < 0.05 from one-way ANOVA with Bonferroni’s test). Staining with Masson’s trichrome showed cardiac fibrosis in the noninfarcted intraventricular septum to be enhanced in CRP-Tg mice than in controls 5 wk after MI (control, 0.17 ± 0.05%; CRP-Tg, 0.29 ± 0.07%, n = 6 for each group; P = 0.01), whereas there was no group difference in percent area of fibrosis in the infarct region (control, 84.8 ± 2.4%; CRP-Tg, 85.6 ± 4.3%, n = 6 for each group; P = 0.68).

CRP-Tg mice have more LV dilation and worse LV function after MI than controls. Table 2 shows echocardiographic findings before MI and 5 wk after MI. Before MI, there were no differences in heart rate, LV dimensions, wall thickness, or LV function between CRP-Tg and control mice. Five weeks after MI, heart rates did not change. Both anterior and posterior wall thickness decreased to the same extent in both groups. However, LV end-diastolic and end-systolic diameters were larger in CRP-Tg mice, indicating increased chamber dilation. In addition, CRP-Tg mice had lower LV EFs than control mice. To investigate the effects of increased CRP expression on LV remodeling and function at an earlier time point, a different group of animals underwent echocardiography 1 wk after MI. When compared with that of control mice, CRP-Tg mice had larger LV end-diastolic diameter (control, 4.4 ± 0.4 mm, n = 11; CRP-Tg, 5.3 ± 0.4 mm, n = 10; P < 0.0001) and LV end-systolic diameter (control, 3.7 ± 0.5 mm, n = 11; CRP-Tg, 4.6 ± 0.4 mm, n = 10; P = 0.0002) and lower LV EFs (control, 21 ± 5%, n = 11; CRP-Tg, 15 ± 4%, n = 10; P = 0.009). Therefore, the differences in LV size and function between the two groups were already apparent 1 wk after MI. These data suggest that increased CRP expression is associated with more LV dilation and worse LV function after MI.

Hemodynamic measurements were conducted 5 wk after MI. Heart rate, aortic pressure, and LV systolic and end-diastolic pressures did not differ between the CRP-Tg and control groups (Table 3). LV +dP/dt was lower in the CRP-Tg group than in the control group (P = 0.006). LV −dP/dt was also reduced in CRP-Tg mice (P = 0.002). These data indicate that increased CRP expression adversely affects LV contractility and relaxation after MI.

Increased apoptosis in the border zones of infarcted hearts from CRP-Tg mice. There were very few TUNEL-positive nuclei in LV sections from uninfarcted mice, and no group difference in the number of TUNEL-positive nuclei was found (control, 12 ± 5 nuclei per 10⁵ cells; CRP-Tg, 14 ± 6 nuclei per 10⁵ cells, n = 6 for each group; P = 0.46). This finding indicates that myocardial apoptosis is not induced by human CRP expression in mice under physiological conditions. At 1 wk post-MI, TUNEL-positive nuclei were observed more frequently in the border and infarct zones than in the remote zones in the both groups, which is consistent with our previous findings (30). The rate of myocardial apoptosis in the border zone was higher in the CRP-Tg group than in the control group 1 wk after MI induction (control, 596 ± 275 nuclei per 10⁵ cells; CRP-Tg, 1,434 ± 789 nuclei per 10⁵ cells, n = 6 for each group; P = 0.03), whereas the apoptotic rates in the remote and the infarct zones were similar in the two groups (remote: control, 50 ± 12 nuclei per 10⁵ cells, and CRP-Tg, 63 ± 24 nuclei per 10⁵ cells, n = 6 for each group, P = 0.57; and infarct: control, 723 ± 314 nuclei per 10⁵ cells, and CRP-Tg, 781 ± 435 nuclei per 10⁵ cells, n = 6 for each group, P = 0.81; Fig. 3, A–C). We also assessed expression of apoptosis-related genes such as Bcl-2, Bcl-xL, Bax, and Bag in homogenates from the remote, the border, and the infarct zones by real-time RT-PCR (n = 6 for each group). Bcl-2 expression in the border zone was decreased by 41% in the CRP-Tg group compared with the control group (P < 0.05; Fig. 3D). Bcl-2 expression was lower in the infarct zone than in the remote zone, but no group difference in Bcl-2 levels was found in the infarct zone. In contrast, Bag expression in the border zone was increased by 56% in the CRP-Tg group (P < 0.05; Fig. 3E). Bax expression

Table 2. Echocardiographic data of control and CRP-Tg mice before and after MI

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<tr>
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<th>CRP-Tg</th>
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<tr>
<td>n</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>485 ± 56</td>
<td>454 ± 43</td>
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<tr>
<td>LVEDD, mm</td>
<td>3.3 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.7 ± 0.3</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>AWh, mm</td>
<td>0.69 ± 0.04</td>
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<tr>
<td>PWh, mm</td>
<td>0.68 ± 0.04</td>
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</tr>
<tr>
<td>LVEF, %</td>
<td>67 ± 6</td>
<td>67 ± 8</td>
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Table 3. Hemodynamic data of control and CRP-Tg mice after MI

<table>
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<tr>
<td>n</td>
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<td>9</td>
</tr>
<tr>
<td>HR, beats/min</td>
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<tr>
<td>SBP, mmHg</td>
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<td>LVEDP, mmHg</td>
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<td>13 ± 3</td>
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<td>LV +dP/dt, mmHg/s</td>
<td>3.788 ± 656</td>
<td>2.974 ± 509*</td>
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<tr>
<td>LV −dP/dt, mmHg/s</td>
<td>−2.890 ± 480</td>
<td>−2.230 ± 143*</td>
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</table>

Values are means ± SD; n, number of animals studied. SBP, systolic blood pressure; DBP, diastolic blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV +dP/dt, left ventricular positive change in pressure over time; LV −dP/dt, left ventricular negative change in pressure over time. *P < 0.01, CRP-Tg vs. control (Student’s unpaired t-test, 2-tails).
was higher in the infarct zone than in the remote zone, but no group difference in Bcl-2 levels was found in the infarct zone. There were no significant group differences in border zone Bcl-xL expression (control, 1.7 ± 0.8 arbitrary unit; CRP-Tg, 1.0 ± 0.4 arbitrary unit; \( P = 0.05 \)) and Bad expression (control, 2.7 ± 0.7 arbitrary unit; CRP-Tg, 4.5 ± 2.1 arbitrary unit; \( P = 0.11 \)). These data suggest that alterations in Bcl-2 and Bax expression are associated with promotion of apoptosis in the border zones of infarcted hearts from CRP-Tg mice. In addition, Western blot analysis showed protein content of cleaved (active form) caspase-3 to be increased in the border zone samples from CRP-Tg mice compared with control mice. Values are normalized against the corresponding GAPDH levels. Data are means ± SD; \( n = 6 \) for both groups. *\( P < 0.05 \), border CRP-Tg vs. border control; †\( P < 0.05 \), border vs. remote; infarct vs. remote (nonparametric test).

Increased macrophage infiltration, MCP-1 expression, and MMP-9 activity in the border zones of infarcted hearts from CRP-Tg mice. In histological studies, apparent inflammation was seen in the border and the infarct areas but not in the remote region of 1-wk old infarcted hearts. Immunohistochemical studies revealed infiltrating macrophages, as demonstrated by staining with anti-F4/80 antibodies, were predominantly found in the border and the infarct zones. More abundant macrophages (brown stained) were observed in the border zones of infarcted hearts from CRP-Tg mice compared with those from controls (\( P = 0.01 \); Fig. 4, A–C), although the number of macrophages in the infarct zone was comparable between the two groups (\( P = 0.30 \)). The infiltration of neutrophils in the border and the infarct zones, which was a relatively low percentage of infiltrating inflammatory cells at 1 wk post-MI, was similar in the two groups (border: control, 66 ± 18 cells/mm²; CRP-Tg, 50 ± 11 cells/mm², \( P = 0.85 \); and infarct: control, 64 ± 10 cells/mm²; CRP-Tg 73 ± 10 cells/mm², \( n = 6 \) for each group, \( P = 0.43 \)).

Increased MCP-1 expression in the border zones of infarcted hearts from CRP-Tg mice. Immunohistochemical studies identified macrophages infiltrating in the border and the
infarct zones as a main source of MCP-1 (brown stained; Fig. 4, D and E). More MCP-1 positive cells were seen in the border zones of infarcted hearts from CRP-Tg mice than those from control mice, although there was no group difference in the number of MCP-1 positive cells in the infarct zone (Fig. 4 F).

Western blot analysis also showed MCP-1 protein expression to be increased by 1.5-fold in the border zones, but not in the infarct zone, of infarcted hearts from CRP-Tg mice compared with those from control mice (P < 0.05; Fig. 5). MCP-1 expression in remote zone samples was too weak to be detected as clear bands by immunoblotting (data not shown). Serum MCP-1 levels at 1 wk following MI were similar in the two groups (control, 56 ± 24 pg/ml; CRP-Tg, 62 ± 23 pg/ml, n = 6 for each group; P = 0.64).

Increased MMP-9 activity in the infarct and the border zones of infarcted hearts from CRP-Tg mice. Immunohistochemical studies revealed that MMP-9 expression (brown stained) was predominantly found in leucocytes and macrophages infiltrating in the border and the infarct zones (Fig. 4, G and H). MMP-9 expression was more frequently observed in the infarct zone than in the border zones. In addition, more MMP-9 positive cells were found in the border zones of infarcted hearts from CRP-Tg mice than those from control mice (Fig. 4 I). Gelatin zymography showed MMP-9 activity to be higher in border and infarct zones samples from CRP-Tg mice than in those from control mice at 1 wk following MI (Fig. 6, A–C). Although MMP-2 activity was enhanced in the border and the infarct zones of infarcted hearts, no group difference in MMP-2 activity was found in either region (Fig. 6 D).

DISCUSSION

We found that CRP-Tg mice showed greater LV dilation and had poorer LV function with more prominent cardiomyocyte hypertrophy and fibrosis in the viable intraventricular septum after MI than control mice, although survival and infarct size were similar in the two groups. This adverse effect of CRP on LV remodeling was already apparent 1 wk after MI, suggesting a pathogenic role of CRP in the early phase of the post-MI remodeling process. The current study also showed higher

Fig. 4. Immunohistochemical studies stained with antibodies against F4/80 (macrophages), monocyte chemotactic protein (MCP)-1, and matrix metalloproteinase (MMP)-9. A and B: representative photomicrographs showing brown-stained F4/80 positive cells in a section of the border zone from a control mouse (A) and a CRP-Tg mouse (B), 400×. C: infiltration of F4/80 positive cells was more abundant in the border zones of CRP-Tg mice than in those of control mice 1 wk after MI. D and E: representative photomicrographs showing brown-stained MCP-1 positive cells in a section of the border zone from a control mouse (D) and a CRP-Tg mouse (E), 400×. F: MCP-1 expression was predominantly found in infiltrating macrophages in the border and infarct zones. More MCP-1 positive cells were seen in the border zones of infarcted hearts from CRP-Tg mice than those from control mice. G and H: representative photomicrographs showing brown-stained MMP-9 positive cells in a section of the border zone from a control mouse (G) and a CRP-Tg mouse (H), 400×. I: MMP-9 expression was predominantly found in leucocytes and macrophages infiltrating in the border and infarct zones. MMP-9 positive cells were more frequently observed in the infarct zone than in the border zones. More MMP-9 positive cells were seen in the border zones of infarcted hearts from CRP-Tg mice than those from control mice. Data are means ± SD; n = 6 for each group. *P < 0.05, border CRP-Tg vs. border control; †P < 0.05, infarct vs. border (Bonferroni’s test).
apoptotic rates, more macrophage infiltration, and increased MCP-1 expression and MMP-9 activity in the border zones of infarcted hearts from CRP-Tg mice.

CRP is an acute-phase reactant, which responds to various pathological stimuli including infection, inflammation, tissue damage, and neoplasm. Recent experimental studies indicate that CRP per se has multiple biological activities that may be involved in the pathophysiology of various cardiovascular diseases. For instance, CRP induces expressions of adhesion molecules and chemokines such as MCP-1 in in vitro studies (10). CRP reportedly induces apoptosis in human vascular smooth muscle cells as well as rat cardiac myocytes (6, 33). CRP was also reported to attenuate survival, differentiation, and the functions of endothelial progenitor cells (32). CRP-Tg mice that constitutively produce human CRP provide a useful model for studying the biological activities of human CRP in vivo. Some investigators using a crossbreeding method have reported that human CRP expression accelerates the progression of atherosclerosis in apoE-deficient mice (22, 26), although conflicting data have been presented by other laboratories (12, 14, 31).

In acute MI, CRP peaks at 2 to 3 days after the onset, and the peak level is a strong predictor of adverse clinical outcomes (2, 3, 29). Although the pathogenic importance of CRP remains undetermined in the acute MI setting, CRP colocalizes with activated complement in human infarcted tissues (15). Griselli et al. (9) reported that injection of human CRP after coronary artery occlusion increases infarct size in rats. In the present study, however, increased CRP expression did not affect infarct size in our transgenic mouse model. This difference may be explained by the different species (mouse vs. rat) used, the different experimental conditions (transgene expression vs. protein injection), and the extent of infarct size. The average infarct size was ~50% in our study, whereas it was 17% for the vehicle-treated group and 25% for the CRP-treated group in their study (9). It is noteworthy that, in our study, increased CRP expression was associated with more LV dilation and worse LV function after MI without altering infarct size. To our knowledge, no study has shown direct in vivo effects of CRP on the development of adverse LV remodeling after MI. We therefore asked how increased CRP expression could modulate the post-MI inflammatory response and healing process as well as apoptosis, which might be responsible for deterioration of cardiac remodeling after MI in our CRP-Tg mice.

The post-MI inflammatory response plays a critical role in the pathophysiology of infarct expansion and LV remodeling (8). We previously reported post-MI peripheral monocytosis to be associated with LV dysfunction and LV aneurysm (17) and that granulocyte-macrophage colony-stimulating factor induction in a rat MI model resulted in exaggerated LV remodeling...
with increased infiltration of monocyte-derived macrophages and dendritic cells and impaired reparative fibrosis in the infarcted area (18, 20). In the present study, immunohistochemical studies have shown more pronounced macrophage infiltration in the border zones of infarcted hearts from CRP-Tg mice than in those from control mice. In addition, MCP-1 expression was predominantly found in infiltrating macrophages. Western blot analysis also showed MCP-1 expression in the border zones to be increased in CRP-Tg mice. These results indicate that increased CRP expression intensifies the inflammatory response after MI through local macrophage infiltration and activation, although the precise mechanisms by which CRP enhances this infiltration and activation in the present context remain uncertain. A recent clinical study with endomyocardial biopsies has shown the evidence for colocalization of CRP with complement and macrophages in the myocardium of patients with dilated cardiomypathy (34). Since CRP is reported to upregulate MCP-1-mediated chemotaxis in human monocytes (10), CRP might be directly involved in increased macrophage infiltration and activation around the necrotic tissues.

Myocardial apoptosis is one of the key factors contributing to the progression of LV remodeling and heart failure after MI (21, 25). Moreover, apoptosis of cardiac myocytes is reported to peak during the first several days following MI in the ischemic area (5, 16), which is consistent with our previous data showing that myocardial apoptosis frequently occurs in the infarct border zone in the early phase after MI (30). In the present study, apoptotic rates were higher in the border zones of infarcted hearts from CRP-Tg mice compared with those from control mice, where proapoptotic gene expression profiles were noted. One report showed that CRP induces ischemia-related apoptosis of cardiac myocytes in vitro (33). Thus increased CRP expression may promote the development of LV remodeling after MI through increased apoptosis in the border zone.

MMPs, major regulators of extracellular matrix degradation, have been shown to be involved in the pathogenesis of LV remodeling after MI. Several studies in genetically manipulated mice documented that gelatinases such as MMP-2 and MMP-9 play a critical role in the remodeling process and contributing to LV rupture (11, 19). MMP-9 is predominantly found in leukocytes and macrophages in infarcted regions and targeted deletion of MMP-9 prevents LV rupture in the acute MI setting (11). In the present study, more infiltrating macrophages with increased MMP-9 expression were seen in the border zones of infarcted hearts from CRP-Tg mice compared with those from control mice. Moreover, MMP zymography showed increased MMP-9 activity in the border and the infarct zones of infarcted hearts from CRP-Tg mice. Abe et al. (1) have demonstrated that CRP increases gene expression and activity of MMP-9 in human macrophages, suggesting a direct effect of CRP on MMP-9 activity in macrophages. Thus increased MMP-9 activity in association with macrophages infiltration in CRP-Tg mice may impair the healing process after MI, resulting in adverse LV remodeling.

The current study provides the first evidence for the direct in vivo effect of human CRP on the post-MI remodeling process in an animal model. Although there is considerable variability among species with respect to the biological and pathological functions of CRP, one would assume that CRP partially involves in the pathogenesis of the post-MI remodeling process in human hearts. Further studies are needed to confirm this deleterious effect of CRP on adverse LV remodeling in a clinical post-MI setting.

In conclusion, increased human CRP expression exacerbates LV dysfunction and adverse LV remodeling after MI without affecting infarct size in mice.

ACKNOWLEDGMENTS

We thank Shigeyuki Kasai (Oriental Yeast) and Tomomichi Kanabayashi (Biopathology Institute) for technical assistance.

GRANTS

This work was partly supported by the Medical School Faculty and Alumni Grant from Keio University Medical Science Fund (to T. Anzai) and the Keio Gijuku Academic Development Fund (to T. Takahashi) and the Global Center of Excellence (G-COE) at Keio University (to H. Kaneko).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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