Cardiac dysfunction induced by experimental myocardial infarction impairs the host defense response to bacterial infection in mice because of reduced phagocytosis of Kupffer cells

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Objective: This study was undertaken to investigate the effects of cardiac dysfunction induced by experimental myocardial infarction on the host defense response to bacterial infection and the role of Kupffer cells in mediating this response.

Methods: Myocardial infarction was induced in C57BL/6 mice by ligation of the left anterior descending coronary artery. Mice were challenged with *Escherichia coli* intravenously 1, 5, and 14 days after myocardial infarction or sham operation. Thereafter, the cytokine production and the function of their Kupffer cells were assessed.

Results: Mice with myocardial infarction showed remarkable cardiac dysfunction and had a significantly lower survival than sham mice after bacterial challenge at 5 days after surgery; bacterial challenge at 1 or 14 days after surgery resulted in no difference in survival between myocardial infarction and sham mice. The phagocytic activity of Kupffer cells, assessed by fluorescein isothiocyanate microspheres, remarkably decreased in mice with myocardial infarction 5 days after surgery. Serum peaks of tumor necrosis factor and interferon-γ after bacterial challenge were also suppressed in mice with myocardial infarction at 5 days. Production of these cytokines and immunoglobulin-M from liver mononuclear cells was also impaired in mice with myocardial infarction. Enhancement of the phagocytic activity of Kupffer cells by C-reactive protein significantly improved survival after infection in mice with myocardial infarction, although neither interleukin-18 nor immunoglobulin-M treatment improved survival.

Conclusions: Cardiac dysfunction induced by myocardial infarction renders mice susceptible to bacterial infection and increases mortality because of a reduced ability of Kupffer cells to clear infectious bacteria. C-reactive protein-enhanced phagocytic activity of Kupffer cells may improve the poor prognosis after bacterial infection in mice with myocardial infarction. (J Thorac Cardiovasc Surg 2010;140:624-32)

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Continued advances in cardiac surgery, such as improvements in myocardial protection, surgical techniques, and perioperative care, have extended surgical indications for patients with severe left ventricular (LV) dysfunction.[1] However, patients with low ejection fraction (<20%) still have a higher incidence of perioperative complications, such as respiratory failure, renal failure, or bacterial infections, compared with those with greater ejection fraction (>40%).[2] In particular, patients who have congestive heart failure are susceptible to bacterial infection after cardiac surgery, leading to a poor prognosis.[3] Although the incidence of perioperative infection in cardiac surgery ranges from 2.7% to 9%,[3-5] patients who have perioperative infections, including mediastinitis, thoracotomy or vein harvest site infection, or septicemia, have significantly higher mortality and length of hospital stay than patients without infection.[3-5]

Severe surgical stress renders the host susceptible to bacterial infections.[6,7] We previously demonstrated that burn injury markedly impairs not only interferon (IFN)-γ–mediated cellular immunity[7] but also immunoglobulin (Ig)-M–mediated humoral immunity in mice,[6] thereby decreasing survival from postburn bacterial infections. In our clinical study, the capability of peripheral blood mononuclear cells (MNCs) to produce IFN-γ significantly decreased in patients after gastrointestinal surgery.[8] However, there are few reports on the host defense response to bacterial infection in hosts with congestive heart failure.

C-reactive protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae*, is an exquisitely sensitive marker of inflammation and tissue damage.[9] Increased serum CRP levels are associated with patient outcome in acute coronary syndromes.[10] It was recently revealed that CRP binds to Fcγ receptors I and

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Abbreviations and Acronyms

CRP = C-reactive protein
FITC = fluorescein isothiocyanate
IFN = interferon
Ig = immunoglobulin
IL = interleukin
IV = intravenously
LAD = left anterior descending
LV = left ventricular
MI = myocardial infarction
MNC = mononuclear cell
PBS = phosphate-buffered saline
TTC = triphenyltetrazolium chloride

II,[11] and thereby activates Fcγ receptor-mediated opsonization,[12] resulting in enhanced phagocytosis of microorganisms.[13] Although there are several reports that CRP-activated opsonization might exacerbate the acute phase of myocardial infarction (MI),[14] CRP-enhanced phagocytic activity may also improve the host defense response to bacterial infection, especially when the host is immunocompromised,[15] because bacterial phagocytosis is crucial to eliminate invading bacteria. The present study investigated the innate immune response to bacterial infection in mice with cardiac dysfunction induced by experimental MI.

MATERIALS AND METHODS

This study was conducted according to the guidelines of the Institutional Review Board for the Care of Animal Subjects at the National Defense Medical College, Tokorozawa, Japan.

Animals and Experimental Myocardial Infarction

Male C57BL/6 mice were studied (8 weeks old, 20 g, Japan SLC, Shizuoka, Japan). Mice were initially anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg, Abbott Laboratories, North Chicago, Ill). They were then intubated and sedated with 2% sevoflurane (Maruishi Pharm, Osaka, Japan) mixed with air and oxygen using a vaporizer (Shinano Mfg Co Ltd, Tokyo, Japan). Ventilation was performed with a rodent volume-controlled mechanical ventilator (Animal Ventilator KN-55, Natume Co Ltd, Tokyo, Japan). The heart was exposed through a left thoracotomy, and the electrocardiogram was monitored. The proximal left anterior descending (LAD) coronary artery was permanently ligated with 8-0 polypropylene to produce an infarct (MI group).[16,17] Myocardial ischemia was confirmed by regional cyanosis and ST-segment elevation. The incision was closed in layers with 5-0 polyester sutures. Sham-operated mice underwent the same procedure without ligation of the artery, but rather the suture was placed behind the LAD coronary artery (sham group) (Figure 1, *A, B*). In the MI group, 70% of the mice survived until 14 days after surgery (Figure 2, *A*).

Reagents

Escherichia coli strain B (ATCC 11303, Sigma-Aldrich Co, St Louis, MO) was grown in brain heart infusion broth (Difco Co Ltd, Detroit, Mich) and used for the experiments. Mouse recombinant interleukin (IL)-18 (Medical & Biological Laboratories, Nagoya, Japan) and mouse IgM (pp50; Chemicon International Inc, Temecula, Calif) were also used for the experiments. Synthetic CRP (174-185) was purchased from Sigma-Aldrich Co. Carboxylate microspheres (75 nm diameter; Polysciences Europe, Epipelheim, Germany) (hereafter called ''fluorescein isothiocyanate [FITC] microspheres'') were used to analyze the phagocytic activity of Kupffer cells.

Systemic *Escherichia coli* Challenge and Collection of Blood Samples

Mice were challenged intravenously (IV) with 5×10^8 colony-forming units of *E. coli* at 1, 5, or 14 days after cardiac surgery or sham operation (Figure 2, *B*). Blood samples were obtained from the retro-orbital plexus of mice at the indicated times and then stored at –80°C until assays were performed.

Interleukin-18, Immunoglobulin-M, and C-Reactive Protein Treatments

Mice were injected intraperitoneally with IL-18 (0.1 μg/0.5 mL/body) at 2 days, 4 days, and 1 hour before *E. coli* challenge, and 1, 3, and 5 days after *E. coli* challenge, which was performed 5 days after surgery (Figure 2, *C*). Mice were also subjected to surgery and then injected IV with IgM (330 μg/0.5 mL/body) 1 hour before *E. coli* challenge and 2 days after challenge, or injected IV with CRP (500 μg/0.5 mL/body) 1 hour before *E. coli* challenge (Figure 2, *C*). Sham treatment was performed by injection with phosphate-buffered saline (PBS) (0.5 mL) in the same manner as the IL-18, IgM, and CRP treatment.

Blood Culture and Histologic Examinations

Blood culture and histologic examinations of the heart, lung, liver, and kidney were performed as described.[6,7,15]

Isolation of Mononuclear Cells and Cell Cultures

MNCs were obtained from the liver, spleen, and femurs as previously described.[6,7,18] After counting cells, 5×10^5 of liver, spleen, or bone marrow MNCs in 200 μL 10% fetal bovine serum-Roswell Park Memorial Institute 1640 medium were cultured in 96-well flat-bottom plates in 5% CO_2 at 37°C for 24 hours.[6,7,18]

Measurements of Cytokine and Immunoglobulin-M Levels in Culture Supernatants and Sera

Cytokine levels of the sera or the culture supernatants were measured using commercially available enzyme-linked immunosorbent assay kits (IL-18, MBL, Nagoya, Japan; others, Endogen, Woburn, Mass). IgM levels of sera or culture supernatants were measured using an enzyme-linked immunosorbent assay quantification kit (Bethyl Laboratories Inc, Montgomery, Tex).

Determination of Microsphere Phagocytic Activity of Kupffer Cells

Mice were injected IV with FITC microspheres (20 μL/0.5 mL/body). After 20 minutes, they were euthanized to remove the livers. The liver MNCs, including Kupffer cells, were stained with phycoerythrin-conjugated anti-mouse F4/80 Ab (eBioscience, San Diego, Calif). Thereafter, phagocytosis of FITC microspheres by $F4/80^+$ hepatic macrophages, namely, Kupffer cells, was analyzed using the EPICS XL (Beckman Coulter Inc, Miami, Fla). Because FITC fluorescence intensity is dependent on the number of ingested microspheres, the peaks of the histogram correspond to Kupffer cells that contained no ingested microspheres (peak 0) and 1 (peak 1), 2 (peak 2), and more (peak ≥ 3) microspheres from left to right, respectively.

Assessment of Myocardial Infarction

Echocardiography. Two-dimensionally guided M-mode recordings were obtained from the short-axis view at the level of the papillary muscles using an HD11XE ultrasound system and a 15-MHz linear-array transducer (Royal Philips Electronics, Eindhoven, The Netherlands). LV end-systolic and end-diastolic dimensions, as well as systolic and diastolic wall thickness,

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