

Original article

# The role of natural IgM in myocardial ischemia–reperfusion injury

Ming Zhang<sup>a,b,1</sup>, Lloyd H. Michael<sup>c</sup>, Sandrine A. Grosjean<sup>d</sup>, Ralph A. Kelly<sup>d,e</sup>,  
Michael C. Carroll<sup>a,b,f</sup>, Mark L. Entman<sup>c,\*</sup>

<sup>a</sup> The CBR Institute for Biomedical Research, Inc., Harvard Medical School, Boston, MA 02115, USA

<sup>b</sup> The Department of Pathology, Harvard Medical School, Boston, MA 02115, USA

<sup>c</sup> Section of Cardiovascular Sciences, The Methodist Hospital and the DeBakey Heart Center, Baylor College of Medicine, Houston, TX 77030, USA

<sup>d</sup> Cardiovascular Division, Brigham and Women's Hospital, Boston, MA 02115, USA

<sup>e</sup> Genzyme Corporation, Framingham, MA 01701, USA

<sup>f</sup> The Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA

Received 8 February 2006; accepted 24 February 2006

Available online 16 June 2006

## Abstract

Myocardial ischemia–reperfusion injury represents a combination of factors, namely the intrinsic cellular response to ischemia and the extrinsic acute inflammatory response. Recent studies in mesenteric and skeletal muscle reperfusion models identified natural IgM as a major initiator of pathology through the activation of the complement system and inflammatory cells. To determine whether a similar mechanism is involved in myocardial tissues, mice bearing an altered natural IgM repertoire (Cr2<sup>-/-</sup>) were examined in a murine model of coronary artery ischemia. Notably, these mice were significantly protected based on the reduced infarct size, limited apoptosis of cardiomyocytes, and decreased neutrophil infiltration. Protection was IgM-dependent as reconstitution of these mice with wild-type IgM restored myocardial reperfusion injury. These results support a model in which natural IgM initiates the acute inflammatory response in the myocardium following ischemia and reperfusion.

© 2006 Elsevier Inc. All rights reserved.

**Keywords:** Natural antibody; Complement; Myocardial ischemia–reperfusion injury; Infarction; Apoptosis; Neutrophil

## 1. Introduction

Acute myocardial ischemia and myocardial infarction are a leading cause of mortality in today's society. Restoration of blood flow (reperfusion) is essential for survival of the myocardium, but, paradoxically, it also induces an acute inflammatory response referred to as ischemia–reperfusion injury (I/R). Full injury represents a manifestation of both the intrinsic cellular response of myocardium and the extrinsic acute inflammatory response [1–3].

While numerous studies have revealed the decisive contribution of active cellular responses to injury resulting from ischemic insults [4–10], evidence is emerging that acute inflammatory responses also play an important role in

myocardial I/R [2,11]. Studies on the serum complement system, a major component of natural immunity, indicate that it may be a major factor [2,12–15]. Early observations that transient depletion of the central complement, i.e. C3, reduced inflammation in a rat model of myocardial infarction suggested a role for complement in I/R [12]. Weisman et al. [13] subsequently demonstrated in a rat myocardial model that I/R injury was dramatically reduced by pretreatment with a modified form of a natural inhibitor of complement, soluble CR1 (sCR1). Since then, a similar approach has shown that I/R injury in multiple tissues could be reduced or blocked by pretreatment with sCR1 [16,17]. These studies led to the examination of how complement was activated during the early stages of I/R injury.

Three pathways leading to activation of the complement system have been identified, i.e. the classical, lectin, and alternative pathways. Each is activated by different initiators, but all converge to complement protein C3 and are followed by a common cascade [18]. Although antibody–antigen interaction

\* Corresponding author. Tel.: +1 713 798 4188.

E-mail address: [mentman@bcm.med.edu](mailto:mentman@bcm.med.edu) (M.L. Entman).

<sup>1</sup> Current address: Department of Anesthesiology, SUNY-Downstate Medical Center, Brooklyn, NY 11203, USA.

is traditionally recognized as activating via the classical pathway, new evidence suggests that certain antibodies also activate the lectin pathway [19,20].

The first indication that serum antibody may be involved in complement-mediated I/R injury came from studies of hind limb I/R by Weiser et al. [21]. Using knockout mice deficient in complement proteins C3 or C4, they found that an intact pathway of complement was required for full I/R injury in hind limb. Thus, mice deficient in C4, the protein which is activated in the classical and lectin pathways, were protected to a degree similar to C3-deficient mice or to wild-type mice (WT) treated with sCR1. The finding that C4 was involved suggested that the complement system might be activated by serum antibody. Subsequent experiments confirmed a role for serum immunoglobulin and identified IgM as the important isotype. Thus, mice bearing a genetic deficiency in the Recombination Activation Gene-1 (RAG-1<sup>-/-</sup>), and thus do not develop mature B and T lymphocytes, failed to develop full injury, but reconstitution with fresh pooled serum [21] or with pooled WT IgM [22] restored full injury.

The suggestion that specific, pre-existing, or natural IgM was important in I/R came from studies using mice bearing a genetic deficiency in the Cr2 locus which encode complement receptors CD21 and CD35 [23,24]. Cr2<sup>-/-</sup> mice have an impaired humoral response to T-dependent and T-independent antigens due to both an intrinsic defect in B cell activation and reduced antigen trapping on FDC [23,24]. Although the mice have normal levels of circulating IgM, it was proposed that their repertoire might be limited given the impaired responsiveness of their B cells. Examination of the mice in the intestinal I/R model revealed a significant reduction in injury that was reversed by reconstitution with pooled IgM prepared from WT mice but not Cr2<sup>-/-</sup> mice [25,26]. By contrast, reconstitution with pooled WT IgG did not cause histological injury, although it did augment neutrophil infiltration when combined with IgM [25]. Thus, it was suggested that injury is mediated by specific clone (s) of natural IgM [25,26]. More recently, a single IgM clone isolated from a panel of natural IgM-producing hybridoma cells was identified that was sufficient to reconstitute I/R injury in both the intestinal and hind limb I/R models [27,28].

To determine if specific natural IgM mediates the acute inflammatory response in the myocardial I/R, we examined Cr2<sup>-/-</sup> and RAG-1<sup>-/-</sup> mice in a left anterior descending (LAD) coronary artery model [29,30]. Significantly, myocardial infarct size, frequency of apoptotic cardiomyocytes and number of infiltrating neutrophils relative to the WT controls were found to be dependent on WT IgM. Reconstitution of Cr2<sup>-/-</sup> and RAG-1<sup>-/-</sup> mice prior to ischemia with WT IgM restored full injury. These results suggest a pathogenic mechanism by which myocardial I/R injury is mediated by naturally occurring IgM.

## 2. Methods

### 2.1. Animals

Cr2<sup>-/-</sup> mice were constructed as described using the approach of homologous recombination in embryonic stem

cells which introduces a frameshift mutation and a stop codon disrupting the coding for Cr2 coding region and bred onto the C57BL/6 background for at least 10 generations [23]. RAG-1<sup>-/-</sup> mice (C57BL/6 background) which were purchased from Jackson Laboratory (Bar Harbor, ME) were originally developed by homologous recombination as described [31], as a result a 1356 bp deletion which occurred in the 5' end of RAG-1 gene coding sequence. Animals were maintained in the animal facilities of Harvard Medical School and Baylor College of Medicine according to Federal and State regulations.

### 2.2. Mouse model of myocardial ischemia–reperfusion injury

An established murine myocardial model was used in this study [29]. Briefly, animals are anesthetized by an intraperitoneal injection of pentobarbital sodium, 40 mg/kg weight. Reconstitution with IgM is achieved by intravenous administration 30 min prior to surgery. Midline sternotomy followed by sternal retraction is performed to permit the visualization and ligation of the left anterior descending (LAD) coronary artery under a microscope (Stemi 2000-C, Carl Zeiss, Thornwood, NY) as described by Michael et al. [29]. Reperfusion of the previously occluded coronary bed is confirmed by visual inspection and surface electrocardiogram changes. The chest is then closed, and the animal allowed to recover under a heating lamp. Physiologic variables, including heart rate, core body temperature, and ECG, were monitored and carefully controlled within the normal range throughout the experiments. To assess the infarct size, the heart is harvested 24 h after LAD occlusion. The aorta is cannulated with a 22-gauge Luer stub, and LAD is re-occluded before 1% Evans Blue is perfused into the aorta and coronary arteries with distribution throughout the ventricular wall proximal to the coronary artery ligation. After this procedure, the heart is sectioned transversely into four sections, with one section being made at the site of the ligation. Sections of the ventricle are then incubated in 1.5% triphenyltetrazolium chloride (TTC). After TTC staining, viable myocardium is brick red and the infarct appears pale white. The sections are weighed. The apical side of each slice is imaged, and the area of infarction for each slice is determined by computerized planimetry using an image analysis software program (Image Tools, Houston, TX). The size of infarction is determined by the following equations:

$$\text{Weight of infarction} = (A_1 \times Wt_1) + (A_2 \times Wt_2) + (A_3 \times Wt_3) + (A_4 \times Wt_4)$$

where  $A$  is percent area of infarction by planimetry and  $Wt$  is the weight of each section. Percentage of infarcted LV = (weight of infarction/weight of LV)  $\times$  100. Area at risk (AAR) as a percentage of LV = (weight of LV – weight of LV stained blue)/weight of LV  $\times$  100).

### 2.3. Immunohistochemistry for neutrophils

Neutrophils in the infarcts were analyzed after 24 h reperfusion as previous studies showed neutrophil infiltration peak after 24 h reperfusion [32]. Tissues are fixed in 10%

paraformaldehyde or Z-fix (Anatech, Battle Creek, MI) for at least 3 h, dehydrated with ethanol (50–100%), cleared with xylene, and embedded in paraffin. The sections (5  $\mu\text{m}$  thick) were rehydrated with deionized water in preparation for immunohistochemistry. Staining for neutrophils was performed as described previously [33]. Briefly, rat anti-mouse neutrophil monoclonal antibody AMU-0021 (Biosource, Camarillo, CA) was used as a primary antibody and anti-rat IgG biotinylated monoclonal antibody as a secondary antibody (Vectastain, ABC Kit Peroxidase Rat IgG PK-4004, Vector Laboratories, Burlingame, CA) [32]. Neutrophils were counted manually using an Axioskop microscope (Carl Zeiss) at a power of 400 $\times$ . Neutrophil numbers in each section were expressed per 400 $\times$  power field of section.

#### 2.4. Assessment of cardiomyocyte apoptosis

To determine the prevalence of apoptosis after LAD occlusion, an in situ DNA ligase technique (Serologicals, Norcross, GA) was used that identifies only double-stranded DNA breaks with single 3' base pair overhangs that are more characteristic of DNA breaks that occur during apoptosis [34]. Studies were performed in myocardial sections ( $n = 10/\text{heart}$ ) obtained 6 h after LAD ligation. Previous studies showed that the maximal frequency of apoptosis occurred at 6 h in the WT mice, and there was no significant difference in the frequency of apoptosis between 6 and 24 h [34,35]. Apoptotic cell nuclei detected by the ligase assay were stained with fluorescein. Sections were counterstained with the nucleic acid binding dye DAPI (4',6-diamidino-2-phenylindole) to visualize the entire population of cell nuclei within each myocardial section. Two different sections per slice and per heart were analyzed for each experiment. To determine the fraction of myocyte nuclei that were labeled, we determined the total number of myocyte nuclei per unit area of the myocardium (10,000  $\mu\text{m}^2$ ) by enumerating the number of DAPI (4,6-diamidino-2-phenylindole)-stained myocyte nuclei; final results were expressed as follows: (number of positively labeled nuclei/total number of DAPI stained nuclei per 10,000  $\mu\text{m}^2$ )  $\times$  100%.

### 3. Results

To examine the role of natural IgM in myocardial infarction, mice bearing either a selective ( $\text{Cr}2^{-/-}$ ) or total deficiency ( $\text{RAG-1}^{-/-}$ ) in IgM were examined in a myocardial I/R model [29]. Previous study of peripheral blood leukocytes in  $\text{Cr}2^{-/-}$  mice found no inherent reduction in either the total cell numbers or the ratios of peripheral mononuclear cells to granulocytes compared with that of WT mice [23]. In contrast, natural IgM-producing B-1a cells reduce 50% in  $\text{Cr}2^{-/-}$  mice [23]. In the present myocardial I/R study, infarct size was significantly reduced two-fold in  $\text{Cr}2^{-/-}$  mice versus WT controls after 1 h LAD occlusion and 24 h of reperfusion (% infarct/AAR: 32.7  $\pm$  3.4 vs. 16.8  $\pm$  3.8 respectively,  $P < 0.05$ ,  $n = 6$  per group) (Fig. 1). A similar reduction was observed in  $\text{RAG-1}^{-/-}$  mice (% infarct/AAR: 8.7  $\pm$  2.3,  $P < 0.01$  compared with WT controls,  $n = 6$  per group) (Fig. 1). To further determine that natural IgM can directly

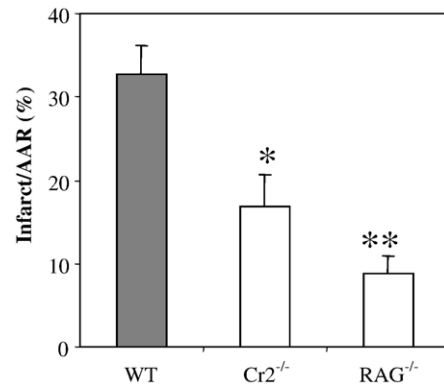


Fig. 1. Decreased myocardial infarct size in  $\text{Cr}2^{-/-}$  and  $\text{RAG}^{-/-}$  mice. Animals were subjected to LAD occlusion for 1 h followed by 24 h reperfusion. Myocardial infarcts were delineated by Evans Blue and TTC staining. Infarct size is expressed as a percentage infarct of area at risk (AAR). Results represent means  $\pm$  SEM (\* $P < 0.05$ , \*\* $P < 0.01$  compared with WT control,  $n = 6$  per group).

mediate myocardial infarction in WT mice,  $\text{Cr}2^{-/-}$  and  $\text{RAG-1}^{-/-}$  mice were reconstituted with pooled WT IgM 30 min prior to I/R surgery (i.v. 400  $\mu\text{g}$  WT IgM). Notably, infarct sizes similar to that of WT mice were observed (%infarct/AAR:  $\text{Cr}2^{-/-}$  with WT IgM = 24.4  $\pm$  5.5;  $P = 0.171$  compared with WT controls;  $\text{RAG-1}^{-/-}$  with WT IgM = 24.3  $\pm$  2.7;  $P = 0.054$  compared with WT controls; WT mice = 34.8  $\pm$  4;  $n = 6$  per group) (Fig. 2). Thus, similar to the intestinal and hind limb models, natural IgM can directly mediate myocardial damage in infarction.

Myocardial I/R injury in this model is characterized by both necrosis and apoptosis [6]. To determine if natural IgM is involved in apoptosis of cardiac myocytes, an in situ DNA ligase assay was performed to assess the frequency of apoptotic cells in the AAR at 6 h post-reperfusion [34,36]. A two-fold reduction in the frequency of apoptotic cardiomyocytes was observed in hearts prepared from  $\text{Cr}2^{-/-}$  mice relative to that of WT controls (% Positive Ligase Nuclei: 2.5  $\pm$  0.4 vs. 5.2  $\pm$  1.1, respectively,  $P < 0.05$ , number of WT mice = 11, number of  $\text{Cr}2^{-/-}$  mice = 7) (Fig. 3). Reconstitution of  $\text{Cr}2^{-/-}$  mice with WT IgM restored apoptosis to the WT level (4.9  $\pm$  1.3,  $n = 4$ ) (Fig. 3).

A hallmark of myocardial I/R injury is neutrophil infiltration [2,11,37,38]. To determine if natural IgM operates upstream of neutrophil infiltration, heart sections were characterized by immunohistochemistry after LAD occlusion of 1 h and reperfusion for 24 h. Approximately two-fold reduction of the neutrophil number in the infarct zone was observed in hearts prepared from  $\text{Cr}2^{-/-}$  mice (neutrophil number/power field: WT = 101  $\pm$  36;  $\text{Cr}2^{-/-}$  = 55  $\pm$  24,  $P < 0.05$ ; number of WT mice = 11, number of  $\text{Cr}2^{-/-}$  mice = 7) (Fig. 4). Furthermore, reconstitution of  $\text{Cr}2^{-/-}$  mice with WT IgM prior to ischemia restored neutrophil infiltration to that of WT level (102  $\pm$  26,  $n = 4$ ) (Fig. 4).

### 4. Discussion

Our results identified a pathogenic role for natural IgM in myocardial reperfusion injury. We found more than two-fold of

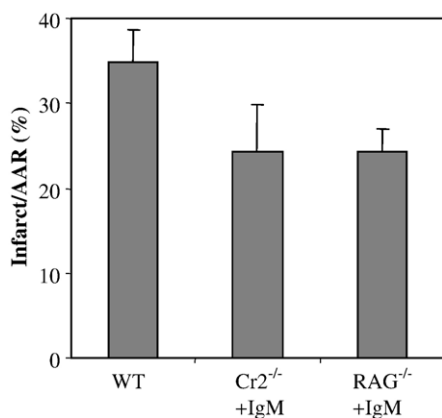


Fig. 2. Restoration of myocardial reperfusion injury in Cr2<sup>-/-</sup> and RAG1<sup>-/-</sup> mice by WT IgM. Animals were reconstituted with 400 µg WT IgM by intravenous injection 30 min prior to myocardial I/R surgery. Infarct size was assessed as described in Fig. 1 ( $n = 6$  per group). Results represent means  $\pm$  SEM.

reduction in infarct size in both Cr2<sup>-/-</sup> and RAG1<sup>-/-</sup> mice compared with WT controls in the LAD occlusion model (Fig. 1). Moreover, neutrophil infiltration and apoptosis of cardiomyocytes were reduced in Cr2<sup>-/-</sup> versus WT mice (Figs. 3 and 4). The limited injury is due to IgM as reconstitution of the IgM deficient mice with pooled WT IgM restored full injury (Fig. 2).

One interpretation of the results is that neoepitope(s) are expressed or exposed during myocardial ischemia and recognized by specific natural IgM following reperfusion. This possibility is supported by similar findings in our studies of I/R injury in Ig deficient mice in both intestinal and hind limb models [27,28].

The molecular events leading to the exposure of self-antigen during I/R are unknown. One possibility is that organ ischemia, thus, hypoxia to cells, leads to cell membrane disturbance revealing intracellular components. It was reported that, during the earliest phase of reperfusion (minutes), cardiomyocytes could develop hypercontracture and concomitantly rupture the cellular membrane [4]. In addition, the generation of reactive oxygen species (ROS) during ischemia followed by a ROS burst

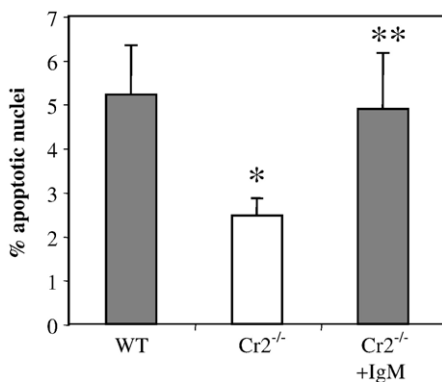


Fig. 3. Reduced apoptosis of cardiac myocytes in Cr2<sup>-/-</sup> mice and restoration of apoptosis by reconstitution of WT IgM. Apoptosis frequencies of cardiac myocytes were examined after 6 h of reperfusion. Numbers of experimental animals: WT = 11, Cr2<sup>-/-</sup> = 7, Cr2<sup>-/-</sup> + IgM = 4 ( $*P < 0.01$  comparing Cr2<sup>-/-</sup> with WT mice,  $**P < 0.05$  comparing Cr2<sup>-/-</sup> mice with or without WT IgM). Results represent means  $\pm$  SEM.

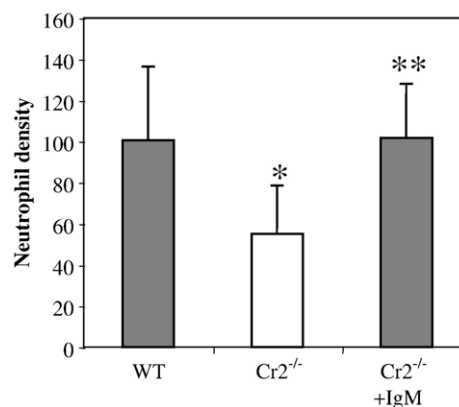


Fig. 4. Decreased neutrophil infiltration in Cr2<sup>-/-</sup> mice and restored by WT IgM reconstitution. Neutrophil density in the infarct was quantified after 24 h reperfusion. Numbers of experimental mice: WT = 11, Cr2<sup>-/-</sup> = 7, Cr2<sup>-/-</sup> + IgM = 4 ( $*P < 0.01$  comparing Cr2<sup>-/-</sup> with WT mice,  $**P < 0.05$  comparing Cr2<sup>-/-</sup> mice with or without WT IgM). Results represent means  $\pm$  SEM.

during reperfusion induces lipid peroxidation [39] and alteration of cytoskeletal structures [40], thus, possibly exposes specific self-antigen. Alternatively, apoptosis during ischemia could expose self-antigen resulting in binding of IgM in reperfusion.

How natural IgM induces direct apoptosis in reperfused myocardium remains unclear; however, IgM-dependent apoptosis was also observed in normal and tumor epithelial cells [41,42]. Presumably in the latter two cases, cell death pathway was triggered by direct IgM binding to a neoepitope on the cell membrane.

Acute inflammation could be induced by IgM–antigen interaction through activation of the complement system which produces the potent anaphylatoxins C3a and C5a, activating mast cells [43], and neutrophils [2,11]. In a recent report, Krijnen et al. showed that IgM colocalizes with complement and C reactive protein in infarcted human myocardium, indicating that IgM targets complement locally to jeopardized cardiomyocytes after acute myocardial infarction [44]. Evidence of complement activation in myocardial I/R includes deposition of terminal complement complex within reperfused heart tissues [45,46] and reduction in myocardial injury in complement deficient animals [47] and in animals treated with complement inhibitors [48,49].

Recent studies also suggested a role lectin pathway of complement in both intestinal [50] and myocardial [51] I/R models. In those reports, inhibition of mannose binding lectin (MBL) blocked complement activation and led to a reduction in I/R injury. Given our results with IgM and the observation that MBL binds IgM [19,20], a unifying model explaining both sets of results is that IgM first recognizes altered cell surface providing a binding site for MBL which subsequently leads to activation of the lectin-complement pathway. Indeed, preliminary results identifying IgM binding to ischemic intestinal tissue in MBL-a/c double knockout mice support this speculation (Zhang and Carroll, unpublished observation).

In summary, the current study supports a novel mechanism of myocardial reperfusion injury mediated by natural IgM, and

further understanding of this mechanism may help develop clinical strategies for ischemic heart disease.

## Acknowledgments

We thank Jennifer Pocius for excellent scientific support; Isaac Chiu for helpful discussions and critical comments. Research was supported by grants from NIH: P50 GM52585 (MCC) and HL-42550 (MLE).

## References

- [1] Garcia-Dorado D. Myocardial reperfusion injury: a new view. *Cardiovasc Res* 2004;61:363–4.
- [2] Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res* 2002;53:31–47.
- [3] Eltzschig HK, Collard CD. Vascular ischaemia and reperfusion injury. *Br Med Bull* 2004;70:71–86.
- [4] Piper HM, Abdallah Y, Schafer C. The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovasc Res* 2004;61:365–71.
- [5] Schulz R, Kelm M, Heusch G. Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004;61:402–13.
- [6] Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, et al. Role of apoptosis in reperfusion injury. *Cardiovasc Res* 2004;61:414–26.
- [7] Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004;61:461–70.
- [8] Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—A target for cardioprotection. *Cardiovasc Res* 2004;61:372–85.
- [9] Armstrong SC. Protein kinase activation and myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004;61:427–36.
- [10] Szabo G, Liaudet L, Hagl S, Szabo C. Poly(ADP-ribose) polymerase activation in the reperfused myocardium. *Cardiovasc Res* 2004;61:471–80.
- [11] Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res* 2004;61:481–97.
- [12] Hill JH, Ward PA. The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats. *J Exp Med* 1971;133:885–900.
- [13] Weisman HF, Bartow T, Leppo MK, Marsh Jr HC, Carson GR, Concino MF, et al. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 1990;249:146–51.
- [14] Park JL, Lucchesi BR. Mechanisms of myocardial reperfusion injury. *Ann Thorac Surg* 1999;68:1905–12.
- [15] Hart ML, Walsh MC, Stahl GL. Initiation of complement activation following oxidative stress. In vitro and in vivo observations. *Mol Immunol* 2004;41:165–71.
- [16] Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore Jr FD. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia–reperfusion in the rat. *J Immunol* 1992;149:1723–8.
- [17] Lindsay TF, Hill J, Ortiz F, Rudolph A, Valeri CR, Hechtman HB, et al. Blockade of complement activation prevents local and pulmonary albumin leak after lower torso ischemia–reperfusion. *Ann Surg* 1992;216:677–83.
- [18] Carroll MC. The role of complement and complement receptors in induction and regulation of immunity. *Annu Rev Immunol* 1998;16:545–68.
- [19] Nevens JR, Mallia AK, Wendt MW, Smith PK. Affinity chromatographic purification of immunoglobulin M antibodies utilizing immobilized mannan binding protein. *J Chromatogr* 1992;597:247–56.
- [20] Arnold JN, Wormald MR, Suter DM, Radcliffe CM, Harvey DJ, Dwek RA, et al. Human serum IgM glycosylation: identification of glycoforms that can bind to mannan-binding lectin. *J Biol Chem* 2005;280:29080–7.
- [21] Weiser MR, Williams JP, Moore FD, Kobzik L, Ma M, Hechtman HB, et al. Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J Exp Med* 1996;183:2343–8.
- [22] Williams JP, Pechet TT, Weiser MR, Reid R, Kobzik L, Moore FD, Carroll MC, Hechtman HB. Intestinal reperfusion injury is mediated by IgM and complement. *J Appl Physiol* 1999;86:938–42.
- [23] Ahearn JM, Fischer MB, Croix D, Goerg S, Ma M, Xia J, et al. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. *Immunity* 1996;4:251–62.
- [24] Molina H, Holers VM, Li B, Fung Y, Mariathasan S, Goellner J, et al. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc Natl Acad Sci USA* 1996;93:3357–61.
- [25] Fleming SD, Shea-Donohue T, Guthridge JM, Kulik L, Waldschmidt TJ, Gipson MG, et al. Mice deficient in complement receptors 1 and 2 lack a tissue injury-inducing subset of the natural antibody repertoire. *J Immunol* 2002;169:2126–33.
- [26] Reid RR, Woodcock S, Shimabukuro-Vornhagen A, Austen Jr WG, Kobzik L, Zhang M, et al. Functional activity of natural antibody is altered in Cr2-deficient mice. *J Immunol* 2002;169:5433–40.
- [27] Zhang M, Austen Jr WG, Chiu I, Alicot EM, Hung R, Ma M, et al. Identification of a specific self-reactive IgM antibody that initiates intestinal ischemia/reperfusion injury. *Proc Natl Acad Sci USA* 2004;101:3886–91.
- [28] Austen Jr WG, Zhang M, Chan R, Friend D, Hechtman HB, Carroll MC, et al. Murine hindlimb reperfusion injury can be initiated by a self-reactive monoclonal IgM. *Surgery* 2004;136:401–6.
- [29] Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, et al. Myocardial ischemia and reperfusion: a murine model. *Am J Physiol* 1995;269:H2147–54.
- [30] Nossuli TO, Lakshminarayanan V, Baumgarten G, Taffet GE, Ballantyne CM, Michael LH, et al. A chronic mouse model of myocardial ischemia–reperfusion: essential in cytokine studies. *Am J Physiol, Heart Circ Physiol* 2000;278:H1049–55.
- [31] Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 1992;68:869–77.
- [32] Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, et al. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. *Am J Pathol* 2004;164:665–77.
- [33] Briaud SA, Ding ZM, Michael LH, Entman ML, Daniel S, Ballantyne CM. Leukocyte trafficking and myocardial reperfusion injury in ICAM-1/P-selectin-knockout mice. *Am J Physiol, Heart Circ Physiol* 2001;280:H60–7.
- [34] Kurrelmeyer KM, Michael LH, Baumgarten G, Taffet GE, Peschon JJ, Sivasubramanian N, et al. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. *Proc Natl Acad Sci USA* 2000;97:5456–61.
- [35] Misra A, Haudek SB, Knuefermann P, Vallejo JG, Chen ZJ, Michael LH, et al. Nuclear factor-kappaB protects the adult cardiac myocyte against ischemia-induced apoptosis in a murine model of acute myocardial infarction. *Circulation* 2003;108:3075–8.
- [36] Engel D, Peshock R, Armstrong RC, Sivasubramanian N, Mann DL. Cardiac myocyte apoptosis provokes adverse cardiac remodeling in transgenic mice with targeted TNF overexpression. *Am J Physiol, Heart Circ Physiol* 2004;287:H1303–11.
- [37] Engler RL, Dahlgren MD, Peterson MA, Dobbs A, Schmid-Schonbein GW. Accumulation of polymorphonuclear leukocytes during 3-h experimental myocardial ischemia. *Am J Physiol* 1986;251:H93–H100.
- [38] Jolly SR, Kane WJ, Hook BG, Abrams GD, Kunkel SL, Lucchesi BR. Reduction of myocardial infarct size by neutrophil depletion: effect of duration of occlusion. *Am Heart J* 1986;112:682–90.
- [39] Nanobashvili J, Neumayer C, Fugl A, Punz A, Blumer R, Prager M, et al. Ischemia/reperfusion injury of skeletal muscle: plasma taurine as a measure of tissue damage. *Surgery* 2003;133:91–100.
- [40] VanWinkle WB, Snuggs M, Miller JC, Buja LM. Cytoskeletal alterations in cultured cardiomyocytes following exposure to the lipid peroxidation product, 4-hydroxynonenal. *Cell Motil Cytoskeleton* 1994;28:119–34.

- [41] Vollmers HP, Brandlein S. Death by stress: natural IgM-induced apoptosis. *Methods Find Exp Clin Pharmacol* 2005;27:185–91.
- [42] Li CX, Wan YH, Chi SM, Wang G, Sun LC, Zhang YG, et al. Purification of natural antikeratin autoantibodies from normal human serum and their effect on human keratinocytes cultured in vitro. *Br J Dermatol* 2001;145:737–48.
- [43] Abonia JP, Friend DS, Austen Jr WG, Moore Jr FD, Carroll MC, Chan R, et al. Mast cell protease 5 mediates ischemia–reperfusion injury of mouse skeletal muscle. *J Immunol* 2005;174:7285–91.
- [44] Krijnen PA, Ciurana C, Cramer T, Hazes T, Meijer CJ, Visser CA, et al. IgM colocalises with complement and C reactive protein in infarcted human myocardium. *J Clin Pathol* 2005;58:382–8.
- [45] Schafer H, Mathey D, Hugo F, Bhakdi S. Deposition of the terminal C5b-9 complement complex in infarcted areas of human myocardium. *J Immunol* 1986;137:1945–9.
- [46] Robert-Offerman SR, Leers MP, van Suylen RJ, Nap M, Daemen MJ, Theunissen PH. Evaluation of the membrane attack complex of complement for the detection of a recent myocardial infarction in man. *J Pathol* 2000;191:48–53.
- [47] Kilgore KS, Park JL, Tanhehco EJ, Booth EA, Marks RM, Lucchesi BR. Attenuation of interleukin-8 expression in C6-deficient rabbits after myocardial ischemia/reperfusion. *J Mol Cell Cardiol* 1998;30:75–85.
- [48] Vakeva AP, Agah A, Rollins SA, Matis LA, Li L, Stahl GL. Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: role of the terminal complement components and inhibition by anti-C5 therapy. *Circulation* 1998;97:2259–67.
- [49] Roos A, Ramwadhoebe TH, Nauta AJ, Hack CE, Daha MR. Therapeutic inhibition of the early phase of complement activation. *Immunobiology* 2002;205:595–609.
- [50] Hart ML, Ceonzo KA, Shaffer LA, Takahashi K, Rother RP, Reenstra WR, et al. Gastrointestinal ischemia–reperfusion injury is lectin complement pathway dependent without involving C1q. *J Immunol* 2005;174:6373–80.
- [51] Walsh MC, Bourcier T, Takahashi K, Shi L, Busche MN, Rother RP, et al. Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. *J Immunol* 2005; 175:541–6.