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Atherosclerosis and Leukocyte-Endothelial Adhesive Interactions are Increased Following Acute Myocardial Infarction in Apolipoprotein E Deficient Mice

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Abstract

Objective—To determine the effect of myocardial infarction (MI) on progression of atherosclerosis in apolipoprotein E deficient (*ApoE*^{−/−}) mice.

Methods and Results—MI was induced following left anterior descending coronary artery (LAD) ligation in wild-type (WT) (n=9) and *ApoE*^{−/−} (n=25) mice. Compared to sham-operated animals, MI mice demonstrated increased intravascular leukocyte rolling and firm adhesion by intravital microscopy, reflecting enhanced systemic leukocyte-endothelial interactions. To determine if MI was associated with accelerated atherogenesis, LAD ligation was performed in *ApoE*^{−/−} mice. Six weeks following surgery, atherosclerosis was quantitated throughout the arterial tree by microdissection and Oil- Red-O staining. There was 1.6-fold greater atherosclerosis burden present in *ApoE*^{−/−} MI mice compared to sham-operated mice.

Conclusions—Acute MI accelerates atherogenesis in mice. These results may be related to the increased risk of recurrent ischemic coronary events following MI in humans.

Keywords

Myocardial infarction; atherosclerosis; TNF α ; *ApoE*^{−/−}; intravital microscopy

Introduction

Patients who survive a myocardial infarction (MI) are at increased risk of subsequent ischemic events for weeks to months following the acute infarction (1,2). This peri-infarct risk period is associated with biomarker evidence of a systemic pro-inflammatory state as demonstrated by higher levels of C-reactive protein, serum amyloid A, and tumor necrosis factor alpha (TNF- α) (3,4). This apparent inflammatory response to acute myocardial injury may trigger a period of accelerated atherosclerotic lesion growth and/or plaque instability (5). However, the mechanism(s) by which acute myocardial infarction predisposes to recurrent ischemic cardiac events is unclear. The purpose of this study was to determine the effect of acute myocardial infarction on the progression of atherosclerosis in *ApoE*^{−/−} mice.

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Methods

Mice

Male wild type (WT) and *ApoE*^{-/-} mice of the C57BL/6J background strain were used. WT mice were maintained on a normal chow diet (LabDiet 5001, 57.9% Kcal from carbohydrates, 28.5% Kcal from protein, and 13.5% Kcal from fat) (6) throughout the study. *ApoE*^{-/-} mice were placed on a Western diet (Teklad TD88137, 42% Kcal from fat, 42.7% Kcal from carbohydrate, 15.2% Kcal from protein, and 0.2% cholesterol) at 8 weeks of age, then switched to normal chow for 3 days prior to surgical treatment at 11 weeks of age and continued on normal chow for 2 weeks following surgery. These mice were then switched to the Western diet until sacrifice, 4 weeks later. The Western diet was used to produce a robust atherosclerosis phenotype in a short period of time in *ApoE*^{-/-} mice. The switch to normal chow in the perioperative period was done to aid in recovery following the operation. This is based on previous experience from our laboratory that severe hyperlipidemia may promote inflammatory reactions at wound sites and impair healing. All procedures complied with the Principles of Laboratory and Animal Care established by the National Society for Medical Research and were approved by the University of Michigan Committee on Use and Care of Animals.

Myocardial Infarct Model

Eleven week old male WT (n=14) and *ApoE*^{-/-} (n=39) mice were subjected to either LAD ligation or sham surgery. LAD ligation and sham surgery was performed as described previously (7,8). Ischemia was confirmed by blanching downstream of the ligation and by persistent ST segment elevation on the electrocardiogram.

Quantification of Infarct Size

Echocardiography—Echocardiography was performed as described previously 5 weeks following surgery (9,10).

Histology—To measure infarct size, the mouse hearts were embedded in paraffin and cut transversally into 8, 0.75 mm thick slices. 4 µm thick sections were then cut and mounted on positively charged glass slides. Sections were stained with hematoxylin/eosin and Masson trichrome. Infarct size was determined using a midline length measurement technique as described previously (10). For assessments of the aortic roots, hearts were embedded in paraffin and 5 µm thick cross sections were cut and stained with anti-mouse Mac3 monoclonal antibody (BD Biosciences). Mac3-positive lesion area was quantitated using Image-Pro Plus software (11).

Intravital Microscopy

Intravital microscopy was used to characterize leukocyte-endothelial interactions in the microcirculation of the cremaster muscle in WT and *ApoE*^{-/-} mice 6 weeks following LAD ligation. This technique was performed as described previously (12). Rolling leukocytes were defined as leukocytes that rolled at a velocity slower than red blood cells. Firm leukocyte adhesion was detected if leukocytes remained stationary for 30 seconds or longer (12).

Atherosclerosis Quantification

Six weeks following induction of MI or sham protocol, *ApoE*^{-/-} mice were euthanized and total arterial tree atherosclerosis was quantitated as described previously (6). Quantification of total aortic root atherosclerosis was performed as described previously (11).

TNF- α and Cholesterol Measurements

Serum was prepared from retro-orbital blood samples obtained from *ApoE*^{-/-} mice 2 and 6 weeks following surgery. TNF- α and cholesterol levels were measured as previously described at sacrifice (6).

Statistical Analysis

Values are expressed as mean $\pm$ SEM. The statistical significance of differences between groups was determined by two tailed Student's *t* test. In our analysis of serum TNF- α levels, and aortic root measures, a one tailed Student's *t* test was used, as our hypothesis anticipated an increase in these measures following myocardial infarction surgery. Values of *P*<0.05 were considered significant.

Results

Mortality and Body Weight

All sham-operated mice survived the entire protocol. Of the WT and *ApoE*^{-/-} mice that underwent LAD ligation surgery, 5/10 (50%) and 13/25 (52%), respectively, survived during the 6 week period of the study. Observed mortality was similar to other published studies of myocardial infarction in mice (13,14). 15 deaths were due to left ventricle (LV) rupture within 4-6 days following surgery and 2 deaths were due to development of congestive heart failure 2-3 weeks following surgery. There were no differences in body weight between sham and MI groups of WT and *ApoE*^{-/-} mice at time of surgery or at sacrifice (data not shown).

Echocardiography and histological assessment of MI

Mice that received LAD ligation surgery all displayed large akinetic segments of the anterior left ventricle, consistent with large anterior myocardial infarctions (Figure 1a, b). No wall motion abnormalities were observed in sham-operated mice. Left ventricular ejection fractions were 26.0 $\pm$ 6.1% in MI mice vs. 65.5 $\pm$ 5.5% in sham-operated mice, *p*=0.00003 (see Figure 1c for ECHO measurements). Histological analyses revealed average infarct size of 37 $\pm$ 1.93% of total LV area in infarcted *ApoE*^{-/-} mice.

Effect of Myocardial Infarction on Leukocyte-Endothelial Interactions

To determine whether MI affects leukocyte-endothelial interactions, the number of adherent and rolling leukocytes was measured in MI and sham-operated WT and *ApoE*^{-/-} mice. Among WT mice subjected to MI or sham surgeries (*n*=5 and *n*=4, respectively), analysis of intravital microscopy data revealed a significantly higher number of rolling leukocytes per mm of vessel length in MI animals compared to controls (8.21 $\pm$ 1.43 cells/mm in MI mice vs. 3.50 $\pm$ 1.08 cells/mm in control mice, *p*=0.030). The number of adherent cells/mm of vessel trended higher in WT MI mice compared to controls (17.18 $\pm$ 6.16 cells/mm in MI mice vs. 3.44 $\pm$ 1.41 cells/mm in control mice, *p*=0.078). Among *ApoE*^{-/-} mice subjected to MI or sham surgeries (*n*=6 and *n*=4), intravital microscopy revealed a significantly higher number of adherent leukocytes per mm of vessel length in MI animals compared to controls (20.64 $\pm$ 4.32 cells/mm in MI mice vs. 7.25 $\pm$ 2.21 cells/mm in control mice, *p*=0.014). The number of rolling leukocytes per mm of vessel length in *ApoE*^{-/-} MI animals compared to controls was not significantly different (110.37 $\pm$ 6.99 cells/mm in MI mice vs. 112.41 $\pm$ 12.06 cells/mm in control mice, *p*=0.881).

Effect of Myocardial Infarction on Development of Atherosclerosis

To test the hypothesis that MI directly contributes to atherogenesis we performed LAD ligation or sham surgery to *ApoE*^{-/-} mice. Analysis of total atherosclerotic lesion area on aortic trees revealed significantly greater atherosclerotic burden in the MI group compared with the sham group (*n*=6 and *n*=9, respectively) (Figure 2a-c). Total surface area covered by oil-red-O

positive atherosclerotic lesions was $10.37 \pm 1.12\%$ in MI mice vs. $6.16 \pm 0.61\%$ in sham-operated mice, $p=0.0043$. Atherosclerotic lesion area in aortic valves was significantly higher in MI mice compared to sham-operated mice (area per valve: 315.6 ± 60.3 vs. 158.2 ± 3.6 mm², respectively, $p<0.05$). The macrophage-rich lesion area in aortic valves was also higher in MI mice compared to sham-operated mice (Mac3-positive area per valve: 188.5 ± 31.5 vs. 100.0 ± 17.3 mm², respectively, $p=0.045$).

Effect of Myocardial Infarction on Circulating TNF- α and Cholesterol levels

ApoE^{-/-} mice that received MI had significantly higher circulating levels of TNF- α compared to sham-operated control animals at 6 weeks following operation (MI mice 61.2 ± 23.0 pg/ml vs. control mice 21.8 ± 9.5 pg/ml, $p=0.039$). Cholesterol levels were not different between *ApoE*^{-/-} MI and sham-operated mice at 2 weeks (96.9 ± 13.5 vs 96.6 ± 12.6 mg/dL) and 6 weeks (258.9 ± 4.4 vs. 268.5 ± 9.8 mg/dL) following operation.

Discussion

There is a heightened risk for recurrent coronary events following MI (1,2). The mechanisms responsible for this increased susceptibility to coronary events are unclear although a heightened pro-inflammatory state has been found to correlate with increased risk for future cardiovascular events (3,4). These clinical findings suggest that the underlying substrate for ischemic events, atherosclerosis, may be affected following MI.

To further characterize the pro-inflammatory state following MI, we first examined leukocyte-endothelial interactions in a mouse model of MI induced by LAD coronary artery ligation. Our results in WT mice demonstrated increased leukocyte rolling 6 weeks following MI. Increased leukocyte infiltration contributes to atherosclerosis and may decrease stability of advanced atherosclerotic plaques through several mechanisms (15). Thus, enhanced systemic leukocyte-endothelial interactions may represent a contributory process toward the increased risk of peri-infarct ischemic events in humans.

To determine if acute MI affected atherosclerosis, we next examined the effect of MI on the progression of atherosclerosis in atherosclerotic-prone mice. Experimental MI increased the atherosclerotic surface area by 67% compared to sham-operated mice. To our knowledge, this is the first description of a direct proatherogenic effect of acute MI. *ApoE*^{-/-} mice also showed evidence of increased leukocyte-endothelial interactions with increase in leukocyte firm attachment.

TNF- α is an important regulator of inflammatory processes (16) and is elevated for several weeks following acute myocardial infarction in humans (4) and rodents (17). Several studies have also shown an increase in local expression of TNF- α within damaged and undamaged myocardium following acute MI in rats (17,18). TNF- α promotes adhesive interactions between the endothelium and leukocytes and may contribute to atherosclerosis, especially in the setting of systemic inflammation. Since the heart is a relevant source of TNF- α in the setting of acute MI, TNF- α may contribute to the proinflammatory response following acute MI. This is consistent with a recent study implicating TNF- α as the mediator of post-MI enhanced smooth muscle cell hyperplasia in response to femoral artery wire injury (8). Our findings of increased atherosclerosis following MI were also associated with elevated levels of circulating TNF- α following MI in *ApoE*^{-/-} mice. Systemic increases in TNF- α as well as other inflammatory mediators may play an important role in the vascular consequences of MI, although causal relationships remain to be determined.

In summary, the current study demonstrates that MI leads to acceleration of atherosclerosis. These findings may explain the increased vascular risk observed in the period following acute

MI and why some therapeutic interventions are particularly efficacious at reducing recurrent events when applied in the peri-infarct setting (2). Furthermore, this mouse model of MI in *ApoE*^{-/-} mice will allow further characterization of the inflammatory mediators involved in accelerated atherosclerosis following MI.

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1C	Sham	MI	P-Value
2D-Loop			
EDV (ul)	81.4±6.7	171.9±23.3	0.0004
ESV (ul)	28.4±6.7	126.8±18.4	0.00005
EF %	65.5±5.5	26.0±6.1	0.00003
CO (ml/min)	26.2±1.3	21.7±7.8	0.36
M-Mode			
LVM (mg)	127.7±19.6	190.0±39.7	0.04
LVDd (mm)	4.3±0.2	5.4±0.4	0.002
LVDs (mm)	3.0±0.1	4.7±0.5	0.002
FS(%)	28.7±1.9	13.0±7.1	0.007

Figure 1.

Quantitation of histological and echocardiographic changes following MI in *ApoE*^{-/-} mice. (A) Representative section of heart from a sham operated *ApoE*^{-/-} mouse. (B) Representative section of heart from an *ApoE*^{-/-} mouse that received experimentally-induced MI. The infarcted area is the thinned region stained blue with Masson trichrome stain. Images represent hearts removed from mice 6 weeks following surgical treatment. (C) Echocardiography analysis of MI and sham-operated *ApoE*^{-/-} mice 5 weeks following surgery. EDV and ESV: end diastolic and systolic volume, EF: ejection fraction, CO: Cardiac output, LVM: Left ventricular mass, LVDd and LVDs: left ventricular end diastolic and systolic dimension.

Figure 2.

Effect of MI on atherosclerosis in *ApoE*^{-/-} mice. Representative en face view of aortic tree stained with Oil-Red-O of (A) sham-operated and (B) MI *ApoE*^{-/-} mice. (C) *ApoE*^{-/-} mice that received MI (solid circles) had more total atherosclerotic lesion area than sham-operated (solid diamonds) mice, black bars indicate means (P=0.0043).