Int J Clin Exp Pathol 2015;8(3):3238-3249
www.ijcep.com /ISSN:1936-2625/IJCEP0005639

Original Article

Role of CD11b$^+$Gr-1$^+$ myeloid cells in AGEs-induced myocardial injury in a mice model of acute myocardial infarction

Tongqing Yao[1], Wenbin Lu[2], Jian Zhu[2], Xian Jin[1], Genshan Ma[2], Yuepeng Wang[1], Shu Meng[1], Yachen Zhang[1], Yigang Li[1], Chengxing Shen[1]

[1]*Department of Cardiology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China;* [2]*Department of Cardiology, Zhongda Hospital Affiliated to Southeast, 89#, Dingjiaqiao Road, Nanjing 210009, China*

Received January 7, 2015; Accepted February 27, 2015; Epub March 1, 2015; Published March 15, 2015

Abstract: Aims: Polymorph neutrophils are the predominant inflammatory cells and play a crucial role on the pathogenesis of myocardial injury at the early stage of acute myocardial infarction (AMI). However, the precursors and the differentiation of neutrophils are not fully understood. Here we explored the role of CD11b$^+$Gr-1$^+$ myeloid-derived suppressor cells (MDSCs) on myocardial injury in the absence and presence of advanced glycation end-products (AGEs) in a mice model of AMI. Methods and Results: Male C57BL/6J mice were selected. Fluorescent actived cell sortor (FACS) data demonstrated significantly increased CD11b$^+$Gr-1$^+$ MDSCs both in peripheral blood circulation and in the ischemic myocardium at 24 hours post AMI. Quantitative-real-time PCR results also revealed significantly upregulated CD11b and Ly6G mRNA expression in the ischemic myocardium. AGEs treatment further aggravated these changes in AMI mice but not in sham mice. Moreover, AGEs treatment also significantly increased infarction size and enhanced cardiomyocyte apoptosis. The mRNA expression of pro-inflammatory cytokine IL-6 and iNOS2 was also significantly increased in AMI + AGEs group compared to AMI group. Conclusion: These data suggest enhanced infiltration of MDSCs by AGEs contributes to aggravated myocardial injury in AMI mice, which might be one of the mechanisms responsible for severer myocardial injury in AMI patients complicating diabetes.

Keywords: Acute myocardial infarction (AMI), inflammation, myeloid-derived suppressor cells (MDSCs), CD11b$^+$Gr-1$^+$ myeloid cells, advanced glycation end-products (AGEs), pro-inflammatory cytokines

Introduction

Acute myocardial infarction (AMI) is the leading cause of morbidity and mortality all over the world. Inflammation, as a result of AMI, is regarded as a driving force in the disease progression post AMI. Epidemiological studies have demonstrated that leukocyte counts, especially peripheral neutrophil counts, serve as an independent risk factor for coronary artery disease, and a prognostic marker of future adverse events in patients with cardiovascular disease [1]. Accumulating evidence indicates that neutrophils are activated and infiltrated into the myocardium following ischemic insult and are capable of releasing substances injurious to the myocardium [2-5]. Recent studies suggested myeloid-derived suppressor cells (MDSCs), a heterogeneous population of early myeloid progenitors including immature granulocytes, macrophages, and dendritic cells at different stages of differentiation [6], which are broadly defined as CD11b$^+$Gr-1$^+$ cells in mice, might play a decisive role in the pathogenesis of AMI [7].

Cardiovascular complications are the major causes of morbidity and mortality for patients with diabetes [8]. The proposed mechanisms linking accelerated atherosclerosis and increased cardiovascular risk in this population are not fully understood. Hyperglycemia and intracellular metabolic changes induced oxidative stress, low-grade inflammation, endothelial dysfunction, epigenetic factors, co-existed obesity, dyslipidemia, and hypertension all account-

ed for the potential pathomechanisms [9]. Recent studies also suggest that formation of advanced glycation end-products (AGEs) in diabetic patients might contribute to the development of cardiovascular disease and serum AGEs level independently predicts obstructive coronary artery disease and the severity of coronary atherosclerosis [10]. AGEs are produced by non-enzymatic reactions between proteins and carbonyl compounds (e.g. sugars), especially during hyperglycemia [11]. The deleterious link of AGEs with diabetic vascular complications has been suggested and it is known that AGEs could induce oxidative stress and inflammation while oxidative stress and inflammation could also accelerate formation of AGEs, both leading to vessel wall injury and plaque development [12]. Thus, AGEs might be a crucial mediator between diabetes and diabetic cardiovascular complications.

The interaction between AGEs and MDSCs during myocardial injury remains largely unknown now and in this study, we explored if AGEs induced myocardial injuries were partly mediated by MDSCs and tested the hypothesis that AGEs could enhance MDSCs myocardial infiltration and increase the circulating MDSCs, thereby aggravate myocardial injury in a mouse AMI model.

Results and discussion

Animals and AMI model

AMI was generated in Male C57BL/6J mice (8-10 weeks old, n = 25) via permanent left coronary ligation as previously described [13, 14]. Briefly, mice were anesthetized using 50 mg/kg intra-peritoneal injection of sodium pentobarbital (Sigma), intubated and connected to a respirator (TKR200C Jiangxi Teli, China). According to the frequency of heartbeat and breathing on its own, we monitored the depth of anesthetic during the acute myocardial infarction surgery. The heart was quickly exteriorized through left thoracotomy and pericardial incision, descending coronary artery was permanently ligated with a silk suture (6.0). Successful establishment of AMI was suggested with a pale appearance in the anterior wall of the left ventricle. To minimize postoperative pain caused by the procedure, we used 15 mg/kg intra-peritoneal injection of sodium pentobarbital (Sigma) until death or been sacrificed. Infarction was further identified through morphological and pathological examination after been sacrificed. All mice were sacrificed in deep anesthesia with overdose sodium pentobarbital (Sigma) (130 mg/kg intraperitoneal injection). Sham operated animals (n = 12) underwent an identical surgical operation without ligating the coronary artery. AGEs (25 mg/kilogram/day via peritoneal injection for three days, Biological reagents sales company of Shanghai Anyan) or equal volume saline was applied at 24 hours before coronary ligation or sham operation. Control animals (n = 6) had no treatment.

The animal experiment was approved by the medical ethics committee of Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, and conforms to the Principles of Laboratory Animal Care (National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (NIH). Approval No: XHEC-2013F-017.

Single-cell preparations for FACS analysis and sorting

Bone marrow-derived cells (BMDCs) from the femurs of euthanized mice were flushed with ice-cold PBS with 2% FBS (GIBCO USA) and depleted of red blood cells (RBCs) using RBC lysing buffer (BD Biosciences). Spleen was cut into small pieces and rubbed following single-cell filtration through 40 μm strains. Total nucleated cells in peripheral blood were isolated after erythrocyte lysis. Single-cell suspensions were made by filtrating cells through 40 μm strains. For FACS analysis, single-cell suspensions were stained with antibodies and evaluated or sorted by multi-color flow cytometry using a flow cytometer (EPICS® ALTRA, Beckman Coulter). Data were analyzed using FlowJo7 software (Tree Star). Similar protocols were used for immune cells isolated from mouse heart.

Antibodies and immunohistochemical staining

The following antibodies were used: antibody to CD11b (Abcam); PE-conjugated lineage specific antibodies (Ly6G), FITC-conjugated antibodies to CD11b; PercP-conjugated antibodies to Ly6C, and Gr1 (Ly6G and Ly6C) (all from BD Pharmingen). Texas Red-conjugated rabbit-specific secondary antibody and Texas red-conju-

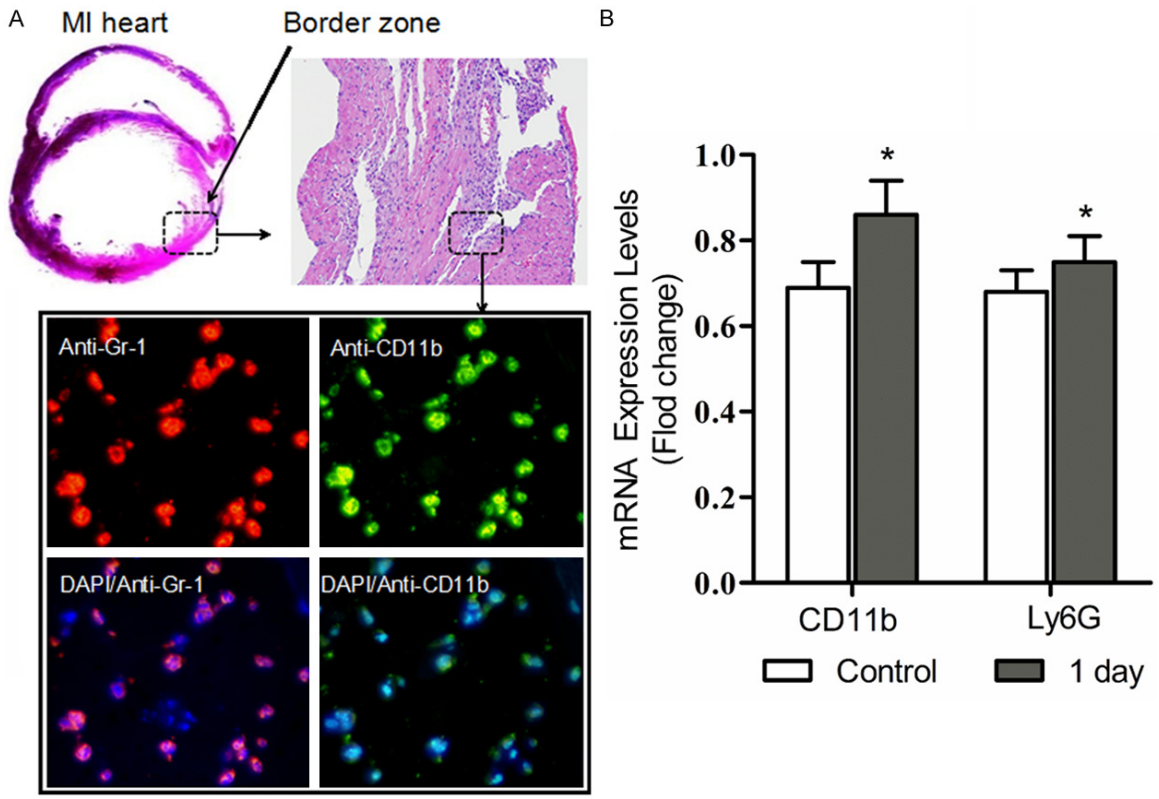

Figure 1. Mouse Myeloid cells were examined in ischemic myocardium of AMI mice. A. The immunofluorescence image of CD11b+Gr1+ MDSC at marginal zone along infarction area of 24 h post AMI mice heart (n = 6). Arrows indicate where infarct area was. Arrows indicate where infarct area was detected by immune-fluorescence staining. B. CD11b and Ly6G mRNA expression in the ischemic myocardium at 24 h post operation (n = 6) compared with sham (n = 6) (student t-tests, *$P < 0.05$ compared with control groups). AMI = acute myocardial infarction; MI = myocardial infarction.

gated rat-specific secondary antibody (Vector Laboratories) were used for immunohistochemistry analysis. Tissue samples (heart and spleen) from AMI and sham mice were fixed with 4% paraformaldehyde for 12 hours followed by 30% sucrose overnight. Fluorescence expression was directly observed using an inverted fluorescence microscope (Zeiss). Images were analyzed with Photoshop.

mRNA expression of CD11b, Ly6G, IL-6 and iNOS in ischemic myocardium of mice

mRNA levels of various factors including CD11b, Ly6G, IL-6 and iNOS were detected by RT-PCR at border zone near infarction area of mouse heart post enzymatic dissociation with 0.25% trypsin including EDTA (HyClone). Total RNA was extracted (TRIzol Reagent, Invitrogen) from the cells, and reverse transcribed using Revert Aid First Strand cDNA synthesis Kits (RT-PCR; Fermentas). Reactions were performed using Platinum SYBR Green qPCR Super Mix UDG (Applied Invitrogen) in a Mini Opticon RT-PCR System (BIO-RAD). As an internal control, levels of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were quantified in parallel with target genes. Normalization and fold changes were calculated using BIO-RAD CFX Manager. The following primers were used: Ly6G forward, 5'-GAT GGA TTT TGC GTT GCT CT G-3'; Ly6G reverse, 5'-TTG TCC AGA GTA GTG GGG CAG A-3'; CD11b forward, 5'-GTG AGC CCC ATA AAG CAG CT-3'; CD11b reverse, 5'-TCT CCA TCT GTG ATG ACA ACT AGG AT-3'; IL6 forward, 5'-CTT CCC TAC TTC ACA AGT CCG G-3'; IL6 reverse, 5'-GCC ACT CCT TCT GTG ACT CCA G-3'; iNOS forward, 5'-ATT CAC AGC TCA TCC GGT ACG-3'; iNOS reverse, 5'-CAC TTC AAC CCG AGC TCC TG-3'; GAPDH forward, 5'-GCC TCA AGA TCA TCA GCA AT-3'; GAPDH reverse, 5'-GGA CTG TGG TCA TGA GTC CT-3'.

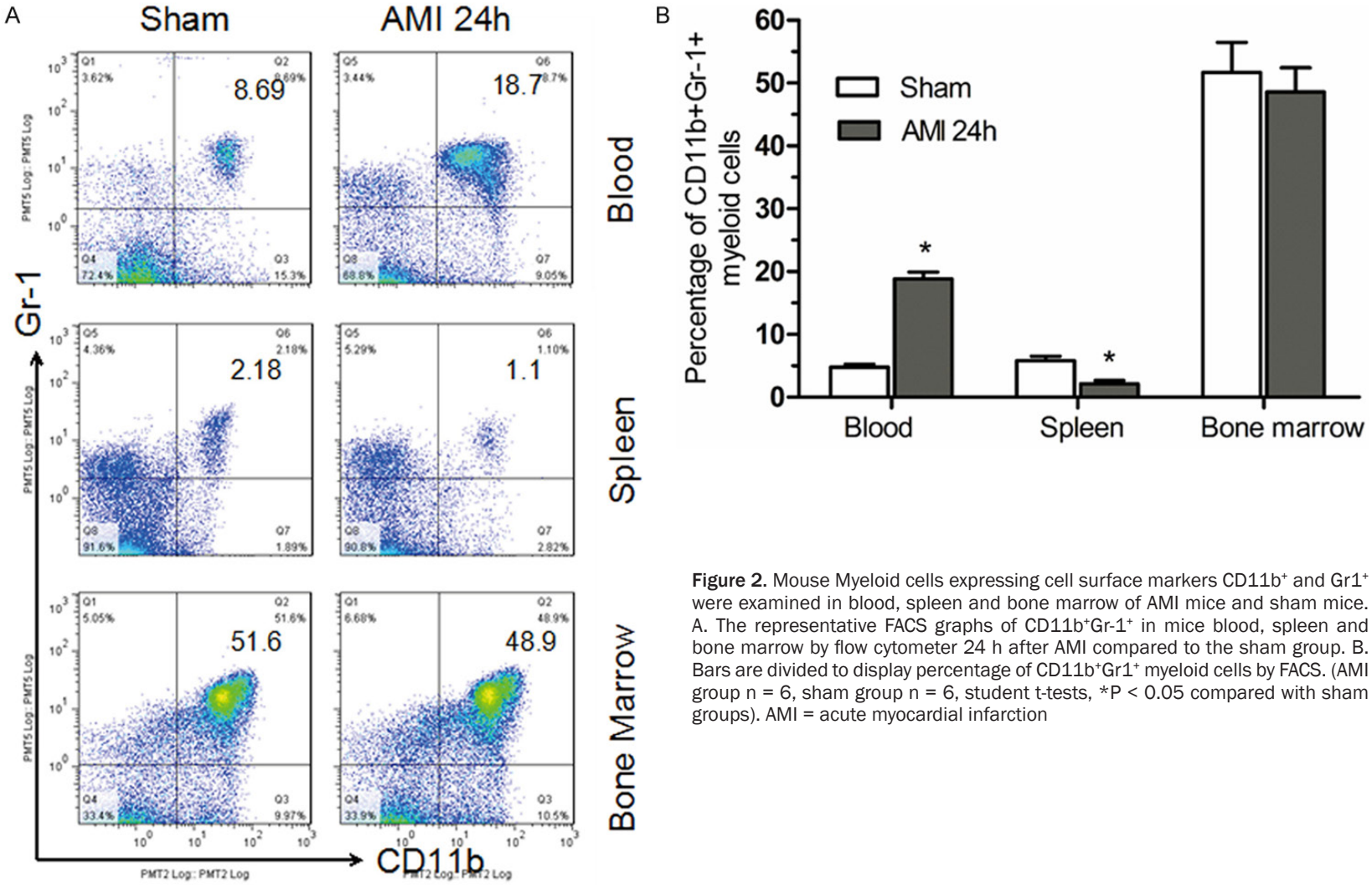

Figure 2. Mouse Myeloid cells expressing cell surface markers CD11b+ and Gr1+ were examined in blood, spleen and bone marrow of AMI mice and sham mice. A. The representative FACS graphs of CD11b+Gr-1+ in mice blood, spleen and bone marrow by flow cytometer 24 h after AMI compared to the sham group. B. Bars are divided to display percentage of CD11b+Gr1+ myeloid cells by FACS. (AMI group n = 6, sham group n = 6, student t-tests, *P < 0.05 compared with sham groups). AMI = acute myocardial infarction

TUNEL immunohistochemistry (IHC) staining for caspase3

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) was performed to detect apoptotic nuclei by using terminal deoxynucleotidyl transferase-mediated in situ fluorescein conjugated, dUTP nick end-labeling technique according to the manufacturer's protocol (Roche, Indianapolis, IN). The sections were incubated with mouse monoclonal antibody (Abcam, Inc, San Francisco, CA) to recognizing cardiac myosin heavy chain to specifically recognize apoptotic cardiomyocytes. The fluorescence staining was viewed with a confocal laser-scanning microscope. The number of apoptotic cells with TUNEL-positive nuclei was counted by 2 independent observers blinded to treatment group and expressed as a percentage of total myocyte population.

Data analysis

Quantitative data were expressed as mean ± standard deviation (SD). Student T-test was used for two groups Comparison. Multi-group comparison was performed by one-way ANOVA followed by a Tukey's test for post hoc analysis. $P < 0.05$ was considered statistically significant.

Results

Increased CD11b$^+$Gr1$^+$ myeloid cells in peripheral circulation and heart in the AMI mice

Mouse myeloid cells expressing cell surface markers CD11b$^+$ and Gr1$^+$ were examined by immune-fluorescence staining in ischemic myocardium of AMI mice at 24 h post operation (**Figure 1A**). Quantitative-RT-PCR results revealed significantly up-regulated CD11b and Ly6G (cell surface marker for granulocytes) mRNA expression in the ischemic myocardium at 24 h post AMI operation (**Figure 1B**). Taken together, these data demonstrated increased presence of CD11b$^+$Gr1$^+$ myeloid cells in the circulation and in ischemic myocardium post AMI.

To address the cellular source of infiltrated CD11b$^+$Gr-1$^+$ myeloid cells, we examined the expression pattern of multiple inflammatory cells, in particular CD11b$^+$Gr1$^+$ Myeloid cells in the peripheral blood, spleen, and bone marrow which shared the same cell surface markers with myeloid cells in AMI mice 24 h post operation. FACS data showed that the percentage of circulating CD11b$^+$Gr1$^+$ myeloid cells were significantly higher in this early stage in AMI mice than in sham mice (18.7% vs. 8.69%; $P < 0.05$; at 24 h). Data related to the origin of myeloid cells or Gr1$^+$ granulocytic cells in various tissues were as follows: the percentage of CD11b$^+$Gr1$^+$ myeloid cells in the spleen was significantly decreased (1.1% vs. 2.18%), whereas the percentage of CD11b$^+$Gr1$^+$ myeloid cells just slightly decreased in the bone marrow at 24 h post AMI (**Figure 2A** and **2B**). These results were consistent with previous study by Swirski et al [15]. The results from dynamic measurements of CD11b$^+$Gr1$^+$ myeloid cells in the blood, spleen, and bone marrow post AMI in mice are suggestive of the splenic reservoir of myeloid cells. In addition, activation and mobilization of CD11b$^+$Gr1$^+$ IMCs within the bone marrow might also contributes to the majority source of myeloid cells infiltrated in ischemic heart in this AMI mice model.

Impact of AGEs on mobilization of CD11b$^+$Gr-1$^+$ myeloid cells in AMI mice

Previous study showed that receptor of advanced glycation end-product (RAGE) deficiency markedly reduced migration of CD11b$^+$Gr1$^+$ myeloid cells in DSS-treated RAGE KO mice [16]. In this study, FACS data presented approximate four-fold increase of myeloid cells in the peripheral blood ($P < 0.05$) and the percentage of CD11b$^+$Gr1$^-$ monocytic cells slightly increased from 6.66% to 8.26% ($P > 0.05$), while CD11b$^+$Gr1$^+$ and CD11b$^+$Gr1$^-$ myeloid cells in the spleen were significantly reduced and the proportion of CD11b$^+$Gr1$^+$ and CD11b$^+$Gr1$^-$ myeloid cells remained unchanged in the bone marrow at 72 hours after the last AGEs injection in control group (**Figure 3A** and **3B**). CD11b$^+$Gr1$^+$ myeloid cells in the peripheral blood of sham+AGEs mice doubled than in sham mice (17.7% vs. 8.01% **Figure 3C** and **3D**). Furthermore, higher expression of RAGE mRNA in CD11b$^+$Ly6G$^+$ granulocytic myeloid cells were detected in the AGEs treated sham mice by Q-RT-PCR (**Figure 3E**). Pathological examination showed that the number of CD11b$^+$ myeloid cells in the heart tended to be higher in sham+AGEs group than in sham group ($P > 0.05$, **Figure 4A** and **4B**). These results suggest

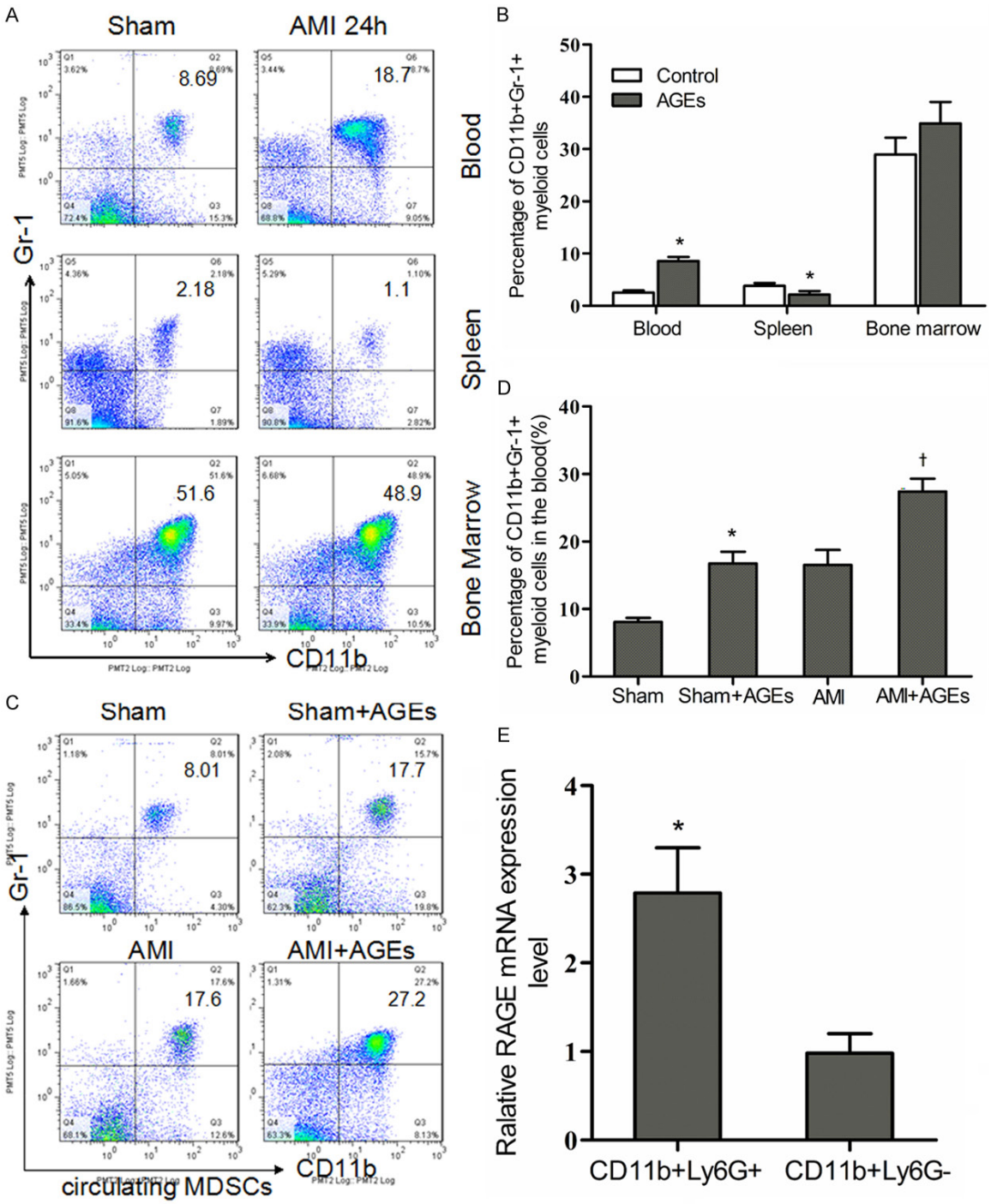

Figure 3. AGEs enhanced infiltrating of CD11b$^+$Gr1$^+$ Myeloid cells in mice. A. The representative FACS graphs of CD11b$^+$Gr-1$^+$ MDSC in mice blood, spleen and bone marrow by flow cytometer 72 hours after AGEs injection compared to the control group. B. Bars are divided to display percentage of CD11b$^+$Gr1$^+$ myeloid cells changes between control group (n = 6) and AGEs injection group (n = 6) by FACS (student t-tests, *P < 0.05 compared with control). C. The representative FACS graphs of CD11b$^+$Gr1$^+$ MDSC in mice blood of sham (n = 6), sham+AGEs (n = 6), AMI (n = 6) and AMI+AGEs (n = 6) groups by flow cytometer one day after AMI. D. Bars are divided to display percentage of CD11b$^+$Gr1$^+$ myeloid cells changes among the four groups by FACS (one-way ANOVA followed by a Tukey's test, *P < 0.05 compared with sham, †P < 0.05 compared with AMI). E. mRNA levels of RAGE in CD11b$^+$Ly6G$^+$ and CD11b$^+$Ly6G$^-$ cells of AGEs treated sham mice (n = 6) detected by real time RCR (student t-tests, *P < 0.05 compared with CD11b$^+$Ly6G$^-$). AMI = acute myocardial infarction; AGEs = advanced glycation end products; RAGE = receptor for advanced glycation end-product.

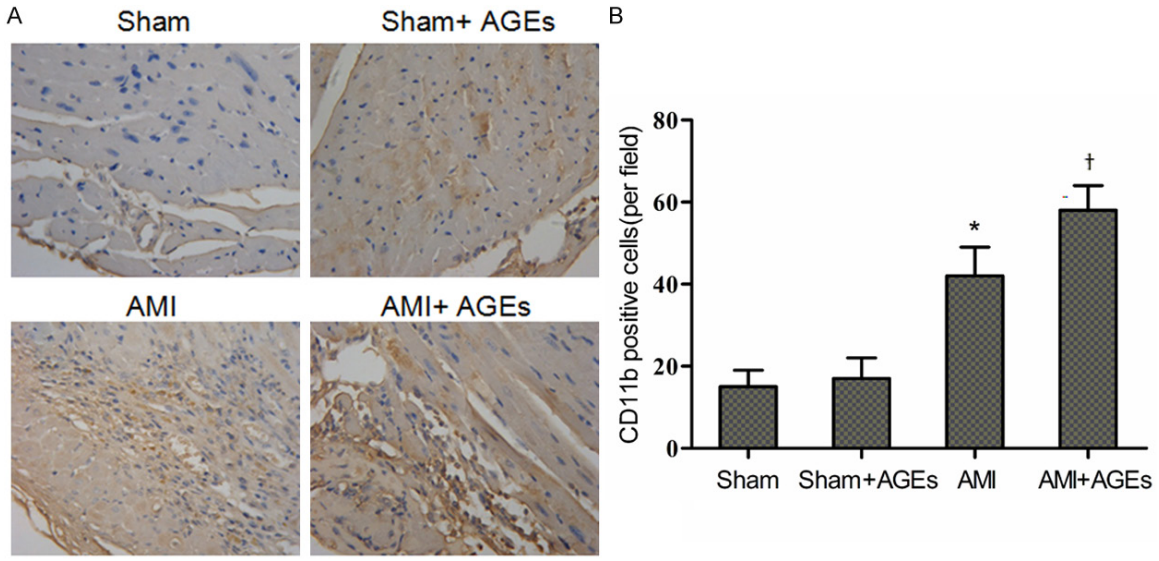

Figure 4. AGEs enhanced infiltrating of CD11b positive cells in mice. A. CD11b positive cells count by immunohistochemistry in the ischemic myocardium of sham (n = 6), sham+AGEs (n = 6), AMI (n = 6) and AMI+AGEs (n = 6) groups. B. Statistical of bar chart from immunohistochemistry (one-way ANOVA followed by a Tukey's test, *P < 0.05 VS sham group, †P < 0.05 VS AMI group). AMI = acute myocardial infarction; AGEs = advanced glycation end products.

that activation of RAGE signaling pathway might be critical in releasing of CD11b+Gr1+ myeloid cells in AGEs treated sham mice.

We further observed the impact of pretreatment with AGEs on CD11b+Gr1+ myeloid cells mobilization in AMI mice. FACS analysis demonstrated that peripheral CD11b+Gr1+ myeloid cells were significantly higher in AMI+AGEs mice than in AMI mice (27.2% vs. 17.6%, **Figure 3C** and **3D**). Markedly increased CD11b+ myeloid cells in the infarcted myocardium were evidenced in the AMI+AGEs group compared with AMI group by immunohistochemistry staining (IHC, P < 0.05 **Figure 4A** and **4B**). These data suggested that AGEs enhanced CD11b+Gr1+ immature myeloid cells (IMCs) release in blood and myocardial infiltration in AMI mice.

Increased CD11b+Gr-1+ myeloid cells by AGEs promote cardiomyocyte apoptosis and exacerbation of infarct size in AMI mice

To determine whether recruitment of CD11b+ Gr-1+ MDSCs aggravates the infarction of AMI, we applied AMI operation on mice which pretreated with AGEs injection. As we expected, decreased cardiac function was measured in the AMI plus AGEs injection group compared with single AMI group and sham group (**Figure 5A** and **5B**). Furthermore, TUNEL results demonstrated the highest number of apoptotic cardiomyocytes in AMI+AGEs group (approximately 6 times higher than sham group, 1.5 times higher compared with AMI group) while apoptotic cardiomyocytes were similar between sham and sham + AGEs groups (**Figure 5C** and **5D**). IHC staining with anti-Caspase3 evidenced significantly activated Caspase3 expression in the border zone of AMI+AGEs mice (**Figure 5E** and **5F**). Overall, these data suggest that increased myocardial CD11b+Gr1+ myeloid cells infiltration post pretreatment with AGEs might be responsible for the enhanced ischemic injury in the infarcted myocardium of AMI mice, which was possibly mediated through promoting myocardial CD11b+Gr-1+ myeloid cells infiltration in this model.

Increased pro-inflammatory cytokines and inducible nitricoxide synthase (iNOS) released by CD11b+Gr-1+ myeloid cells

To further explore the underlying mechanism of enhanced myocardial ischemic injury in this model by AGEs, we compared myocardial mRNA expression of inflammatory cytokines among various groups. Quantitative-RT-PCR detected significant increases of pro-inflammatory cyto-

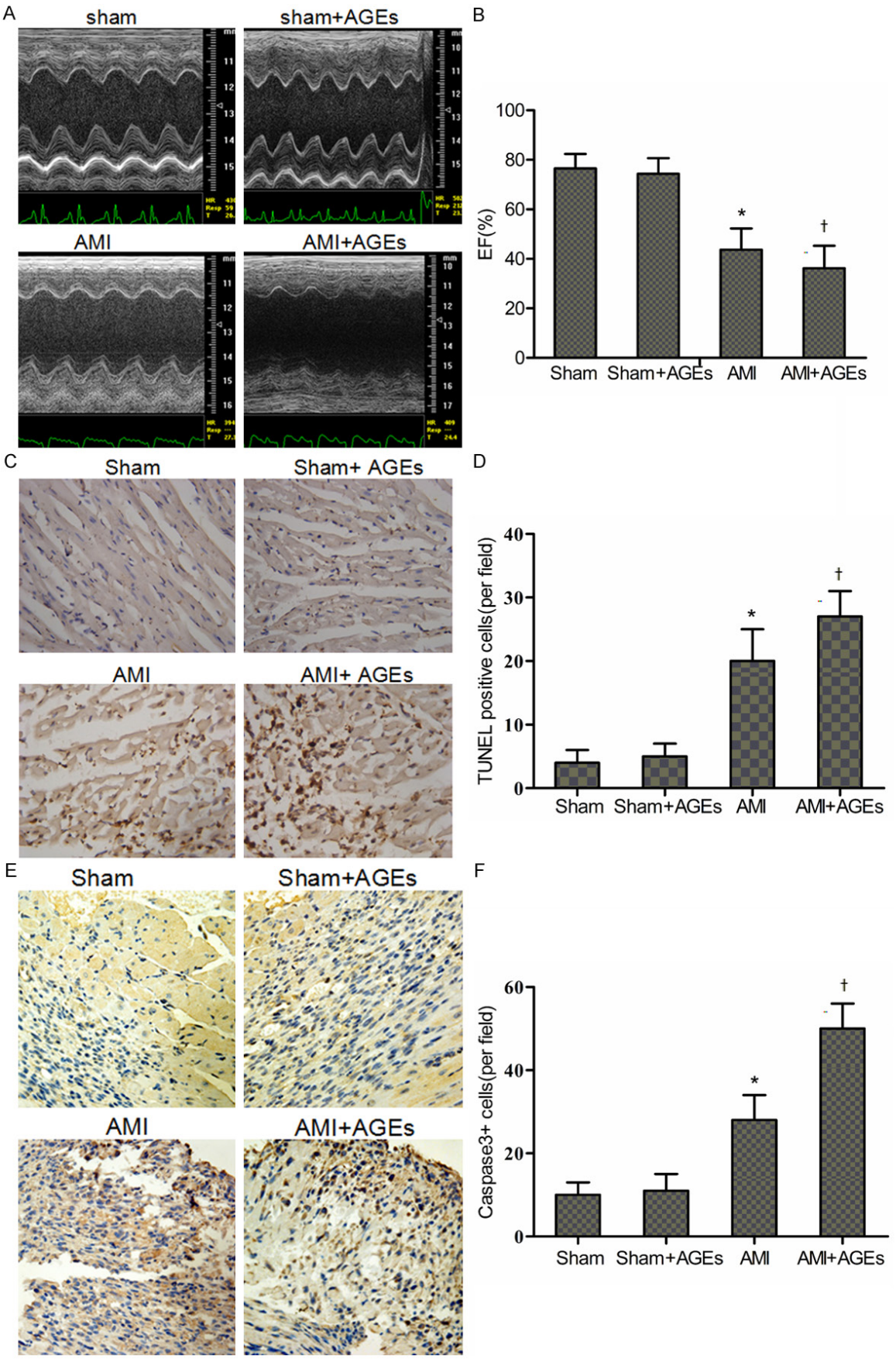
A
sham
sham+AGEs
AMI
AMI+AGEs
B
EF(%)
100
80
60
40
20
0
*
†
Sham
Sham+AGEs
AMI
AMI+AGEs
C
Sham
Sham+ AGEs
AMI
AMI+ AGEs
D
TUNEL positive cells(per field)
40
30
20
10
0
*
†
Sham
Sham+AGEs
AMI
AMI+AGEs
E
Sham
Sham+AGEs
AMI
AMI+AGEs
F
Caspase3+ cells(per field)
60
40
20
0
*
†
Sham
Sham+AGEs
AMI
AMI+AGEs

Figure 5. AGEs aggravate myocardial ischemic injury. A. Ultrasonic data showed worse ventricular Systolic function in AMI + AGEs group compared with AMI group. Images are representative of 6 mice analyzed. B. Left Ventricular Ejection Fraction was detected in sham (n = 6), sham + AGEs (n = 6), AMI (n = 6) and AMI + AGEs (n = 6) groups. (one-way ANOVA followed by a Tukey's test, EF%: 36.2 ± 9.1 vs. 43.7 ± 8.6, *$P < 0.05$, compared with sham group, †$P < 0.05$, compared with AMI group). C. TUNEL results: apoptotic cardiomyocytes were no obvious change between sham and sham + AGEs groups. markedly increased apoptotic cardiomyocytes in the infarcted myocardium in the AMI + AGEs injection group. D. Bars are divided to display the positive cells by TUNEL. Images are representative of 6 mice analyzed. (one-way ANOVA followed by a Tukey's test, *$P < 0.05$, compared with sham group, †$P < 0.05$, compared with AMI group). E. Caspase3+ cells count by immunohistochemistry sham, sham + AGEs, AMI and AMI + AGEs groups. The microscope was 400 times. F. Statistical of bar chart display the number of caspase3+ cells. Images are representative of 6 mice analyzed. (one-way ANOVA followed by a Tukey's test, *$P < 0.05$, compared with sham group, †$P < 0.05$, compared with AMI group). AMI = acute myocardial infarction; AGEs = advanced glycation end products; EF = Ejection Fraction; TUNEL = Terminal deoxynucleotidy ltransferase-mediated dUTP nick-end labeling.

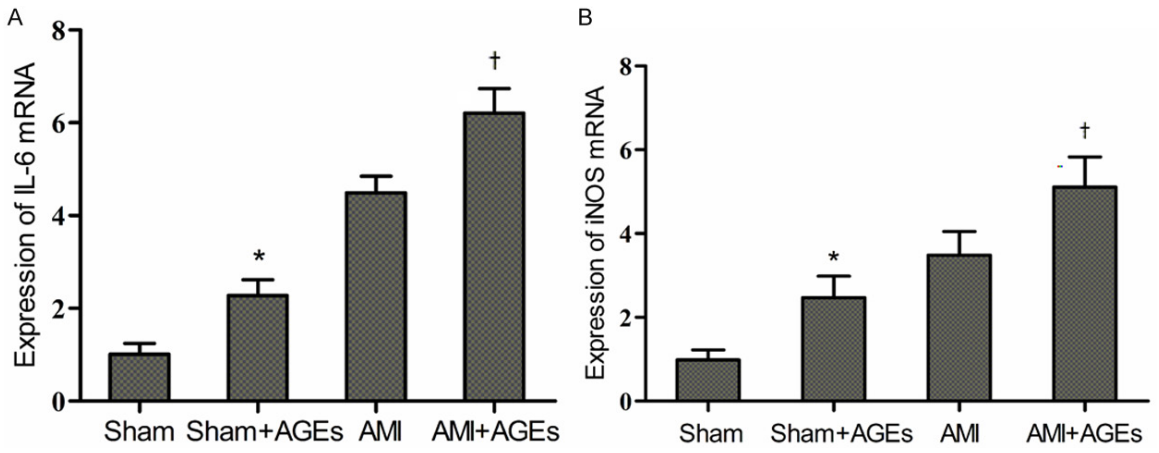

Figure 6. AGEs increased pro-inflammatory cytokines IL-6 and iNOS releasing. A and B. mRNA levels of IL-6 in myocardium of sham (n = 6) and sham + AGEs (n = 6) mice, or in infarction myocardium of AMI (n = 6) and AMI + AGEs (n = 6) mice, detected by real time RCR. mRNA levels of IL-6 in infarction heart of AMI mice detected by real time RCR (one-way ANOVA followed by a Tukey's test, *$P < 0.05$, compared with sham group, †$P < 0.05$, compared with AMI group). AMI = acute myocardial infarction; AGEs = advanced glycation end products; iNOS = inducible nitricoxide synthase.

kine IL-6 and iNOS mRNA at 24 h post AMI in ischemic myocardium which were further enhanced in AMI+AGEs group (**Figure 6A** and **6B**). These data suggested that increased myocardial CD11b+Gr1+ myeloid cells infiltration in AMI mice post AGEs could further promote releasing of pro-inflammatory cytokines and iNOS, and subsequently enhance cardiomyocytes apoptosis.

Discussion

The major findings of our study include that there are significantly increased CD11b+Gr1+ myeloid cells in the peripheral circulation and in the heart at 24 hours post AMI, this phenomenon is joined by significantly upregulated CD11b and Ly6G mRNA expression in the ischemic myocardium. Moreover, pretreatment with AGEs significantly enhanced these changes and aggravated the ischemic injury and cadiomyocytes apoptosis in AMI mice possibly through up-regulating RAGE signaling pathway and myocardial expression of pro-inflammatory cytokines and iNOS. These results suggested enhanced infiltration of myeloid cells plays a pivotal role in the pathogenesis of AMI. Moreover, pretreatment with AGEs further aggravated ischemic injury in this AMI mice model via enhancing infiltration of myeloid cells both in peripheral circulation and in the ischemic myocardium and AGEs mediated pathogenic responses might be responsible for the clinically observed severer myocardial injury in AMI patients complicating with diabetes.

The majority of current MDSC studies have focused on their role on tumor associated immunosuppression, acute and chronic inflammation or infection and so on [17-19], while the

pathogenic role of these immature myeloid cells in cardiovascular diseases remains largely unknown. In the current study, we showed that CD11b$^+$Gr1$^+$ myeloid cells were significantly increased in the blood and in the ischemic myocardium in AMI mice. Together with upregulated CD11b and Ly6G mRNA expression in the ischemic myocardium, these data collectively suggested a potential role of CD11b$^+$Gr1$^+$ myeloid cells in the ischemic pathogenesis of AMI. Previous studies demonstrated that neutrophils and monocytes/macrophages were the predominant inflammatory cells in response to AMI in the acute and subacute stages after AMI [20, 21]. Myeloid cells can be divided into two major subsets according to differentiation antigen Gr1 and CD11b: granulocytic myeloid cells with the phenotype of CD11b$^+$Ly6G$^+$Ly6C^{low} and monocytic myeloid cells with the phenotype of CD11b$^+$Ly6G$^-$Ly6C^{high} [22]. In our study, the expression of CD11b and Ly6G mRNA was examined in the infarcted myocardium. We found the mRNA of CD11b$^+$ and Ly6G$^+$ were significantly upregulated in the ischemic myocardium. CD11b$^+$Ly6G$^+$ myeloid cells were mobilized and recruited to the infarcted myocardium in the acute stage of AMI, acting as the primary infiltrated myeloid cells and leading to inflammatory response in infarcted area. Since the inflammatory role of CD11b$^+$Ly6G$^+$ myeloid cells are related to oxidative burst, secretion of proteolytic enzymes, and promotion of cardiomyocyte death [23, 24], our finding is consistent with the recent studies showing that the Ly6G$^+$ is increased in circulation of AMI mice [25] and increased CD11b$^+$Ly6G$^+$ myeloid cells in both peripheral circulation and ischemic myocardium contributed directly to the ischemic and inflammatory process post AMI.

Another novel finding of this study is that pretreatment with AGEs further aggravated ischemic injury in this AMI mice model via enhancing infiltration of myeloid cells in both in peripheral circulation and in the ischemic myocardium, and increased CD11b$^+$Gr-1$^+$ myeloid cells by AGEs also promoted cardiomyocyte apoptosis and increased infarct size in AMI mice, AGEs also upregulated pro-inflammatory cytokine IL-6 and iNOS mRNA at 24 h post AMI. All these findings are suggestive for the potential pathogenic role of AGEs in ischemic heart diseases and might be responsible for the clinically observed severer and diffused coronary vessel diseases in AMI patients complicating diabetes [26].

It is known that AGEs bind to monocytes via the receptor for AGEs (RAGE) [27]. Recent studies indicated that AGEs might contribute to cardiovascular disease and serum AGEs level independently predicts obstructive coronary artery disease and the severity of coronary atherosclerosis [28, 29]. Previous studies also showed that AGEs could regulate RAGE and intratumor RAGE contributed to accumulation of MDSCs and tumor progress [30-32]. In our study, we evidenced significantly upregulated RAGE-mRNA in CD11b$^+$Ly6G$^+$ cells in ischemic myocardium. We further found that infarction area was increased in AGEs injection group. The expression of IL-6 and iNOS was also increased in ischemic myocardium, which is consistent with the previous study revealing positive association between iNOS and IL-6 andMDSCs in various disease conditions [33, 34]. Thus, activation of RAGE signal, promoted emigration of CD11b$^+$Gr1$^+$ myeloid cells early from spleen and bone marrow post AGEs could lead to exacerbated apoptosis of myocardial cells and aggravate ischemic injury in AMI mice, and therefore contribute to the aggravated ischemic process in AMI patients complicating diabetes. In the current study, we observed significant increased myeloid cells in the peripheral blood and ischemic myocardium in this AMI mice model, however, CD11b$^+$Gr1$^+$ cells in the spleen was reduced post AMI. This phenomenon was further aggravated in AGEs pretreated AMI mice. Increased myeloid cells in the peripheral blood and ischemic myocardium and reduced myeloid cells in the bone marrow and spleen suggest that bone marrow-derived CD11b$^+$Gr1$^+$ cells were mobilized to the peripheral and myocardium after heart injury and bone marrow and spleen could serve as important IMCs reservoir. Spleen and bone marrow reservoirs liberated IMCS thus contributed to progression of AMI. The mechanisms mediated the migration of CD11b$^+$Gr1$^+$ myeloid cells from the spleen and bone marrow need incisive investigation.

In conclusion, CD11b$^+$Gr1$^+$ myeloid cells play a crucial role in the pathogenesis post AMI and AGEs further aggravated ischemic injury by increasing the peripheral and myocardial CD11b$^+$Gr1$^+$ myeloid cells and promoting

inflammatory cytokine release and cardiomyocytes apoptosis, which could partly explain the severer myocardial dysfunction and diffused coronary artery vessel diseases observed in AMI patients complicating diabetes.

Acknowlegements

This work was supported by Nature Science Foundation of China Grant 81170193.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chengxing Shen, Department of Cardiology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China. Tel: 0086-18501664545; Fax: 0021-65791783; E-mail: shencx@sjtu.edu.cn

References

[1] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998; 279: 1477-1482.

[2] Cochain C, Auvynet C, Poupel L, Vilar J, Dumeau E, Richart A, Récalde A, Zouggari Y, Yin KY, Bruneval P, Renault G, Marchiol C, Bonnin P, Lévy B, Bonecchi R, Locati M, Combadière C, Silvestre JS. The chemokine decoy receptor D6 prevents excessive inflammation and adverse ventricular remodeling after myocardial infarction. Arterioscler Thromb Vasc Biol 2012; 32: 2206-2213.

[3] Kyne L, Hausdorff J.M, Knight E, Dukas L, Azhar G, Wei JY. Neutrophilia and congestive heart failure after acute myocardial infarction. Am Heart J 2000; 139: 94-100.

[4] Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S, Yoshiyama M, Takeuchi K, Yoshikawa J, Becker AE. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 2002; 106: 2894-2900.

[5] Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. Cardiovasc Res 2004; 61: 481-497.

[6] Ueha S, Shand FH, Matsushima K. Myeloid cell population dynamics in healthy and tumor-bearing mice. Int Immunopharmacol 2011; 11: 783-788.

[7] Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, Iwamoto Y, Thompson B, Carlson AL, Heidt T, Majmudar MD, Lasitschka F, Etzrodt M, Waterman P, Waring MT, Chicoine AT, van der Laan AM, Niessen HW, Piek JJ, Rubin BB, Butany J, Stone JR, Katus HA, Murphy SA, Morrow DA, Sabatine MS, Vinegoni C, Moskowitz MA, Pittet MJ, Libby P, Lin CP, Swirski FK, Weissleder R, Nahrendorf M. Myocardial infarction accelerates atherosclerosis. Myocardial infarction accelerates atherosclerosis. Nature 2012; 487: 325-329.

[8] Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: The framingham study. Diabetes Care 1979; 2: 120-126.

[9] El-Omar MM, Yang ZK, Phillips AO, Shah AM. Cardiac dysfunction in the Goto-Kakizaki rat. A model of type II diabetes mellitus. Basic Res Cardiol 2004; 99: 133-141.

[10] Falcão-Pires I, Palladini G, Gonçalves N, van der Velden J, Moreira-Gonçalves D, Miranda-Silva D, Salinaro F, Paulus WJ, Niessen HW, Perlini S, Leite-Moreira AF. Distinct mechanisms for diastolic dysfunction in diabetes mellitus and chronic pressure-overload. Basic Res Cardiol 2011; 106: 801-814.

[11] Brownlee M, Cerami A, Vlassara H. Advanced glycation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988; 318: 1315-1321.

[12] Del Turco S, Basta G. An update on advanced glycation endproducts and atherosclerosis. Biofactors 2012; 38: 266-274.

[13] Imanishi Y, Miyagawa S, Maeda N, Fukushima S, Kitagawa-Sakakida S, Daimon T, Hirata A, Shimizu T, Okano T, Shimomura I, Sawa Y. Induced adipocyte cell-sheet ameliorates cardiac dysfunction in a mouse myocardial infarction model: a novel drug delivery system for heart failure. Circulation 2011; 124: S10-S17.

[14] Abarbanell AM, Herrmann JL, Weil BR, Wang Y, Tan J, Moberly SP, Fiege JW, Meldrum DR. Animal models of myocardial and vascular injury. J Surg Res 2010; 162: 239-249.

[15] Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, Figueiredo JL, Kohler RH, Chudnovskiy A, Waterman P, Aikawa E, Mempel TR, Libby P, Weissleder R, Pittet MJ. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 2009; 325: 612-616.

[16] Yang XD, Ai W, Asfaha. S, Bhagat G, Friedman RA, Jin G, Park H, Shykind B, Diacovo TG, Falus A, Wang TC. Histamine deficiency promotes inflammation-associated carcinogenesis through reduced myeloid maturation and accumulation of CD11b$^+$Ly6G$^+$ immature myeloid cells. Nat Med 2011; 17: 87-95.

[17] Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. Int Immunopharmacol 2011; 11: 802-807.

[18] Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009; 9: 162-174.
[19] Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008; 181: 5791-5802.
[20] Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. Circulation 2010; 121: 2437-2445.
[21] Panizzi P, Swirski FK, Figureueiredo JL, Waterman P, Sosnovik DE, Aikawa E, Libby P, Pittet M, Weissleder R, Nahrendorf M. Impaired infarct healing in atherosclerotic mice with Ly-6C (hi) monocytosis. J Am Coll Cardiol 2010; 55: 1629-1638.
[22] Zhang Y, Lv D, Kim HJ, Kurt RA, Bu W, Li Y, Ma X. A novel role of hematopoietic CCL5 in promoting triple-negative mammary tumor progression by regulating generation of myeloid-derived suppressor cells. Cell Res 2013; 23: 394-408.
[23] Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature 2012; 485: 251-255.
[24] Epelman S, Mann DL. Communication in the Heart: the Role of the Innate Immune System in Coordinating Cellular Responses to Ischemic Injury. J Cardiovasc Transl Res 2012; 5: 827-836.
[25] Sheu JJ, Sung PH, Leu S, Chai HT, Zhen YY, Chen YC, Chua C, Chen YL, Tsai TH, Lee FY, Chang HW, Ko SF, Yip HK. Innate immune response after acute myocardial infarction and pharmacomodulatory action of tacrolimus in reducing infarct size and preserving myocardial integrity. J Biomed Sci 2013; 20: 82-95.
[26] Baidoshvili A, Krijnen PA, Kupreishvili K, Ciurana C, Bleeker W, Nijmeijer R, Visser CA, Visser FC, Meijer CJ, Stooker W, Eijsman L, van Hinsbergh VW, Hack CE, Niessen HW, Schalkwijk CG. N(epsilon)-(carboxymethyl)lysine depositions in intramyocardial blood vessels in human and rat acute myocardial infarction: a predictor or reflection of infarction? Arterioscler Thromb Vasc Biol 2006; 26: 2497-2503.
[27] Li F, Cai Z, Chen F, Shi X, Zhang Q, Chen S, Shi J, Wang DW, Dong N. Pioglitazone attenuates progression of aortic valve calcification via down-regulating receptor for advanced glycation end products. Basic Res Cardiol 2012; 107: 306-319.
[28] Hodgkinson CP, Laxton RC, Patel K, Ye S. Advanced glycation end-product of low density lipoprotein activates the toll-like 4 receptor pathway implications for diabetic atherosclerosis. Arterioscler Thromb Vasc Biol 2008; 28: 2275-2281.
[29] Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products:sparking the development of diabetic vascular injury. Circulation 2006; 114: 597-605.
[30] Vernon PJ, Zeh Iii HJ, Lotze MT. The myeloid response to pancreatic carcinogenesis is regulated by the receptor for advanced glycation end-products. Oncoimmunology 2013; 2: e24184-1-e24184-3.
[31] Sinha P, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. J Immunol 2008; 181: 4666-4675.
[32] Ichikawa M, Williams R, Wang L, Vogl T, Srikrishna G. S100A8/A9 activate key genes and pathways in colon tumor progression. Mol Cancer Res 2011; 9: 133-148.
[33] Jiao ZJ, Gao JJ, Hua SH, Chen DY, Wang WH, Wang H, Wang XH, Xu HX. Correlation between circulating myeloid-derived suppressor cells and Th17 cells in esophageal cancer. World J Gastroenterol 2012; 18: 5454-5461.
[34] Lechner MG, Megiel C, Russell SM, Bingham B, Arger N, Woo T, Epstein AL. Functional characterization of human Cd33+ and Cd11b^{+} myeloid-derived suppressor cell subsets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines. J Transl Med 2011; 9: 90-109.