Bone marrow MyD88 signaling modulates neutrophil function and ischemic myocardial injury

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Feng Y, Zou I, Si R, Nagasaka Y, Chao W. Bone marrow MyD88 signaling modulates neutrophil function and ischemic myocardial injury. Am J Physiol Cell Physiol 299: C760–C769, 2010. First published July 14, 2010; doi:10.1152/ajpcell.00155.2010.—Myeloid differentiation factor 88 (MyD88), an adaptor critical for innate immune function, plays a role in neutrophil recruitment and myocardial injury after transient ischemia. However, how MyD88 signaling modulates neutrophil function and myocardial injury remains unclear. In an in vivo model of neutrophil migration and a chimeric model of MyD88 deletion, we demonstrated that Gr-1-positive (Gr-1+) neutrophil migration was significantly decreased by 68% in MyD88-deficient (Myd88−/−) mice and by 33% in knockout—wild-type (KO—WT; donor→recipient) chimeric mice, which lacked MyD88 in bone marrow cells but maintained normal MyD88 expression in the heart. This marked attenuation in neutrophil migration was associated with decreased peritoneal neutrophil CXCR2 expression and lower peritoneal KC, a neutrophil chemoattractant, in MyD88−/− mice. Moreover, in vitro, KC induces significantly more downregulation of CXCR2 expression in MyD88−/− than WT neutrophils. In an in vivo model of myocardial ischemia-reperfusion (I/R) injury, KO→WT chimeric mice had significantly smaller infarct sizes compared with the WT→WT mice. While there was a marked increase in proinflammatory cytokine/chemokine expression in the myocardium following I/R, there was no significant difference between WT→WT and KO→WT mice. In contrast, Gr-1+ neutrophil recruitment in the myocardium was markedly attenuated in MyD88−/− mice. Deletion of Toll-interleukin-1 receptor (TIR)-domain-containing adaptor protein-induced interferon-β-mediated transcription factor (Trif)-dependent (or MyD88-independent) pathways. The two distinct signaling pathways lead to the production of proinflammatory cytokines and type 1 IFN, respectively (25). All TLRs, with the exception of TLR-3, signal through a common MyD88-dependent pathway (6, 19). MyD88−/− mice (18) lack the ability to respond to lipopolysaccharide although MyD88-independent pathways (e.g., Trif-dependent) exist in TLR-4 signaling (20, 37). Trif is also critical for the TLR-3 signaling pathway (37). In mouse models of myocardial I/R injury, previous studies have established that global TLR-4- or MyD88-deficient mice have reduced myocardial neutrophil infiltration and are partially protected from myocardial I/R injury (10, 14, 29). However, the underlying mechanisms by which TLR signaling modulates neutrophil recruitment and myocardial injury remain incompletely understood. Here we tested the hypotheses 1) that MyD88 signaling controls neutrophil migratory function by modulating tissue chemoattractant production and neutrophil chemokine receptor expression and 2) that MyD88 signaling in bone marrow-derived circulating cells plays an important role in the pathogenesis of myocardial I/R injury.

METHODS

Animals. MyD88−/− mice were generated by Kawai and colleagues (18) and had been backcrossed >10 generations into the C57BL/6J strain. Trif−/− mice were generated by Yamamoto et al. (37). The animal protocol was approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital.

MyD88 chimeric mice. Six- to eight-week-old mice were lethally irradiated with a dose of 10 Gy and transplanted with total of 107 bone marrow cells isolated from either wild-type (WT) or MyD88−/− mice. There was a high mortality among MyD88−/− mice subjected to irradiation and bone marrow trans fusion even at lower dose (6 Gy) of irradiation. Thus, all recipient mice used in the current study were WT. Eight weeks later, MyD88 expression of both bone marrow-derived and myocardial cells in the recipient mice were examined and

NEUTROPHILS REPRESENT THE first line of defense against invading pathogens and play a critical role in host response in both infectious and noninfectious tissue inflammation such as ischemia-reperfusion (I/R) injury (38). Evidence from several lines of investigation suggests that inflammation is an important functional contributor to the development of ischemic myocardial injury (15, 24, 30, 38). Following I/R, there is a robust activation of multiple soluble and cellular factors including activation of endothelial cells, release of chemoattractants, and rapid recruitment of neutrophils into ischemic myocardium, all of which contribute to subsequent myocardial injury (38).
carefully characterized. For details, see Supplemental Methods and Fig. S1; Supplemental Material for this article is available online at the Journal website.

**Western blot analysis.** Homogenates of heart tissues or cell pellets were centrifuged at 12,000 g at 4°C for 30 min. Proteins were separated in 4–20% gradient SDS-PAGE and immunoblotted with MyD88 antibody (1:500 diluted) as described previously (7, 9).

**Quantitative RT-PCR.** Quantitative (q)RT-PCR was performed as described previously (8, 14). Changes in relative gene expression from the peritoneal lavage, bone marrow, and the heart, respectively, were analyzed for statistical significance. The null hypothesis was rejected as means

**Results**

**Neutrophil mobility was decreased in MyD88 knockout (KO) and KO→WT chimeric mice.** We examined the role of MyD88 signaling in neutrophil migratory function in an in vivo model of neutrophil migration. Thioglycollate was used to elicit peritonitis and neutrophil migration into the peritoneal space. As illustrated in Fig. 1, A and B, there was a marked neutrophil migration to the peritoneal space after thioglycollate injection in WT mice. Neutrophil migration was markedly decreased in MyD88−/− mice compared with WT mice (10.9 ± 1.5 × 10^6 vs. 3.5 ± 0.4 × 10^6, 68% reduction) with a decrease in the percentage of neutrophils in the peritoneal cells (66.8 ± 0.2% vs. 41.6 ± 1.7%) as gated on Gr-1 and CXC CR2 in flow cytometry. To dissect the role of MyD88 signaling in bone marrow-derived cells in controlling neutrophil migration, we examined neutrophil peritoneal recruitment in the chimeric MyD88-deficient mice. Compared with WT→WT control, KO→WT chimeric mice had 33% reduction in neutrophil recruitment (Fig. 1E). Of note, the reduction in peritoneal neutrophil recruitment in KO or KO→WT mice was not due to reduced numbers of blood neutrophils in these mice because no difference was observed in the numbers of total neutrophils among the four groups of mice (WT, 1,780 ± 77/µL; KO, 1,303 ± 157/µL; WT→WT, 1,513 ± 296/µL; KO→WT, 1,011 ± 35/µL; n = 3–5).

Taken together, these data suggest that MyD88 signaling, both that of bone marrow-derived and resident parenchymal cells, plays an important role in maintaining normal neutrophil migratory function in vivo.

**MyD88−/− mice had reduced peritoneal cytokine production.** In vivo neutrophil migration is critically affected by chemokine levels at the site of infection or injury. To test whether or not the attenuated peritoneal neutrophil migration observed in MyD88−/− mice could be the result of decreased chemokine production in the peritoneal space, we measured the chemokine and cytokine levels of peritoneal lavage after intraperitoneal injection of thioglycollate. As indicated in Fig. 1G, KC, a potent neutrophil chemoattractant and a CXC ligand for CXC CR2, and IL-6, an important proinflammatory cytokine, were significantly lower in the peritoneal lavage of MyD88−/− mice compared with that in WT mice, whereas the levels of monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 2 (MIP-2), two chemoattractants for macrophages and neutrophils, respectively, and the cytokines TNF-α and IL-10, were not significantly different between WT and MyD88−/− mice.

**CXCR2 expression on the peritoneal neutrophils was downregulated in MyD88 KO and KO→WT chimeric mice.** In addition to chemokine gradients, CXCR2 expression on neutrophils is also critical for normal neutrophil migratory function (31). We examined the impact of MyD88 deletion on CXCR2 expression on both bone marrow neutrophils and peritoneal neutrophils. MyD88−/− mice had the same levels of CXCR2 expression on their bone marrow neutrophils compared with WT mice (data not shown). This was true for both the control mice that received no treatment and the mice with thioglycollate-induced peritonitis. In contrast, there was a marked reduction (>60%) in the peritoneal neutrophil CXCR2 expression in MyD88−/− mice compared with WT mice (Fig. 2, A and B). Interestingly, compared with WT→WT control, the peritoneal neutrophil CXCR2 expression in KO→WT mice was also significantly decreased, but to a lesser degree (30%) (Fig. 2, C).
and D). Taken together, these data suggest that MyD88 signaling, both that of bone marrow-derived cells and of peritoneal resident cells, plays an important role in maintaining CXCR2 expression on neutrophils during active inflammation. The reduced CXCR2 expression may explain in part the attenuated neutrophil mobility in the MyD88−/− mice.

MyD88 signaling modulates KC-induced CXCR2 downregulation in neutrophils. Our pilot study established that bone marrow-derived neutrophils from WT and MyD88−/− mice had the same CXCR2 expression and the same in vitro chemotaxis to KC (data not shown). We hypothesized that the differential CXCR2 expression on the peritoneal neutrophils in
WT and MyD88<sup>−/−</sup> mice had developed after the neutrophils were exposed to cytokines and chemokines in the inflammatory site of the peritoneal space and that MyD88 signaling could modulate the cytokine (such as KC)-mediated CXCR2 downregulation. Cytokines (2, 21) and phagocytosis (12) are known to downregulate the plasma membrane expression of neutrophil CXCR2. We have also reported that marked CXCR2 downregulation occurs in infectious peritonitis (40). To determine whether MyD88 signaling could modulate this process, we tested KC-induced CXCR2 downregulation in both WT and MyD88<sup>−/−</sup> neutrophils in vitro. As illustrated in Fig. 3, incubation with KC induced a dose-dependent CXCR2 downregulation in neutrophils isolated from WT bone marrow. However, compared with the WT neutrophils, MyD88<sup>−/−</sup> neutrophils appeared more prone to the KC-induced downregulation and had significantly lower CXCR2 expression after exposed to 50 nM KC.

KO→WT mice had smaller MI size compared with WT→WT mice after I/R. To determine the role of circulating MyD88 in the development of myocardial ischemic injury, we subjected the four groups of mice, i.e., WT, KO, WT→WT, and KO→WT, to 30 min of ischemia and 24 h of reperfusion (I/R) and examined infarct sizes. Figure 4A shows representative photographs of AAR and MI. The ratios of AAR to left ventricle (LV) were similar among the groups (Fig. 4B). As illustrated in Fig. 4C, similar to MyD88<sup>−/−</sup> (KO) mice, WT mice transplanted with MyD88<sup>−/−</sup> bone marrow cells (KO→WT) had markedly reduced MI size compared with WT mice (20 ± 2% vs. 37 ± 2%, P < 0.01) or with the WT→WT controls (28 ± 1%, P < 0.05), suggesting that MyD88 signaling in bone marrow-derived circulating cells may contribute to myocardial injury during I/R. Surprisingly, the infarct sizes of WT→WT mice were significantly smaller than that of WT mice (28 ± 1% vs. 37 ± 2%, P < 0.05) (Fig. 4C), which may suggest that irradiation and/or bone marrow reconstitution confers protective effects in this group of chimeric animals. Both bone marrow cell transfusion (28, 36) and irradiation (22) have been reported to confer protective benefits against ischemic injury. To dissect the effect of bone marrow cell transfusion from that of irradiation, we transfused WT mice that

**Fig. 1.** Knockout (KO) and KO→wild-type (KO→WT) mice have impaired neutrophil mobility and reduced peritoneal cytokine/chemokine levels. Mice were injected intraperitoneally with thioglycollate. Ten hours after the injection, 3 ml of normal saline was injected and mixed well. The peritoneal lavage fluid and leukocytes were isolated and analyzed. A: total numbers of cells recruited into the peritoneal cavity of WT and KO mice. B: total neutrophils recruited into the peritoneal cavity of WT and KO mice. Each error bar represents mean ± SD of 3 mice. C: a representative example of flow cytometry plots of peritoneal neutrophils from WT and myeloid differentiation factor 88 (MyD88)-deficient mice (MyD88<sup>−/−</sup>). The cells were gated on CXCR2 and Gr-1. C: total neutrophils recruited into the peritoneal cavity of the chimeric mice. E: total neutrophils recruited into the peritoneal cavity of the chimeric mice. Each error bar represents mean ± SD of 3 mice. F: a representative example of flow cytometry plots of peritoneal neutrophils from WT→WT and KO→WT chimeric mice. G: peritoneal lavage was analyzed for cytokine production using Luminex multiplex immunoassay. The bars in each dot plot represent median values of the measured cytokines. Some cytokine values were overlapping. n = 4–7. MCP-1, monocyte chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2.
received no irradiation with WT bone marrow cells and 8 wk later examined the MI sizes of these mice. We found that the WT→WT mice (without irradiation) had similar MI sizes compared with the WT mice (23.0 ± 1.8% vs. 28.8 ± 3.1%, n = 5–7, P > 0.05). These data suggest that bone marrow transfusion per se does not confer cardiac protection against I/R injury and that the MI reduction observed in WT→WT mice after irradiation and bone marrow transfusion may be due to irradiation.

**Myocardial cytokine production after I/R is independent of MyD88 signaling in bone marrow-derived circulating cells.**

Myocardial inflammatory responses such as cytokine production occur during I/R and are known to contribute to myocardial injury as well as dysfunction. We tested whether MyD88 signaling in bone marrow-derived circulating cells was essential in myocardial cytokine and chemokine production after I/R. The control WT→WT chimeric mice had very robust production of IL-6, KC, MCP-1, and MIP-2 proteins in the heart following I/R. To our surprise, there was no significant difference in the cytokine levels between WT→WT and KO→WT mice (Fig. 5) or between the two groups of chimeric mice and WT mice (data not shown). These data strongly suggest that myocardial cytokine expression after I/R is independent of MyD88 signaling in bone marrow-derived circulating cells.

**MyD88−/− mice had attenuated myocardial neutrophil recruitment after I/R.**

Neutrophils play a critical role in acute myocardial injury during I/R. Neutrophil depletion leads to reduced myocardial injury after I/R (13, 30, 32). To examine whether MyD88 deficiency has any impact on myocardial neutrophil recruitment after I/R, we used flow cytometry to quantify the number of neutrophils recruited into the myocardium in response to I/R in WT and MyD88−/− mice. As indicated in Fig. 6, I/R induced a marked increase in Gr-1+ neutrophil recruitment into the myocardium, whereas the sham-operated mice had minimal neutrophils present in their myocardium. Compared with WT mice, MyD88−/− mice had a marked reduction in myocardial Gr-1+ neutrophil recruitment in response to I/R injury (P = 0.001 vs. 5.4 ± 1.2 × 10^4 vs. 2.0 ± 0.2 × 10^5). Gr-1+ neutrophils were further confirmed with flow cytometry gated for Ly-6G, which is more specific for neutrophils (see Supplemental Material for details).

**Trif signaling has no effect on myocardial neutrophil recruitment in vivo and CXCRI2 downregulation in vitro.**

To determine whether or not myocardial neutrophil recruitment after I/R and CXCR2 expression modulation were specific to MyD88-dependent pathway, we examined the effect of Trif-dependent signaling pathway. As shown in Fig. 7, A and B, systemic Trif-deficiency did not impact on myocardial neutrophil recruitment following I/R as demonstrated by Gr-1+ gated flow cytometry. In vitro, isolated Trif−/− neutrophils responded to KC treatment with a marked downregulation in CXCR2 expression, but to the similar degree as WT neutrophils (Fig. 7, C and D).

**DISCUSSION**

Several lines of evidence suggest that innate immune system components such as TLRs may play an essential role in myocardial inflammation and injury during I/R (1, 10, 14, 29). However, the underlying mechanisms of the pathological process remain incompletely understood. In this study, we investigated how MyD88 signaling modulated neutrophil function in two models of tissue inflammation, i.e., thioglycollate-elicited peritonitis and transient ischemia-induced myocardial inflammation, and its impact on myocardial injury induced by I/R. We found that neutrophil mobility was severely impaired in MyD88−/− mice and in mice deficient of MyD88 in their bone marrow-derived circulating cells. MyD88−/− mice had significantly lower CXCR2 expression on their neutrophils that migrated into the peritoneal inflammatory loci in vivo, and their neutrophils were more prone to KC-induced CXCR2 downregulation in vitro. MyD88 chimeric KO→WT mice, which lacked MyD88 in bone marrow-derived circulating cells but maintained normal MyD88 signaling in the heart, had significantly smaller MI sizes compared with WT→WT controls. In contrast, Trif-deficient mice had similar levels of neutrophil infiltration in their hearts after I/R compared with WT mice and had similar levels of neutrophil CXCR2 expression as WT after KC treatment. These data suggest that MyD88 signaling, particularly that of bone marrow-derived circulating cells, plays a critical role in maintaining normal neutrophil migratory function and CXCR2 expression and contributes to the development of myocardial I/R injury.

Our study demonstrates that MyD88 signaling plays an important role in neutrophil migratory function, which is an intricate part of the inflammatory process. MyD88−/− mice had severely impaired neutrophil mobility into the peritoneal space. MyD88 deficiency in bone marrow cells (i.e., in KO→WT mice) also led to impairment of neutrophil mobility,
although to a lesser degree, compared with MyD88$^{-/-}$ mice. These data clearly suggest that neutrophil mobility is dependent on the MyD88 signaling of both the recruited bone marrow-derived circulating cells and the peritoneal resident cells. In response to inflammatory stimuli, the peritoneal resident cells synthesize and secrete chemokines into the peritoneal space, which attract circulating neutrophils into the inflammatory site. The chemoattractant effect is known to be dependent on the chemokine gradient between the blood and the site of inflammation and injury. The markedly decreased peritoneal level of KC and IL-6 in MyD88$^{-/-}$ mice may explain in part the observed reduction in neutrophil migration into peritoneal space. Plasma membrane CXCR2 expression is also critical for neutrophil migratory function. Our finding that MyD88$^{-/-}$ or KO→WT MyD88 chimeric mice had significant reduction in CXCR2 expression on their peritoneal neutrophils strongly indicates that MyD88 signaling, either in bone marrow-derived cells or in peritoneal resident cells, controls neutrophil migratory function by maintaining neutrophil CXCR2 expression. MyD88 deficiency leads to reduced neutrophil CXCR2 expression and hence impaired neutrophil migratory function. We speculate that the impairment of neutrophil function is probably responsible for the marked reduction in myocardial neutrophil recruitment during I/R in MyD88$^{-/-}$ mice.

Our previous study has established that global MyD88 deletion confers cardiac protection against I/R injury and attenuates myocardial inflammation (14). Because myocardial inflammation is an intricate part of I/R injury that involves both myocardial tissue as well as circulating inflammatory cells, we sought to dissect the contribution of myocardial and circulating MyD88 to I/R injury. We generated the chimeric mice that specifically lacked MyD88 expression in their bone marrow-derived hematopoietic cells but maintained normal MyD88 expression in the heart. Similar chimeric models have been validated and widely used to determine the role of hematopoietic versus parenchymal TLR-MyD88 signaling in the host immune response to invading pathogens or in noninfectious tissue injury (1, 3–5, 26). The finding that KO→WT mice have significant reduction in MI sizes compared with WT→WT mice suggests that MyD88 signaling in circulating cells plays an important role in the development of I/R injury. This is consistent with our previous finding that MyD88 deficiency confers cardiac protection against I/R injury only in vivo but not in isolated heart (14). The result is also in agreement with a recent study by Arslan et al. (1), who have demonstrated that myocardial infarction size after I/R is determined by circulating leukocyte TLR-2. It is noteworthy that while MI sizes of KO→WT mice were reduced to the level of KO mice, a 46% reduction compared with WT, part of the cardiac protective benefit observed in the KO→WT chimeric mice might be due to irradiation since the WT→WT chimeric controls also had modest but significant reduction in MI sizes compared with the WT control mice. Both irradiation and bone marrow transplantation have been shown to protect against ischemic injury. Casium-137 irradiation has been recently found to have a protective preconditioning effect against kidney I/R injury via superoxide production and enhanced heat shock protein-27 expression (22). A number of studies in animals and human have demonstrated that intravenous transfusion or direct myocardial injection of bone marrow or enriched bone marrow-derived stem cells markedly improves LV function after I/R (28, 34, 36). However, in our studies, while WT→WT chimeric mice exhibited reduced MI sizes compared with WT animals, bone marrow transfusion without prior irradiation in WT mice did not achieve any MI reduction after I/R. These data suggest that bone marrow transfusion per se does not confer any protection and that the observed cardiac benefit in the WT→WT chimeric mice is probably due to irradiation, rather than bone marrow transfusion. Taken together, these chimeric studies demonstrate that lack of MyD88 signaling in bone marrow-derived circulating cells results in a significant reduction in myocardial MI and that irradiation may confer an...
additional cardiac protection against I/R injury in the chimeric mice.

Neutrophil recruitment and cytokine production are an important part of inflammatory process. Following ischemia and during the reperfusion phase, circulatory neutrophils are recruited to the myocardium in response to a number of proinflammatory parameters such as adhesion molecules and cytokines/chemokines. The recruited neutrophils may con-

Fig. 5. Bone marrow MyD88 deficiency has no impact on myocardial cytokine levels during ischemia-reperfusion (I/R). Both WT→WT and KO→WT chimeric mice were subjected to sham operation or I/R. The hearts were isolated, and myocardial cytokines were measured using Luminex multiplex immunoassay. The bars in each dot plot represent median values of the measured cytokines. Some cytokine values were overlapping. n = 5–6.

Fig. 6. MyD88−/− mice have marked attenuation in myocardial neutrophil recruitment after I/R. Twenty-four hours after 60 min of left anterior descending coronary artery (LAD) ligation, the hearts were isolated, perfused, and digested. After removal of the large cardiomyocytes through filtration, 50% of total cells were loaded onto flow cytometry and gated on Gr-1 and CXCR2. A: total Gr-1+ cells as measured by flow cytometry. Each error bar represents mean ± SD of 4 mice. A small number of neutrophils were recovered from the sham-operated hearts as indicated by the line. B: a representative example of flow cytometry plots of myocardial infiltrating cells from sham, WT-I/R, and KO-I/R mice. FSC, forward scatter; SSC, side scatter.
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Toll-interleukin-1 receptor (TIR)-domain-containing adaptor protein-inducing interferon-β-mediated transcription factor (Trif) deficiency has no impact on myocardial neutrophil recruitment and KC-induced CXCR2 downregulation. Twenty-four hours after 60 min of LAD ligation, the isolated hearts were perfused and digested. After removal of the large cardiomyocytes, 50% of total cells were loaded onto flow cytometry and gated on Gr-1 and CXCR2. A: total Gr-1$^+$ cells as measured by flow cytometry from the hearts subjected to I/R. Each error bar represents mean ± SD of 3 mice. B: a representative example of flow cytometry plots of myocardial infiltrating cells from WT-I/R and Trif-KO-I/R mice. C: accumulated data of neutrophil CXCR2 expression presented as the percentage of the control cells without KC treatment. Each error bar represents mean ± SD of 3 separate neutrophil preparations. D: a representative plot of flow cytometry illustrating neutrophil CXCR2 expression in the control cells and the cells treated with 50 nM KC from WT and Trif-KO mice.

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It is noteworthy that some bone marrow mesenchymal stem cells (MSC) might have been transplanted into the receipt mice and could become nonhematopoietic cells residing in the heart and other parenchymal tissues. The possible contribution of donor bone marrow-derived nonhe-
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matopoietic MyD88 to I/R injury cannot be ruled out and needs to be further investigated. However, the observation that WT→WT and KO→WT chimeric mice have the same myocardial cytokine production seems to suggest that at least those possible MSC-derived noncardiomyocytes have minimal contribution to the myocardial inflammation after I/R injury.

It is important to note that the neutrophil migratory function in the ischemic heart and the KC-mediated modulation of neutrophil CXCR2 expression appear to be highly dependent on and specific to MyD88 signaling. Trif, a key adaptor protein unique to TLR-3 and TLR-4 signaling pathways, seems to have little role in these processes since compared with WT mice, Trif-deficient mice have similar levels of myocardial neutrophil recruitment after I/R in vivo and the CXCR2 expression on isolated neutrophils in vitro.

Of note, thioglycollate-induced peritonitis is a widely used model to measure the migratory function of monocytes and neutrophils in response to inflammation (11, 16, 35, 39). The model is clearly different in many ways from I/R-induced myocardial inflammation, such as the cytokine profiles, the kinetics and pattern of inflammatory cell recruitment, and the causes of inflammation and injury. Thus, the peritonitis model was not used as a model for leukocyte infiltration in ischemic heart, but rather as a model to provide the mechanistic insights into how neutrophil functions were modulated. To this end, the peritonitis model provides some valuable information on the role of MyD88 signaling in regulating the neutrophil migratory function and chemokine expression.

In summary, we have demonstrated that MyD88 signaling is critical for maintaining normal neutrophil migratory function and CXCR2 expression and that MyD88 signaling in bone marrow-derived circulating cells contributes to the development of ischemic myocardial injury.

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DISCLOSURES

No conflicts of interest or otherwise, are declared by the authors.

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