Macrophages modulate cardiac function in lipotoxic cardiomyopathy

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Schilling JD, Machkovech HM, Kim AH, Schwedwener R, Schaffer JE. Macrophages modulate cardiac function in lipotoxic cardiomyopathy. Am J Physiol Heart Circ Physiol 303: H1366–H1373, 2012. First published October 5, 2012; doi:10.1152/ajpheart.00111.2012.—Diabetes is associated with myocardial lipid accumulation and an increased risk of heart failure. Although cardiac myocyte lipid overload is thought to contribute to the pathogenesis of cardiomyopathy in the setting of diabetes, the mechanism(s) through which this occurs is not well understood. Increasingly, inflammation has been recognized as a key pathogenic feature of lipid excess and diabetes. In this study, we sought to investigate the role of inflammatory activation in the pathogenesis of lipotoxic cardiomyopathy using the α-myosin heavy chain promoter-driven long-chain acylCoA synthetase 1 (MHC-ACS) transgenic mouse model. We found that several inflammatory cytokines were upregulated in the myocardium of MHC-ACS mice before the onset of cardiac dysfunction, and this was accompanied by macrophage infiltration. Depletion of macrophages with liposomal clodrolip reduced the cardiac inflammatory response and improved cardiac function. Thus, in this model of lipotoxic cardiac injury, early induction of inflammation and macrophage recruitment contribute to adverse cardiac remodeling. These findings have implications for our understanding of heart failure in the setting of obesity and diabetes.

heart failure; diabetes; inflammation

OBESITY AND DIABETES ARE SIGNIFICANT risk factors for the development of heart failure (11). In many diabetic patients, this can occur in the absence of underlying coronary artery disease or hypertension, a phenomenon known as diabetic cardiomyopathy (1). The pathogenesis of diabetic cardiomyopathy is complex; however, myocardial lipid overload is a pathologic feature of this condition in humans and in animal models (6, 7, 12, 25). The observation that cardiac myocyte lipid overload causes cardiomyopathy in genetic mouse models, without systemic abnormalities of insulin or glucose metabolism, supports the notion that excess lipids can be cardiotoxic (4, 5, 7, 32). Membrane lipid remodeling, endoplasmic reticulum and oxidative stress, and production of toxic lipid species, such as ceramides, have been implicated as potential mechanisms of cardiac lipotoxicity (12, 23).

Systemic inflammation is another hallmark of obesity and diabetes and has been shown to correlate with the risk of heart failure in patients with these metabolic conditions (2). This raises the intriguing possibility that lipid-induced inflammatory responses might contribute to cardiac dysfunction in obesity and diabetes. Much of what is known regarding the link between diabetes and inflammation has come from investigations of adipose tissue. In humans and animals, obesity triggers the recruitment of macrophages to white adipose tissue, which is followed by release of inflammatory mediators and the onset of insulin resistance (26, 29). When this response is interrupted in mice fed a high fat diet, either through macrophage depletion or genetic knock-out of macrophage chemoattractants, the animals are protected against insulin resistance despite an equivalent extent of obesity (19, 21, 28). Macrophages expressing F4/80 and CD11c appear to be a key leukocyte subset involved in the inflammatory and metabolic effects of a high-fat diet (17, 21). Despite the evidence supporting a critical role for adipose tissue macrophages in the pathogenesis of metabolic disease, very little is known about cardiac macrophages in heart failure associated with metabolic stress. However, models of myocardial ischemia and/or infarction have illuminated an important, but complicated, function of these inflammatory cells in cardiac injury and repair. For example, monocytes and macrophages are required for the reparative response to myocardial tissue damage, yet overexuberant inflammation and cytokine production can exacerbate pathologic left ventricular (LV) remodeling (8).

In the present study, we sought to investigate the role of inflammation and macrophages in the pathogenesis of lipotoxic cardiomyopathy. To address this issue, we used the well-established α-myosin heavy chain promoter-driven long-chain acylCoA synthetase 1 (MHC-ACS) transgenic mouse model of heart failure. In these mice, cardiac-selective overexpression (driven by the α-MHC gene promoter) of long-chain acyl CoA synthetase 1 (ACS) leads to marked myocyte lipid accumulation independent of systemic metabolic disturbances and leads to cardiac dysfunction and premature death (5). Our findings reveal that macrophages modulate the myocardial response to this metabolic stress and contribute to adverse cardiac remodeling in response to lipid overload.

MATERIALS AND METHODS

Reagents. Antibodies for flow cytometry were from BD Pharmingen (CD11b-PE, CD 45-FITC, CD11c-APC, and CD16 Fc Block) or eBiosciences (F4/80-PerCP-Cy5.5). Antibodies for MAC3 immunohistochemistry and STAT-3 phospho STAT-3 Western blot were from Santa Cruz Sciences (F4/80-PerCP-Cy5.5). Antibodies for MAC3 immunohistochemistry and STAT-3/3phospho STAT-3 Western blot were from Santa Cruz Biotechnology (sc-81729, sc-8059, sc-7179). Clodrolip and control liposomes were produced as described by Dr. Reto Schwendener (34).

Animal experiments. MHC-ACS mice in FVB inbred genetic background (5) were maintained on a standard chow diet ad libitum (6% fat). For experiments, MHC-ACS and nontransgenic littermates were analyzed at the indicated ages. For clodrolip macrophage depletion, mice were given intraperitoneal injections of 100 mg/kg clodrolip or control liposomes in 150 μl volume at 22 days of age, followed by injections of 30 mg/kg in 100 μl volume every 4 days for the duration of the experiment. All animal experiments were conducted in accor-
dance with National Institutes of Health guidelines for humane treatment of animals and were approved by the Animal Studies Committee of Washington University School of Medicine.

RNA analysis and real-time quantitative PCR. Total cellular RNA was isolated from mouse cardiac ventricles using RNAzol B followed by chloroform extraction. Messenger RNA was reverse transcribed using a high capacity cDNA Reverse Transcription kit according to manufacturer’s instructions (Applied Biosystems). Real-time quantitative RT-PCR (qRT-PCR) was performed using SYBR green reagent (Applied Biosystems) and mouse specific primers on an ABI Prism 7500 Sequence Detection system. Signal intensity was normalized to 36B4. For the qRT-PCR array (SA Biosciences; PAMM-011A), RNA samples were further purified after initial RNAzol B/chloroform using RNase columns (Qiagen). Relative quantification of gene expression was performed using the ΔΔ-CT method.

Echocardiographic studies. Transthoracic M-mode and two-dimensional echocardiography was performed on mice anesthetized with inhaled isoflurane in the Washington University Cardiovascular Phenotyping Core by using a Visual Sonics 770 echocardiography system (5).

Protein analysis. Total cellular protein lysates were prepared from mouse hearts using 250 μl modified radioimmunoprecipitation assay buffer [1% Nonidet P-40, 0.5% SDS, 1 mM phenylmethylsulfonyl fluoride, and 1× Protease Complete (Roche) in PBS] followed by sonication (50% duty cycle, output 4, 30 pulses, Branson Sonifier 250, on ice). Total protein was quantified using the bicinchoninic acid assay method (Thermo Scientific). For Western blots, 25 μg of total protein was run on a 7.5% polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with antibodies specific to mouse hearts using 250 μl of mouse anti-Rat secondary followed by streptavidin-horseradish peroxidase and diaminobenzidine substrate per manufacturer’s instructions (Vector Laboratories). Bright field microscopy was performed using a Zeiss Axioskop.

Flow cytometry. Mice were euthanized by CO2 asphyxiation, and retrograde perfusion of hearts was performed using 30 ml of ice cold PBS. Ventricles were dissected, rinsed in ice-cold PBS, and minced to ~1 mm in size, and 5 ml of collagenase mix (collagenase II; Invitrogen) at 100 units/ml in HBSS with 60 units/ml of DNase 1 was added to samples. Samples were placed on shaker at 37°C for 1 h. After digestion, the hearts were triturated and passed through a 40 μm filter. The cells were pelleted by centrifugation at 200 g for 5 min after they were washed in 5 ml PBS and counted. Antibody staining was performed in FACS buffer (PBS, 2% FBS, 0.02% sodium azide). All samples were incubated with 0.5 μg Fc blocking antibody for 5 min on ice before the addition of primary antibodies. For staining, primary antibodies were added to the cells in a volume of 20 μl and incubated for 15 min on ice in the dark. After incubation, the cells were washed with FACS buffer and resuspended in 300 μl of PBS + 1% paraformaldehyde. The data were collected on a FACSCaliber Flow cytometer and analyzed using FlowJo software.

Myocardial triglyceride determination. Hearts were harvested and perfused with PBS and then frozen at ~80°C until analysis. Tissue was homogenized in chloroform:methanol (2:1), and an aliquot of organic extract was evaporated and analyzed using the Infinity Tri-glyceride Reagent (Thermo Scientific). Concentrations were normalized per milligram of tissue.

Statistics. Statistical analysis was performed using GraphPad Prism software. All results are expressed as means ± SE, where n = number of animals analyzed. Groups were compared by unpaired Student’s t-test, one-way ANOVA, or two-way ANOVA as appropriate. A value of P < 0.05 was considered significant.

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Fig. 1. Inflammatory cytokines are induced in lipotoxic cardiomyopathy. A: transthoracic echocardiograms were performed in lightly anesthetized MHC-ACS (ACS) and nontransgenic (NTG) littermates at 4 and 8 wk of age. The bars represent the mean fractional shortening. B: hearts from 4-wk-old ACS and NTG littermates were harvested and quantitative RT-PCR (qRT-PCR) was performed on total mRNA to assess the expression of inflammatory cytokines. Graphs display mean expression relative to 36B4 ± SE for n = 3 to 4 per group. *P < 0.05 and **P < 0.005 for ACS vs. NTG, ns. Not significant. C: total protein was isolated from the hearts of 4-wk-old NTG or ACS mice. Phosphorylated and total STAT3 levels were determined by Western blotting. MCP-1, monocyte chemotactant protein 1; iNOS, inducible nitric oxide synthase.
RESULTS

Inflammatory phenotype of mice with lipotoxic cardiomyopathy. Increasing evidence suggests that excess lipid can promote toxicity through activation of inflammatory pathways (9). The ACS model of lipotoxic cardiomyopathy is characterized by marked lipid accumulation as early as 18 days (5). Thus we assessed activation of inflammatory pathways by quantifying myocardial expression of several prototypical inflammatory cytokines by qRT-PCR in 4-wk-old transgenic animals and nontransgenic (NTG) littermates. It is important that ACS mice still have preserved cardiac function at this point despite increased cardiac lipid import, allowing us to identify inflammatory changes that precede the onset of cardiomyopathy (Fig. 1A). ACS mice had significantly higher mRNA expression levels of the inflammatory cytokines IL-6 and MCP-1/CCL2 in the heart; however, neither TNF-α nor inducible nitric oxide synthase (iNOS) transcripts were induced (Fig. 1B). Consistent with elevated levels of IL-6, phospho-STAT3, a read out of IL-6 receptor activation, was markedly increased in ACS hearts compared with NTG littermates (Fig. 1C). On the basis of these initial findings, we sought to further characterize the inflammatory phenotype of ACS mice by using a qRT-PCR array of inflammatory target genes. The results of this analysis revealed significant upregulation of several macrophage chemokines/chemokine receptors and related cytokines including osteopontin (OPN), MCP-2/CCL8, MCP-3/CCL7, MCP-5/CCL12, and CCR5 (Table 1).

Macrophage infiltration of the myocardium is an early event in MHC-ACS mice. The inflammatory profile of ACS mice suggested that macrophages might be recruited to heart in response to myocardial lipid overload. Consistent with this prediction, mRNA expression of the macrophage markers CD68 and F4/80 was significantly elevated in the hearts of ACS mice (Fig. 2A). We also observed a profound increase in the expression of CD11c, a marker of dendritic cells and a subset of activated macrophages. The expression of the macrophage chemotactic/migration molecules MCP-2 and OPN was also profoundly upregulated in 4-wk-old ACS mice compared with NTG littermates, consistent with our analysis from the qRT-PCR array (Fig. 2A and Table 1). To confirm the presence of enhanced cardiac macrophage recruitment, MAC3 immunohistochemical staining of myocardial tissue sections was performed. We observed a marked increase in the number

Table 1. Inflammatory markers in MHC-ACS mice at 4 wk of age

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>Induction</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>CCL2/MCP-1</td>
<td>3.0</td>
<td>0.027</td>
</tr>
<tr>
<td>CCL3/MIP-1α</td>
<td>4.8</td>
<td>0.020</td>
</tr>
<tr>
<td>CCL6</td>
<td>3.1</td>
<td>0.007</td>
</tr>
<tr>
<td>CCL7/MCP-3</td>
<td>3.1</td>
<td>0.006</td>
</tr>
<tr>
<td>CCL8/MCP-2</td>
<td>47.6</td>
<td>0.0002</td>
</tr>
<tr>
<td>CCL12/MCP-5</td>
<td>3.4</td>
<td>0.022</td>
</tr>
<tr>
<td>CCR3</td>
<td>5.1</td>
<td>0.045</td>
</tr>
<tr>
<td>CCR5</td>
<td>3.6</td>
<td>0.01</td>
</tr>
<tr>
<td>CXCL5</td>
<td>5.9</td>
<td>0.01</td>
</tr>
<tr>
<td>SPP1/osteopontin</td>
<td>628</td>
<td>0.006</td>
</tr>
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</table>

Induction reflects expression in ACS relative to nontransgenic mice. N = 3 animals/group. MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein.
of macrophages within the myocardium of ACS mice before the development of heart failure (Fig. 2B). Similar to the crown ringed structures observed with macrophage accumulation in adipose tissue of obese mice, the infiltrating macrophages in ACS hearts existed in distinct clusters distributed throughout the myocardium (Fig. 2B). Although robust lipid accumulation in ACS hearts is detected at 3 wk of age (Fig. 2C), we detected only a small increase in expression of the macrophage marker CD68 at that early time point, compared with marked induction of CD68 at 4 wk of age (Fig. 2D). These observations are consistent with a model in which myocardial lipid accumulation leads to macrophage recruitment. Both lipid accumulation and macrophage recruitment precede heart failure, which is not observed until 8 wk (Fig. 1A).

To further define the phenotype of the infiltrating macrophages, cardiac cells from 4-wk-old ACS and NTG mice were disaggregated into single-cell suspensions and analyzed by flow cytometry. CD45+ cells (leukocytes) were analyzed for expression of F4/80, CD11b, and CD11c (Fig. 3). Lipotoxic mice had a higher percentage of myocardial CD45 cells expressing the myeloid marker CD11b compared with NTG littermates. In line with the qRT-PCR data, there was a substantial increase in CD11b+, F4/80+ and CD11b+, CD11c+ cells in ACS mice. Only a small percentage of these myeloid cells coexpressed both CD11c and F4/80 surface markers. It is interesting that neither classic M1 markers, such as TNF and iNOS, nor classic M2 markers such as l-arginase, IL-10, or Chi-313 were significantly upregulated in ACS mice, although M2 markers trended up in ACS mice (Figs. 1B and 2A). Thus the macrophage phenotype in this model of lipotoxicity is not clearly polarized toward M1 or M2.

Depletion of macrophages modulates the cardiac inflammatory response to in MHC-ACS mice. To determine the contribution of macrophage infiltration to the cardiac inflammatory phenotype of ACS mice, macrophages were depleted by intraperitoneal injection of liposomal clodronate (clp), which leads to a reduction in the number of CD11b+ CD11c+ and CD11b+ F4/80+ cells in the myocardium (Fig. 3) and a decrease in myocardial lipid accumulation (Fig. 2C). These observations suggest that macrophage infiltration contributes to the development of cardiac inflammation and lipotoxic cardiomyopathy in ACS mice.
to apoptosis of both tissue macrophages and circulating monocytes (33, 34). When compared with vehicle (veh) liposome-injected ACS mice, clp-treated animals had near complete loss of myocardial F4/80 expression by qRT-PCR (Fig. 4A). Both CD68 and CD11c were also significantly reduced in clp-injected mice; however, the expression of these genes was still increased in ACS myocardium relative to NTG littermates. Induction of the chemokine MCP-2 was completely abrogated, and OPN was significantly attenuated in clp-treated mice (Fig. 4A). In contrast, IL-6 and MCP-1 mRNA levels were unaffected in macrophage-depleted mice compared with controls (Fig. 4A). Consistent with the qRT-PCR data, hematoxylin and eosin and MAC3 staining of LV tissue from these mice revealed a substantial reduction in the number of inflammatory cells in clp-treated ACS mice (Fig. 4B). In line with the IL-6 expression data, clp depletion did not reduce the increase in phospho-STAT3 observed in ACS mice (Fig. 4C). In addition, macrophage
depletion did not alter myocardial triglyceride accumulation in ACS mice (Fig. 4D). Thus treatment of ACS mice with clp dramatically reduces macrophage infiltration and selectively modulates the cardiac inflammatory profile. Together these findings suggest a model in which macrophages act downstream of myocardial lipid accumulation.

**Depletion of macrophages attenuates the cardiomyopathy in MHC-ACS mice.** To determine the effects of macrophage depletion on the development of lipotoxic cardiomyopathy, echocardiograms were performed on 8-wk-old ACS or NTG mice that had been injected with clp or veh as described above. Clp had no effect on baseline cardiac function in NTG mice (Fig. 5). It is interesting that ACS mice treated with veh or clp had a similar degree of cardiac hypertrophy as assessed by LV mass index (LVM; Fig. 5A). However, clp-treated ACS mice had a significant improvement in systolic LV fractional shortening compared with veh-treated ACS mice (Fig. 5B). Moreover, both end-systolic and end-diastolic diameters were reduced in ACS mice treated with clp versus veh (Table 2). These findings demonstrate that in the face of myocyte lipid stress, macrophage depletion attenuates adverse LV remodeling.

**DISCUSSION**

Diabetes is a major risk factor for the development of heart failure through mechanisms involving both direct myocardial toxicity and accelerated atherosclerosis. A key feature of the diabetic heart is the accumulation of myocardial lipid, which is thought to contribute to myocyte dysfunction and cell loss (15, 20, 23, 25). However, the mechanisms involved in cardiac lipotoxicity in this disease are unresolved. We hypothesized that activation of inflammatory pathways in the setting of lipid-stressed cardiomyocytes would accelerate progression of cardiac dysfunction. To address this question, we turned to the ACS transgenic model of cardiac lipotoxicity. By 3 wk of age, these mice reproducibly develop cardiac myocyte lipid overload, which leads to systolic heart failure by 8 wk of age. Using this model, we demonstrated that inflammatory cytokine expression is increased in the hearts of ACS mice before the onset of cardiomyopathy. Moreover, this inflammatory response is accompanied by macrophage infiltration of the myocardium. Depletion of macrophages with liposomal clodrolip reduced the expression of several inflammatory cytokines, decreased leukocyte infiltration, and attenuated the progression of LV dysfunction, independent of effects on cardiac lipid accumulation. Together these data are consistent with a model in which myocardial lipid accumulation leads to recruitment of macrophages in the heart. Moreover, the deleterious effects of lipid overload in the heart are dependent, in part, on this lipid-induced inflammatory response.

Evidence is emerging that inflammation is important in the pathogenesis of metabolic disease (10). It is now well established that macrophage recruitment to adipose tissue in the setting of obesity is a key event in the development of insulin resistance (24). In particular, CD11c-expressing macrophages have been shown to be critical for the metabolic effects of high-fat diet (21). It is interesting that ACS mice have significant macrophage accumulation in the myocardium by 4 wk of age, well before the onset of cardiomyopathy. Because the perturbation of lipid metabolism in ACS mice is cardiac

Table 2. **Echocardiographic measurements in NTG and ACS mice after vehicle or clodronate**

<table>
<thead>
<tr>
<th></th>
<th>NTG</th>
<th>Clodronate</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>587 ± 13</td>
<td>621 ± 19</td>
<td>506 ± 11‡</td>
</tr>
<tr>
<td>Left ventricular diameter, mm</td>
<td>2.91 ± 0.12</td>
<td>2.89 ± 0.05</td>
<td>3.75 ± 0.06‡</td>
</tr>
<tr>
<td>End diastolic</td>
<td>1.28 ± 0.17</td>
<td>1.43 ± 0.20</td>
<td>2.55 ± 0.07‡</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>56.3 ± 5.2</td>
<td>57.9 ± 3.1</td>
<td>31.5 ± 2.1‡</td>
</tr>
<tr>
<td>Left ventricular posterior wall thickness, mm</td>
<td>0.84 ± 0.02</td>
<td>0.88 ± 0.01</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>Left ventricular mass index, mg/weight</td>
<td>3.82 ± 0.12</td>
<td>3.72 ± 0.22</td>
<td>5.89 ± 0.19‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, vehicle vs. clodronate; ‡P < 0.05 NTG vs. ACS.

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and IL-6, whereas M2 cells are characterized by expression of either M1 (inflammatory) or M2 (reparative) subsets based in inflammatory cells. Macrophages are often subdivided into this and other models of lipotoxic cardiomyopathy. 

source of MCP-1 and other macrophage chemoattractants in fatty acids themselves contribute to the influx of macrophages infiltration. It is also possible that myocyte cell death or free production and potentially is the initial trigger for macrophage that a resident, nonmacrophage cell type is responsible for its not impacted by the depletion of macrophages. This suggests that a resident, nonmacrophage cell type is responsible for its production and potentially is the initial trigger for macrophage infiltration. It is also possible that myocyte cell death or free fatty acids themselves contribute to the influx of macrophages (14). Future studies will be required to identify the cellular source of MCP-1 and other macrophage chemoattractants in this and other models of lipotoxic cardiomyopathy.

At present, the mechanism by which macrophages accelerate the pathogenesis of cardiomyopathy is not clear; however, it is most likely related to paracrine factors released by these inflammatory cells. Macrophages are often subdivided into either M1 (inflammatory) or M2 (reparative) subsets based in part on the expression or secretion of distinct molecules (3). M1 cells express proinflammatory genes such as TNF, iNOS, and IL-6, whereas M2 cells are characterized by expression of L-arginase, Chi313, and IL-10. It is interesting that the cytokine profile in ACS hearts does not clearly implicate either of these phenotypes, although M2 markers did trend higher in ACS mice. Thus, despite the established relationship between classical M1 inflammatory cytokines and LV dysfunction due to TNF-α and iNOS, heart failure in this model of lipotoxic cardiomyopathy may involve distinct inflammatory signals. Our cell depletion experiments show that this inflammatory response in the myocardium of ACS mice contributes significantly to adverse myocardial remodeling. The observation that cardiac function is not entirely restored to baseline after macrophage depletion may be consistent with residual myocardial inflammation (e.g., CD68, CD11c, and osteopontin are decreased but not restored to baseline) and/or more direct toxic effects of lipid accumulation, which is not affected by treatment with liposomal clodrolip.

Within the unique cytokine profile observed in ACS mice, OPN, a molecule well known to contribute to macrophage recruitment and function, was profoundly upregulated. Previous studies have shown that expression of OPN is increased in diabetes, where it plays a key role in high-fat diet-induced macrophage recruitment to adipose tissue and the induction of insulin resistance (18). Moreover, OPN is upregulated in many forms of heart failure in which both gain- and loss-of-function experiments have shown that it plays a pathologic role in cardiac dysfunction (22, 27, 31). Of note, the expression of OPN was decreased by ~70% in ACS mice treated with clodrolip, suggesting that it may be an important signaling molecule from macrophages that contributes to heart failure progression in this model. Further investigation of OPN as a pathophysiological mediator of cardiac lipotoxicity is ongoing.

The ACS transgenic model of lipotoxic cardiomyopathy provided an ideal setting in which to evaluate the contributions of inflammation to metabolic stress in the heart. Consistent with our findings in these mice, nontransgenic models of lipid overload using long-term high-fat diet or streptozotocin treatment have also demonstrated increased numbers of macrophages within the hearts of insulin resistant and/or diabetic mice (13, 30). However, assessing the influence of cardiac macrophages on LV function using cell depletion strategies with these models is confounded by the concomitant role of macrophages in promoting the primary dysregulation of systemic metabolism, which is further modified by macrophage depletion (16, 21). The ACS model, in which myocardial lipid accumulation is independent of systemic metabolic perturbations, enabled us to investigate the contribution of macrophages to adverse cardiac remodeling in the setting of metabolic stress. On the other hand, the rapid time course of cardiomyopathy in ACS mice at a relatively young age precluded the use of bone marrow and adoptive transfer experiments to further manipulate the myeloid compartment in this model. Future genetic studies will be required to extend our findings from the ACS model to investigate cardiac myocyte-macrophage interactions in the setting of systemic lipid overload and diabetes.

The present study supports a model in which macrophages can influence the response of the heart to lipid-induced injury, contributing to rapid progression of cardiac dysfunction. This model is supported by the observations that macrophages infiltrate the heart before the onset of cardiomyopathy and their depletion attenuates cardiac dysfunction and remodeling. As such, further research investigating the mechanisms of macrophage recruitment to and activation in the myocardium has the potential to yield novel insights into the pathogenesis of diabetic cardiac disease.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: J.D.S. and J.E.S. conception and design of research; J.D.S., H.M., A.H.K., and R.S. performed experiments; J.D.S., H.M., and A.H.K. analyzed data; J.D.S. and J.E.S. interpreted results of experiments; J.D.S., H.M., A.H.K., and R.S. wrote the final version of manuscript.

REFERENCES
MACROPHAGES AND LIPOTOXIC CARDIOMYOPATHY


