



# Distinct roles of resident and nonresident macrophages in nonischemic cardiomyopathy

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**Nonischemic cardiomyopathy (NICM) resulting from long-standing hypertension, valvular disease, and genetic mutations is a major cause of heart failure worldwide. Recent observations suggest that myeloid cells can impact cardiac function, but the role of tissue-intrinsic vs. tissue-extrinsic myeloid cells in NICM remains poorly understood. Here, we show that cardiac resident macrophage proliferation occurs within the first week following pressure overload hypertrophy (POH; a model of heart failure) and is requisite for the heart's adaptive response. Mechanistically, we identify Kruppel-like factor 4 (KLF4) as a key transcription factor that regulates cardiac resident macrophage proliferation and angiogenic activities. Finally, we show that blood-borne macrophages recruited in late-phase POH are detrimental, and that blockade of their infiltration improves myocardial angiogenesis and preserves cardiac function. These observations demonstrate previously unappreciated temporal and spatial roles for resident and nonresident macrophages in the development of heart failure.**

cardiac macrophage | angiogenesis | pressure overload hypertrophy

Cardiomyopathy is a disease of the heart muscle that decreases the heart's ability to pump blood and meet the body's energetic demands. Cardiomyopathy can be broadly classified as ischemic cardiomyopathy (ICM) or nonischemic cardiomyopathy (NICM). In ICM, interruption of coronary artery blood flow, as seen in myocardial infarction (MI), is the primary cause of cardiac dysfunction. In NICM, cardiac muscle function can be compromised by either intrinsic gene mutation (hypertrophic cardiomyopathy) or extrinsic stress, such as pressure overload secondary to chronic hypertension or valve diseases [pressure overload hypertrophy (POH)]. With respect to NICM, accumulating clinical evidence suggests that small vessel dysfunction is a strong independent predictor of clinical deterioration and death in patients (1–4). Further, experimental studies suggest that inadequate myocardial angiogenesis is a critical determinant of the transition from compensatory hypertrophy to heart failure (5–7).

Macrophages are found in virtually every tissue and are critical for homeostasis and stress-induced responses. Macrophages broadly consist of two classes: resident macrophages and blood-borne infiltrating macrophages. Resident macrophages originate from yolk sac-derived erythromyeloid progenitors (EMPs), reside in tissue, perform homeostatic functions, and self-maintain locally (8–10). In general, infiltrating macrophages arise from circulating classic Ly6C<sup>hi</sup> (inflammatory) monocytes and are recruited after inciting pathology through the CCL2–CCR2

chemotaxis pathway (8, 11). Nonclassic Ly6C<sup>lo</sup> (patrolling) monocytes also patrol the luminal side of the endothelium and extravasate in response to both septic and aseptic tissue injury, playing a protective or antiinflammatory role during tissue injury (12). The adult heart contains two major subsets of Ly6C<sup>lo</sup>/CCR2<sup>−</sup> resident macrophages that express different levels of MHC-II, which are EMP-derived resident macrophages self-maintained through local proliferation at steady state. In addition, there are two minor subsets of Ly6C<sup>hi</sup> macrophages (<2%) that differ in CCR2 levels (13). These Ly6C<sup>hi</sup> subsets are derived from hematopoietic stem cells, are maintained through monocyte infiltration, and help to resolve

## Significance

**Pressure overload triggers responses in cardiomyocytes and noncardiomyocytes, leading to pressure overload hypertrophy (POH). Here, we show that cardiac resident macrophages regulate compensatory myocardial adaptation to POH, while nonresident infiltrating macrophages are detrimental. At early-phase POH, pressure overload induces cardiac resident macrophage proliferation, which is regulated by Kruppel-like factor 4. At late-phase POH, pressure overload also induces Ly6C<sup>hi</sup> monocyte infiltration, and its blockade improves myocardial angiogenesis and preserves cardiac function. Mechanistically, the differential impact of these two macrophage subsets on myocardial angiogenesis may underlie the cardiac phenotype. These findings provide insights regarding the role of cardiac resident and nonresident macrophages, conceptually update the view of myocardial angiogenesis, and identify monocyte infiltration as a therapeutic target for nonischemic cardiomyopathy.**

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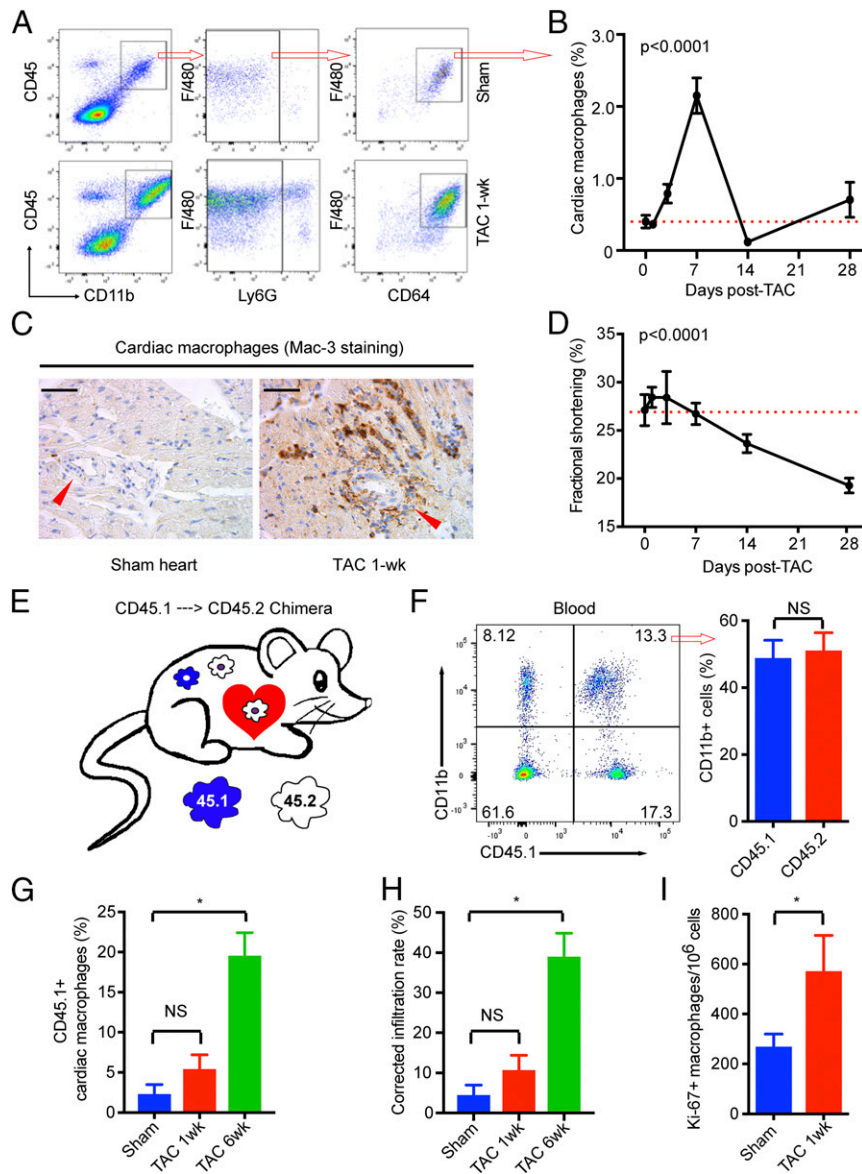
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tissue injury (14). Recent studies suggest that in ICM (i.e., MI), tissue injury induces both local proliferation of resident macrophages and infiltration of monocytes in the site of injury to regulate wound healing and inflammation in the heart (9, 15). Further, macrophages also accumulate in the remote nonischemic zone of the myocardium after MI to mediate post-MI cardiac remodeling (16). By contrast, much less is known in the setting of NICM. For example, whether macrophages are involved in disease progression and, if so, the identity, origin, and function of such cardiac macrophages remain unknown.

Here, using a classic mouse POH model for NICM, we track resident and infiltrating macrophages in the myocardium during the early and late phases of POH and provide multiple lines of evidence showing previously unappreciated and distinct time-dependent roles of different subsets of cardiac macrophages in POH. Using the myeloid-specific Kruppel-like factor 4 (KLF4) mutant mouse, we also shed light on the molecular mechanism that regulates local proliferation of cardiac resident macrophages and a potential role that these cells play in regulating perfusion during POH.

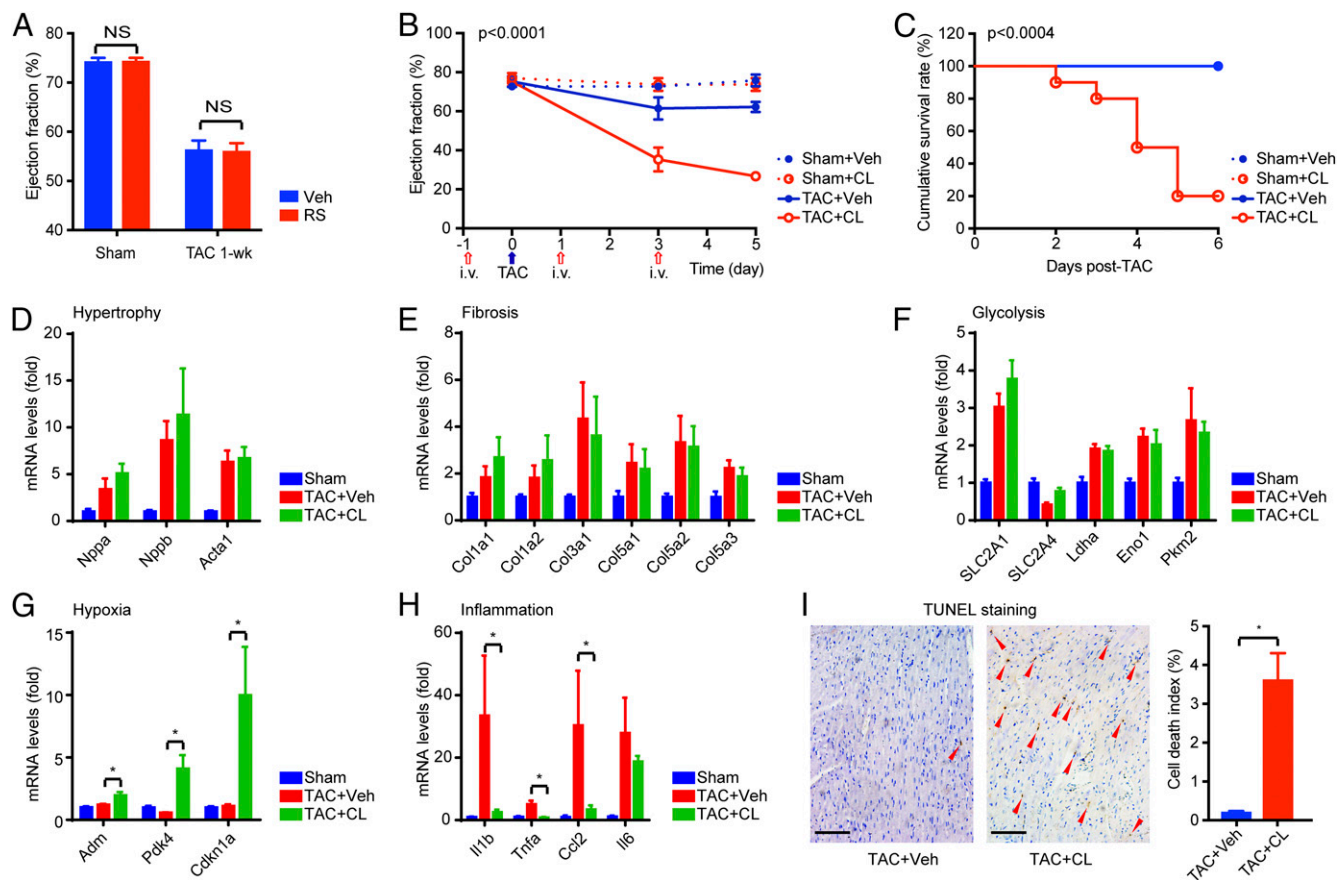


**Fig. 1.** Pressure overload induces local proliferation of cardiac resident macrophages at early phase and infiltration of monocytes at late phase. (A) Gating strategy for flow cytometry. (B) Dynamics of cardiac macrophages following TAC and its correlation with cardiac function. The dashed line indicates baseline. Cardiac macrophages were gated as live (live/dead stain-negative) CD45<sup>+</sup>CD11b<sup>+</sup>Ly6G<sup>-</sup>F4/80<sup>+</sup>CD64<sup>+</sup> cells and are shown as a percentage vs. total heart cells. Cardiac function was expressed as left ventricular fractional shortening. (C) Immunostaining for Mac-3 to reveal macrophages in the myocardium, particularly in the perivascular region. Brown (DAB-positive) cells are macrophages. Arrowheads indicate vessels. (Scale bars, 100  $\mu$ m.) (D) LV dysfunction following TAC. (E) Strategy to generate CD45.1-CD45.2 chimeric mice. Recipient CD45.2 mice were irradiated with their head-chest shielded by lead block and transplanted via tail vein injection with bone marrow cells from CD45.1 donor mice. The resulting chimera mice carry both CD45.2 and CD45.1 cells in the blood but only have CD45.2 cardiac resident macrophages. (F) Blood (50  $\mu$ L) was drawn from the tail vein at 8 wk posttransplantation and analyzed by FACS for CD45.1<sup>+</sup> myeloid cells (CD45<sup>+</sup>CD11b<sup>+</sup>). (G) FACS analysis for CD45.1<sup>+</sup> cardiac macrophages before and after TAC. (H) Corrected monocyte infiltration rate considering that only 50% of monocytes were CD45.1<sup>+</sup> cells. (I) FACS analysis for Ki-67 expression in cardiac macrophages. (B and D) *P* values were calculated from one-way ANOVA. (F–I) \**P* < 0.05 by *t* test after Bonferroni correction (*n* = 5–8 for all data points). NS, not significant.









**Fig. 3.** Cardiac resident macrophages are requisite for cardiac adaptation to POH. (A) Blocking monocyte infiltration by RS had no effect on POH. (B) Left ventricular function following clodronate-mediated macrophage depletion and TAC ( $n = 8-10$  in each group). The  $P$  value was calculated from two-way ANOVA. The blue arrow indicates the time of TAC, and red arrows indicate the time of liposome injections. (C) Postoperation survival rates.  $P$  values were calculated from log-rank between TAC+CL and TAC+Veh groups. (D–H) Gene expression in the heart after 4-d TAC  $\pm$  CL ( $n = 5$ ).  $*P < 0.05$  by  $t$  test with Bonferroni correction. (I) Cell death detected by TUNEL staining in the myocardium after 4-d TAC  $\pm$  CL. Red arrowheads indicate nuclei of dead cells.  $*P < 0.05$  by  $t$  test ( $n = 5$ ). NS, not significant. (Scale bars, 200  $\mu\text{m}$ .)

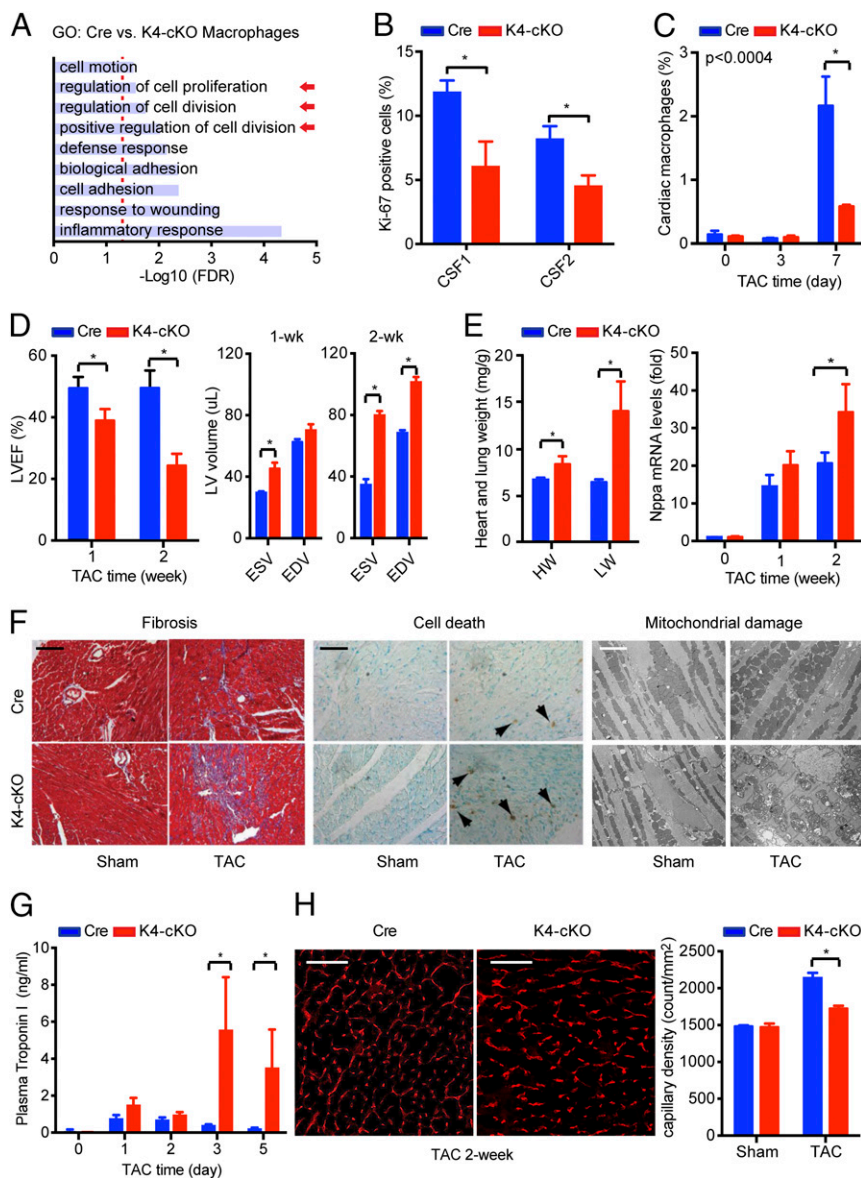
D–F). Because blocking peripheral monocyte infiltration did not promote cardiac dysfunction in early-phase (Fig. 3A) or late-phase-TAC (see Fig. 5), these late-stage macrophage depletion studies implicate cardiac resident macrophages in maintaining cardioprotection throughout the POH disease course.

**KLF4 Regulates Proliferation of Cardiac Resident Macrophages.** Most tissue resident macrophages, including cardiac resident macrophages, are maintained by self-renewal (9, 22). While our understanding of how resident macrophages proliferate is limited, a recent seminal study by Sieweke and coworkers (23) provides critical insights. Specifically, it was found that primary macrophages from *MafB/c-Maf* double-deficient (*Maf*-DKO) mice demonstrated indefinite KLF4-dependent proliferative capacity when cultured with macrophage colony-stimulating factor (M-CSF) (23). Therefore, we utilized myeloid-specific KLF4-deficient mice (*Ly2z-Cre:KLF4<sup>fllox/fllox</sup>*; designated K4-cKO) (24) to investigate if KLF4 regulates cardiac macrophage proliferation in the context of POH.

Given that *Ly2z-Cre* is active in macrophages and neutrophils but not in monocytes (21), we first sought to explore any potential role for neutrophils in this model. The infiltration of *Ly6G<sup>+</sup>* neutrophils was marginal after TAC (Fig. 1A), and depletion of neutrophils using the anti-*Ly6G* antibody (clone 1A8) before TAC demonstrated very little effect on POH (SI Appendix, Fig. S6). Therefore, these data supported our decision to focus on the role of KLF4-deficient macrophages.

We first performed transcriptomic analysis of primary macrophages from *Ly2z-Cre* (designated Cre) and K4-cKO mice via RNA-sequencing (RNA-seq) and found 238 genes that were differentially expressed (false discovery rate  $< 0.05$ ). Gene ontology (GO) analysis demonstrated enrichment in multiple pathways of cell proliferation and division (Fig. 4A). Importantly, when treated in vitro with CSF1 and CSF2, KLF4-deficient primary macrophages showed less accumulation of Ki-67 protein (Fig. 4B and SI Appendix, Fig. S7A), demonstrating impaired proliferative capacity. Next, we subjected the K4-cKO and Cre animals to TAC. FACS analysis demonstrated that TAC-induced cardiac macrophage accumulation was significantly reduced in the K4-cKO group compared with that of Cre (Fig. 4C). Consistent with fewer cardiac macrophages, myeloid KLF4 deficiency impaired cardiac resident macrophage proliferation in vivo following TAC as demonstrated by reduced BrdU incorporation (SI Appendix, Fig. S7B).

Post-TAC serial echocardiography revealed that K4-cKO mice rapidly develop a dilated cardiomyopathy characterized by reduced left ventricular EF (Fig. 4D), elevated heart and lung weight, and expression of the cardiac hypertrophy marker gene *Nppa* (Fig. 4E). Histological analyses exhibited enhanced fibrosis and cell death, as well as severe mitochondrial damage in K4-cKO hearts post-TAC (Fig. 4F). Plasma cardiac troponin I levels were significantly higher in K4-cKO mice post-TAC, indicating myocardial injury (Fig. 4G). K4-cKO hearts exhibited significantly



**Fig. 4.** Myeloid KLF4 regulates cardiac macrophage proliferation in POH. (A) RNA-seq studies with primary peritoneal macrophages from Cre and K4-cKO mice. A total of 283 genes were differentially regulated between the Cre and K4-cKO groups, and gene ontology (GO) term enrichment analysis revealed nine pathways that were functionally enriched [false discovery rate (FDR) < 0.05]. (B) KLF4-deficient peritoneal macrophages exhibited an impaired proliferative response to CSF1/2 stimulation (48 h). \* $P < 0.05$ . (C) KLF4-deficiency impaired TAC-induced cardiac macrophage proliferation ( $n = 10$ ). \* $P < 0.05$ . (D) Myeloid KLF4 deficiency impaired cardiac function after TAC, resulting in dilated cardiomyopathy at 2 wk post-TAC ( $n = 8-10$ ). \* $P < 0.05$ . EDV, end-diastolic volume; ESV, end-systolic volume; LVEF, left ventricular ejection fraction. (E) Myeloid KLF4 deficiency accelerated TAC-induced cardiac hypertrophy. Heart weight (HW; milligrams) and lung weight (LW; milligrams) at 2 wk post-TAC were normalized to body weight (grams) ( $n = 6$  in each group). \* $P < 0.05$  by  $t$  test with Bonferroni correction. (F) TAC induced more fibrosis, cell death, and mitochondrial damage in K4-cKO hearts. Samples were from 1-wk TAC and sham mice. (Black scale bar, 100  $\mu\text{m}$ ; white scale bar, 2  $\mu\text{m}$ .) (G) TAC induced higher plasma cardiac troponin I levels in the K4-cKO group ( $n = 5$ ). \* $P < 0.05$  with Bonferroni correction. (H) Impaired angiogenesis in K4-cKO hearts after TAC as revealed by myocardial capillary density using CD31 immunostaining ( $n = 5$ ). \* $P < 0.05$ . (Scale bars, 100  $\mu\text{m}$ ).

impaired angiogenesis as demonstrated by CD31 immunostaining and the dysregulation of angiogenesis genes (Fig. 4H and *SI Appendix*, Fig. S8 A and B). Although not induced by TAC, macrophage KLF4 appeared to be required for optimal expression of a potent angiogenesis factor, VEGFA, in vitro and in the heart (*SI Appendix*, Fig. S8 C and D). These data, paired with the observation that many cardiac macrophages reside in the perivascular region (Fig. 1C), suggest that cardiac macrophages may have a potential role in angiogenesis or other vascular responses. In addition to impaired angiogenesis, we observed that K4-cKO hearts exhibited enhanced fibrosis after TAC (Fig. 4F). However, qPCR analysis

using a panel of 84 fibrosis-related genes (Qiagen RT<sup>2</sup> Profiler PCR array) revealed no significant difference between Cre and K4-cKO cardiac macrophages (*SI Appendix*, Fig. S8E). These observations, coupled with the appreciation of reduced KLF4-deficient cardiac macrophage numbers (Fig. 4C), suggest that enhanced fibrosis seen in the K4-cKO heart was likely secondary to myocardial injury (Fig. 4 F and G).

Because KLF4 also regulates macrophage M1/M2 polarization (24, 25) and gene expression in KLF4-deficient macrophages exhibited enriched inflammation pathways (Fig. 4A), we tested if the macrophage polarization state contributes to the phenotype

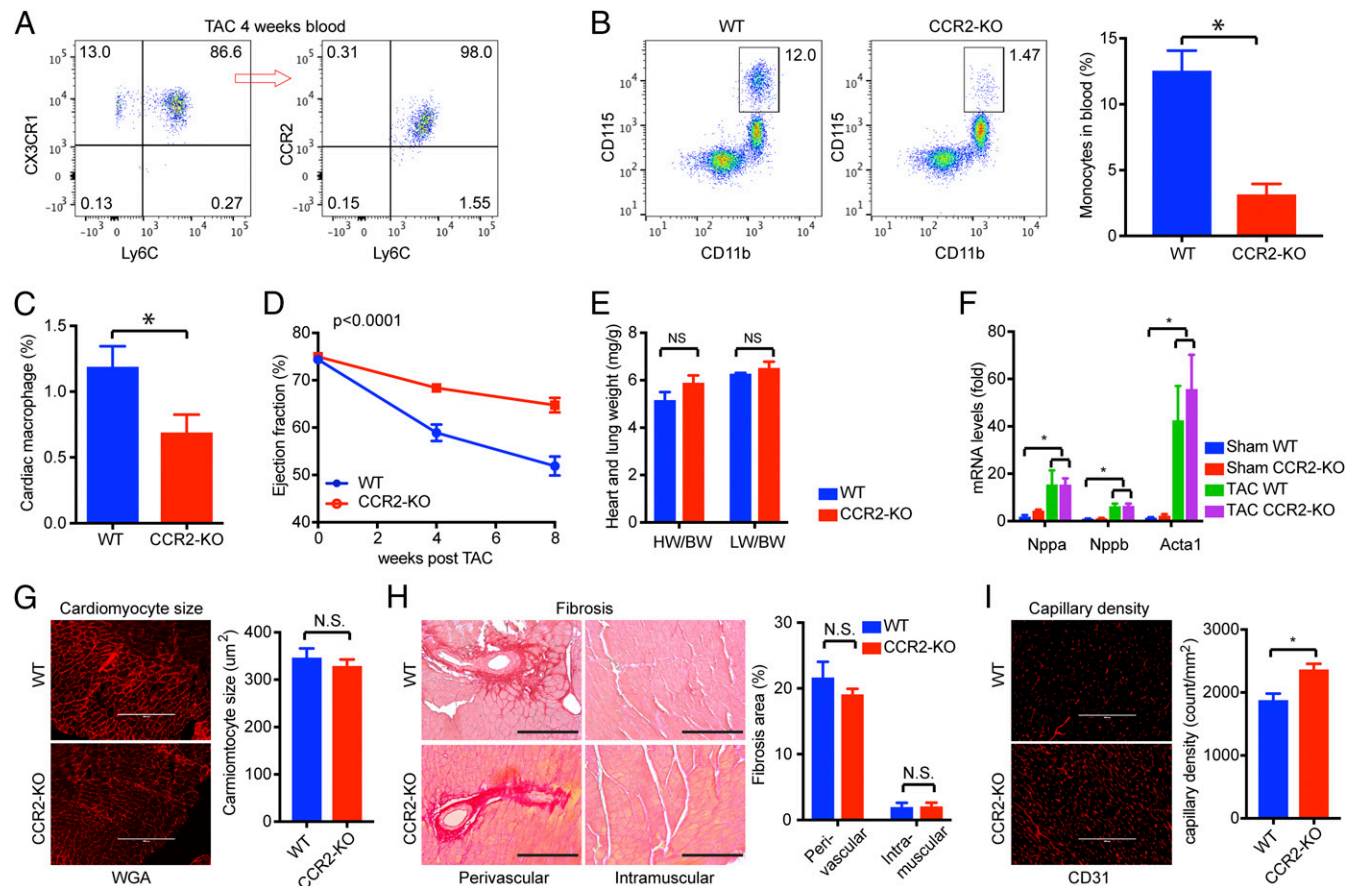


observed after TAC. Consistent with our previous report (24), K4-cKO hearts at 1 wk post-TAC showed lower expression of multiple M2 genes (*SI Appendix, Fig. S9A*). However, the expression of M1 genes in K4-cKO hearts was also lower, indicating the absence of excessive inflammation (*SI Appendix, Fig. S9B*). We attribute this phenomenon to reduced cardiac macrophage numbers in K4-cKO hearts after TAC (Fig. 4C). We further studied two classic M2-deficient animals (26), namely, hematopoietic STAT6-KO mice (via transplantation of STAT6-KO bone marrow to WT recipients) and myeloid IL4Ra-KO mice (Lyz2-Cre:IL4Ra<sup>fllox/fllox</sup>), and found that biased M1/M2 polarization had no effect on POH (*SI Appendix, Fig. S9C and D*). As such, we conclude that the role of KLF4 in cardiac macrophages in POH is not simply defined by M1/M2 polarization but is secondary to its regulation of cardiac resident macrophage proliferation and their angiogenic potential (Fig. 4C and *SI Appendix, Fig. S8D*).

**Infiltrating Macrophages Are Detrimental to the Heart in Late-Phase POH.** While our studies indicate that blood-borne macrophages are not required for the early-phase protective compensatory response to POH, they do infiltrate the myocardium during late-stage POH (Fig. 1G). Because heart failure is often associated with immune cell infiltration and inflammation (27–29), we asked if

blood-borne macrophages contribute to late-phase decompensation of POH. As shown in Fig. 2F, TAC increased circulating Ly6C<sup>hi</sup> monocytes during late-phase POH. These monocytes were further characterized as Ly6C<sup>hi</sup>CX3CR1<sup>+</sup>CCR2<sup>+</sup> classic monocytes (Fig. 5A). To block such monocytes, we utilized the CCR2-KO mouse, which is known to have reduced circulating classic monocytes due to impaired CCL2/CCR2-mediated trafficking from the bone marrow (30, 31). When subjected to 4-wk TAC, the circulating monocytes remained much lower (~25%) in CCR2-KO mice than WT control mice (Fig. 5B). Consistently, overall macrophage numbers in late-phase POH were also lower (~50%) within CCR2-KO myocardium (Fig. 5C).

Serial echocardiography for 8 wk post-TAC demonstrated that WT mice gradually develop cardiac dysfunction during late-phase POH, while CCR2-KO mice maintain relatively normal cardiac function (EF at 8 wk post-TAC: WT: 52 ± 2.0% vs. CCR2-KO: 65 ± 1.5%; *P* < 0.0001) (Fig. 5D). Although there was no difference in cardiac hypertrophy or fibrosis between WT and CCR2-KO groups (Fig. 5E–H), myocardial capillary density was significantly higher in the CCR2-KO group (capillary counts per 1 mm<sup>2</sup> of myocardium: WT: 1,872 ± 113 vs. CCR2-KO: 2,359 ± 98; ~26% increase; *P* < 0.0038) (Fig. 5I), suggesting that CCR2-KO mice were able to maintain superior angiogenesis during the 8 wk of TAC. Finally, blocking CCR2 signaling in WT



**Fig. 5.** Blockade-infiltrating macrophages preserved cardiac function in late-phase POH. (A) Classic Ly6C<sup>+</sup>CCR2<sup>+</sup> monocytes increased in blood after 4 wk of TAC. A representative FACS plot from five samples is shown. (B) Reduced monocyte numbers in CCR2-KO mice after 4 wk of TAC (*n* = 5). \**P* < 0.05 by *t* test. (C) Reduced cardiac macrophage numbers in CCR2-KO hearts after 4 wk of TAC (*n* = 5). \**P* < 0.05 by *t* test. (D) Left ventricular function assessed by echocardiography. The *P* value is shown as *P* (genotype \* time) calculated from two-way ANOVA. (E) Heart weight and lung weight. (F) Expression of hypertrophy marker genes. (G) Cardiomyocyte cross-section area. Cell membranes were outlined by wheat germ agglutinin (WGA) staining. (H) Fibrosis assessed by PicoSirius Red staining, with collagen stained in red. Perivascular and intramuscular areas are shown. Fibrosis was quantified as the percentage of the fibrotic area in total by ImageJ (NIH) software. (I) Myocardial capillary density assessed by CD31 staining. All tissue samples were assessed at 8 wk post-TAC (*n* = 5–8). \**P* < 0.05. NS, not significant. (Scale bars, 200 μm.)

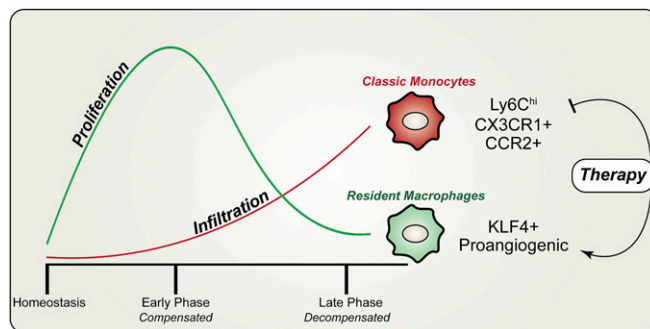
animals using RS preserved cardiac function in an established POH model (RS oral administration 2 wk after TAC) without affecting cardiac hypertrophy (*SI Appendix*, Fig. S10). Collectively, these data demonstrate that blocking monocyte/macrophage infiltration maintains angiogenesis and preserves cardiac function without affecting cardiac hypertrophy or fibrosis in late-phase POH. Taken together, these data demonstrate that late-phase infiltration of Ly6C<sup>hi</sup>CX3CR1<sup>+</sup>CCR2<sup>+</sup> classic monocytes contributes to cardiac dysfunction in POH and, conversely, that blockade of infiltrating macrophages (genetically by CCR2-KO or pharmaceutically by CCR2 antagonist) preserves heart function, in part, through improved angiogenesis.

## Discussion

The heart contains resident macrophages and can recruit infiltrating macrophages upon stress. As cardiac resident and infiltrating macrophages have separate origins and reside in distinct geographic locations, it is not surprising that they have different functions as well. In the present study using a mouse model of NICM, we show that resident macrophage proliferation and angiogenic activity in the initial period following pressure overload stress are critical for cardiac adaptation and function. Furthermore, cardiac resident macrophages are essential to maintain cardiac function even in the late phase of POH. By contrast, in the late phase of POH, infiltrating monocytes/macrophages promote decompensation and blockade of this ingress is ameliorative. These findings are aligned with recent observations that in models of acute and chronic post-MI remodeling, resident macrophages are salutary, while infiltrating macrophages are detrimental (15, 16). Further, in neonatal hearts, tissue macrophages have been shown to regulate cardiac angiogenesis and regeneration after apical resection or MI (15, 32). Collectively, the current work, along with these recent findings, underscores the differential roles of tissue-intrinsic and tissue-extrinsic macrophages in the cardiac response to injury (summarized in Fig. 6).

An important aspect of our work relates to the identification of KLF4 as a critical regulator of resident macrophage proliferation and angiogenic function. We found that myeloid KLF4 deficiency resulted in a marked reduction in local proliferation of cardiac resident macrophages and the total number of cardiac macrophages 7 d after TAC. Pathway analysis of RNA-seq studies in KLF4-deficient macrophages highlighted enrichment for pathways that control cellular proliferation. These findings are particularly intriguing considering prior work linking KLF4 to macrophage proliferation (23). The upstream mechanism following POH that leads to KLF4 induction remains incompletely understood. However, a recent study by Fujii et al. (33) reported that kidney-derived CSF2 activated proliferation of cardiac resident macrophages in response to TAC. We observed that myeloid KLF4 deficiency significantly diminished macrophage proliferation in response to CSF2 (as well as CSF1; Fig. 4B). As such, it is possible that kidney-derived CSF2 induces KLF4-dependent resident macrophage proliferation. Future studies will be needed to delineate the detailed mechanisms by which KLF4 regulates proliferation of resident macrophages in vivo and whether KLF4 is universally required for resident macrophage proliferation in other tissues.

Another important observation relates to myeloid KLF4 control of angiogenesis. We observed that myeloid KLF4 KO animals demonstrated a marked susceptibility to heart failure, likely due to reduced angiogenic capacity leading to cardiomyocyte death (Fig. 4). Consistent with this, myeloid KLF4 KO mice demonstrated significant cardiac troponin levels in plasma and clear evidence of cardiac mitochondrial damage. The basis for myeloid KLF4 control of angiogenesis remains incompletely understood, but we note that expression of a number of proangiogenic factors, such as VEGFA, is reduced



**Fig. 6.** Dynamic changes and distinct roles of cardiac macrophage subsets in POH. Our studies have demonstrated a two-phase response of cardiac macrophages to pressure overload. (i) During the early phase of POH, there is robust local proliferation of resident macrophages that is crucial for cardiac compensation to pressure overload. (ii) During the late phase of POH, the infiltration of inflammatory monocytes increases, which promotes the transition to cardiac decompensation. Mechanistically, transcription factor KLF4 is required for resident macrophage proliferation and the infiltrating monocytes are Ly6C<sup>hi</sup>CX3CR1<sup>+</sup>CCR2<sup>+</sup> classic monocytes. Finally, this model implies that these two subsets of cardiac macrophages can be targeted for therapeutic gain, either by blocking monocytes (i.e., RS administration) or by promoting resident macrophages (i.e., prolonged proliferation), or by simultaneously targeting both subsets for a healthy balance.

and that expression of potent antiangiogenic factors, such as Thbs1, is increased in the K4-cKO hearts. The reduced expression of VEGFA is further confirmed in cardiac macrophages isolated from K4-cKO hearts. An intriguing and unanswered question relates to how the relatively small number of resident macrophages can impact angiogenesis throughout the heart. As macrophages often cluster around blood vessels, it is possible that they may release VEGF into the coronary circulation to impact the heart more broadly. Alternatively, the acute heart failure (<3 d) observed in macrophage-depleted animals (Fig. 3B) suggests that cardiac macrophages may also regulate arteriogenesis in POH as reported previously (34). Additional studies will be required to address these considerations. Finally, while we and others have also shown that KLF4 regulates macrophage polarization (24, 25), the present study excludes the significance of macrophage M1/M2 polarization in POH using two additional M2-deficient mouse models, namely, hematopoietic STAT6-KO and myeloid-specific IL4Ra-KO mice, which exhibited normal cardiac adaptation to POH despite lack of classic M2 macrophages (26, 35). We also note that Aurora et al. (32) have reported that cardiac resident macrophages mainly mediate angiogenesis rather than inflammation, with no clear bias toward M1 or M2 in a neonatal myocardial injury model.

In addition to resident macrophage proliferation in early-phase POH (<7 d), there is significant infiltration of classic Ly6C<sup>hi</sup> monocytes during late-phase POH (>4 wk). Infiltration of these tissue-extrinsic monocytes/macrophages takes place during the cardiac decompensation phase, suggesting a potentially negative role in POH. Consistent with this view, our studies in CCR2-KO mice showed that blockade of infiltrating macrophages (monocytes) preserved cardiac function, likely through improved myocardial angiogenesis (Fig. 5). How infiltrating macrophages affect angiogenesis remains to be fully understood. Infiltrating macrophages may trigger inflammation and other responses that negatively regulate angiogenesis (32). Alternatively, infiltrating macrophages may negatively impact resident macrophages, which are endowed with angiogenic capacity (15). It is noteworthy that cardiac resident macrophages also persist into and clearly are requisite for the late-phase POH (*SI Appendix*, Fig. S5). As such, cardiac resident and infiltrating



macrophage subsets may regulate POH through temporal and spatial activation and interaction between each other. In addition to their roles in angiogenesis as discussed above, cardiac resident macrophages have been reported to regulate electrical conduction of the heart (21). Although CL-mediated macrophage depletion is insufficient to induce arrhythmia in unstressed baseline hearts (21), it is possible that loss of resident macrophages may result in arrhythmia in a more susceptible substrate, such as the hypertrophied heart (36). For instance, some of the post-TAC mortality in CL-treated TAC animals might result from sudden death (Fig. 3C and *SI Appendix*, Fig. S5A).

Heart failure affects more than 26 million people worldwide and (37, 38) is associated with a devastating ~50% mortality within 5 y of diagnosis (39). Current therapies aimed at reducing neurohormonal stress and improving hemodynamics are only partially effective, underscoring the need for orthogonal strategies based on newly identified molecular and cellular mechanisms. The studies here highlight the differential impact of cardiac resident and nonresident macrophages in an experimental model of heart failure. Further, they suggest that inhibition of blood-borne mac-

rophages through blockade of the CCR2 pathway can be exploited for therapeutic gain.

## Materials and Methods

Detailed materials and methods can be found in *SI Appendix*. RNA-seq data are deposited in the Gene Expression Omnibus (accession no. GSE107016). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University School of Medicine and were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (40).

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