



Emerging Function of Cardiac Macrophages Ushers in a New Era for the Electrophysiology of the Heart

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Maintaining a coordinated heart rhythm is essential for maintaining the heart's pumping function and blood circulation. Every heartbeat is generated by electrical impulse propagation that is passing through gap junctions, which are composed of connexin proteins. In mammalian hearts, Cx43, Cx40, Cx45, and Cx30.2 are expressed and regulated by post-translational modification. Cardiac macrophages account for only a small number of total heart cells, but they reside all around the heart. They are primarily established prenatally, and they arise from embryonic yolk sac progenitors. Recently, increasing attention has been directed toward novel roles for cardiac resident macrophages, especially in the heart's electrical impulse conduction. Here, we provide an overview of the recent findings on connexins, with a focus on the emerging function of cardiac macrophages, and we discuss the future directions of treatment for heart disease.

Key Words: Arrhythmia; Atrioventricular node; Connexin43

Heart failure is a chronic disease and accounts for substantial morbidity and mortality worldwide.¹ Its prevalence is increasing because of population aging and improved treatment for acute cardiovascular events that lead to sudden death.² The prevalence of heart failure is estimated to be 1–3% in the adult population at large, and 5–9% selectively in those aged ≥ 65 years;^{3,4} the prevalence in the USA is projected to increase by 25% in the general population in the next 20 years.⁵

Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous disorder that lacks complete understanding, despite the increasing prevalence and attendant clinical and economic burdens. Clinical experience suggests that heart failure death in HFpEF is not classic pump failure, which is the case in heart failure with reduced ejection fraction (HFrEF). Rather, in many cases, it involves progressive pulmonary hypertension, right ventricular failure, and/or renal venous congestion and worsening renal function with ensuing multi-organ dysfunction. Increasing attention has recently been directed toward the role of the right ventricle because its dysfunction is common in HFpEF and is associated with mortality in heart failure patients.^{6,7}

Regarding the cause of death, worsening heart failure accounts for an approximately similar proportion, namely 20–30%, of total cardiovascular deaths both in HFpEF and in HFrEF. Sudden death accounts for up to 25–30% of deaths in the HFpEF population, whereas it constitutes 35–40% of all mortality events in the HFrEF population.⁸

QRS duration and morphology should be considered important prognostic information because it is indicative of more advanced cardiac pathology. Abnormal QRS duration and its morphology frequently identify subjects

with clinically undetected cardiac abnormalities and an increased risk of mortality.^{9,10} Post-myocardial infarction patients with prolonged QRS duration have a significantly increased risk of mortality, although data associating QRS prolongation specifically with sudden death are less supportive. In non-ischemic cardiomyopathy, there is no evidence that QRS duration carries prognostic significance for predicting mortality or sudden death because of limited observations.¹¹ In patients with heart failure and low ejection fraction, however, a significantly prolonged QRS duration, especially with the presence of left bundle branch block, predicts a benefit from cardiac resynchronization therapy.

The heart's electrical conduction system is vital for maintaining normal heart rhythm and cardiac function. Electrical impulses are generated at the sinoatrial node, and they propagate sequentially to the atrioventricular (AV) node for subsequent transmission to the ventricles via specialized conduction pathways. The electrical signals are conveyed from cell to cell through gap junctions, which are non-selective membrane pores that allow molecules $< 1,000$ Da to transit and thus provide electrical continuity between two cells.^{12,13} At the molecular level, gap junction hemichannels, which are called “connexons”, are integral membrane channels that are composed of six connexin molecules. The connexons converge at intercalated discs in clusters of hundreds to thousands and bind end to end with connexon hemichannels of apposing cells to form dense arrays of gap junction plaques. The plaques function as continuous conduits, allowing intracellular ions and other small molecules to pass freely between ventricular cardiomyocytes.

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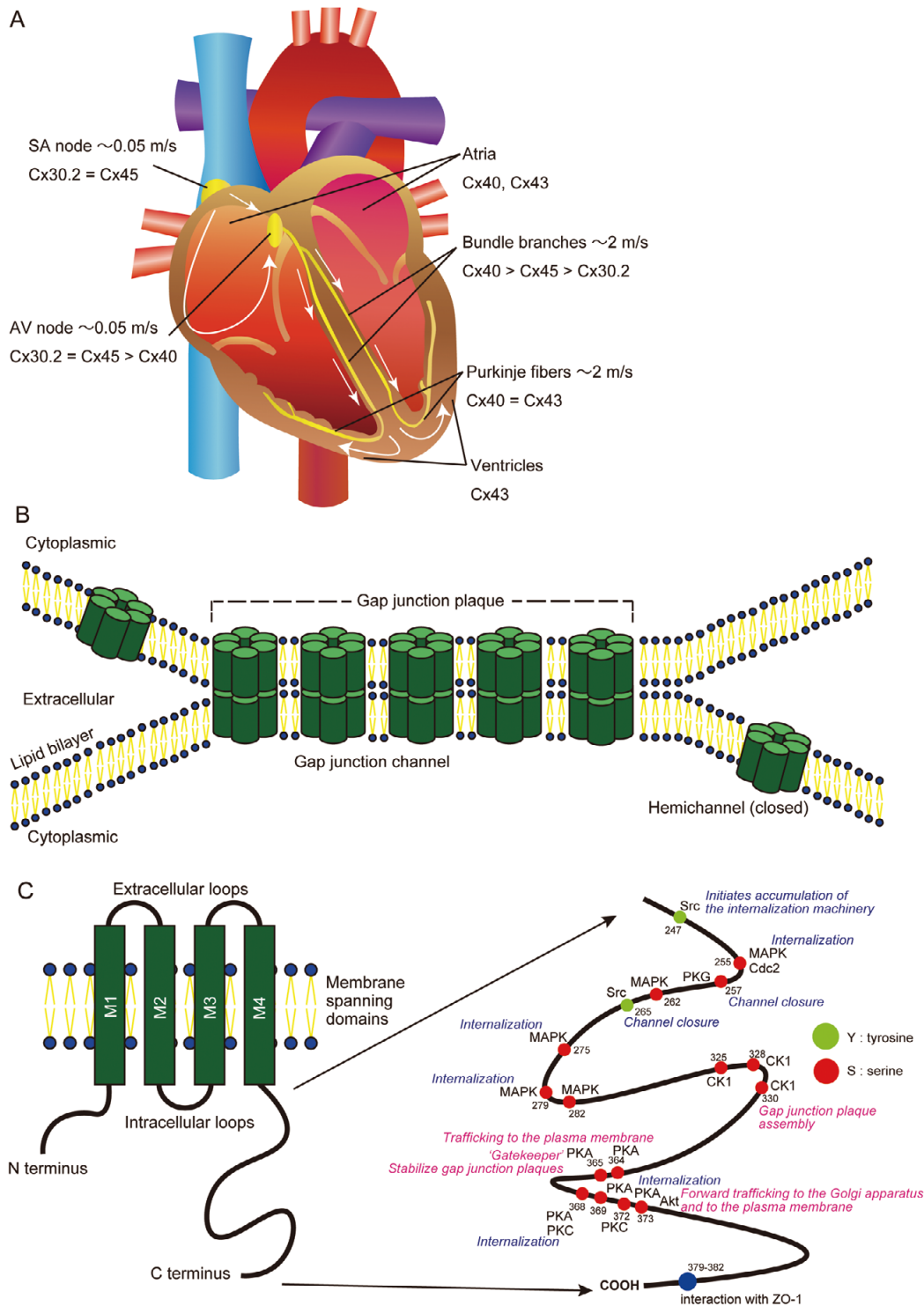


Figure 1. Cardiac connexins and their post-transcriptional modification. **(A)** Schematic diagram of the cardiac conduction system, showing the correlation between conduction velocity and connexin (Cx) expression. Note that connexin expression data are based on rodent experiments.^{21,24,33} **(B)** Schematic diagram of a gap junction plaque joining the cytoplasm of two adjacent cells. The opposed phospholipid bilayers are traversed by connexons that cluster and aggregate in the plane of the membrane to form a gap junction plaque. **(C)** Connexin protein subunits are tetra-spanning membrane proteins that share two extracellular loops. The subunits vary mainly in their cytoplasmic loop and carboxy-terminal regions. Connexin topology with phosphorylation sites and zonula occludens protein 1 (ZO-1) binding region. Green circles, tyrosine; red circles, serine. Pink font, increased gap junctional intercellular communication (GJIC); blue font, decreased GJIC. Cdc2, cyclin-dependent kinase 2; CK1, casein kinase 1; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G.

Connexins are named according to their respective molecular weight. The structural differences between them lie in the cytoplasmic loop and the carboxyl-terminal region. Each connexin subunit contains four hydrophobic transmembrane domains, consisting of two extracellular loops, one cytoplasmic loop, and one cytoplasmic N-terminal as well as a C-terminal region (Figure 1).

More than twenty isoforms of connexin have been recognized,^{14,15} and four of them contribute to the heart: Cx43, Cx40, Cx45, and Cx30.2. Different parts of the heart have varying requirements for the degree of electrical coupling that is needed. The large Purkinje myocytes are strongly coupled because the specialized ventricular conduction system needs to spread the activation wave rapidly over the ventricles. To create a delay between the atrial and ventricular contraction, a very low degree of coupling is required in the AV node. Similarly, to allow pacemaker function, pacemaker myocytes in the sinoatrial node need to be weakly coupled. Otherwise, the pacemaker would be silenced by the surrounding working myocardium.^{16,17}

In cardiac myocytes, connexins are preferentially located at the intercalated disc. They contribute to the anisotropic nature of cardiac impulse conduction.¹⁸ Cx43, which is the most abundant, has been found in almost all parts of the heart, except for the cells of the sinoatrial nodes. In contrast, Cx40 seems to be present specifically in the atrium and the ventricular conduction system. Cx45 is less abundant and is preferentially present in the conduction system.^{19–21} In mice, Cx30.2 is expressed in the cardiac conduction system, predominantly in the sinoatrial node and the AV nodes.²² The human ortholog of Cx30.2, the Cx31.9 protein, however, is not detectable and is unlikely to contribute to either the impulse generation and conduction system or the working myocardium of the human heart.²³ In cardiomyocytes, single-gap junction channel conductance ranges from 9 picosiemens (pS) for Cx30.2²⁴ to approximately 20 pS for Cx45 channels to 45–75 pS for Cx43 channels and approximately 200 pS for Cx40 channels. These conductance values depend on pH,²⁵ on extracellular $[Ca^{2+}]$,^{26–28} on extracellular fatty acid composition,^{29,30} and on the phosphorylation state of the connexins.³¹

Cardiac Cx43 has a relatively short half-life, on the order of 1–5 h, and <2 h at the plasma membrane.^{32,33} This suggests that both the synthesis and the degradation of a gap junction are dynamic processes, and that regulation of protein stability may be a major mechanism of gap junction remodeling. Cx43 is translated in the rough endoplasmic reticulum (ER); it undergoes oligomerization in the post-ER/Golgi compartment, after which Cx43-containing vesicles are thought to be transported to the periphery of existing gap junctions at the plasma membrane. Undocked connexons aggregate into the gap junction in a zonula occludens-1 (ZO-1)-dependent manner.³⁴ Intracellular scaffolding proteins, such as ZO-1, anchor Cx43 via its c-terminus and regulate the gap junction's plaque size. Connexon internalization from the plasma membrane is also an important regulatory step in determining the level of gap junction coupling.

Normal gap junction communication is required for simultaneous initiation of action potentials of cardiomyocytes and an organized heart contraction. Alterations in gap junction coupling occur with many forms of heart disease. These coupling alterations lead to defects in electrical excitation that can result in malignant arrhythmias and sudden cardiac death.

Gap junctional intercellular communication (GJIC) is regulated by post-translational regulation of Cx43, either through direct control of channel activity or by modulating protein-protein interactions and Cx43 localization. Phosphorylation is by far the most well-studied modification, with several studies demonstrating its instrumental role upon regulation of channel gating, trafficking, assembly/disassembly, and degradation of gap junction channels.^{35–37} At the C-terminus of Cx43, 21 phosphorylation sites (19 serine residues and 2 tyrosine residues) that are regulated by the action of more than 10 kinases and phosphatases, including protein kinase C (PKC), protein kinase A (PKA), mitogen-activated protein kinases, Src kinases, and protein phosphatase 2A, have been described.^{38,39}

The lifecycle of Cx43 is regulated in a well-balanced manner by specific phosphorylation and dephosphorylation. For example, phosphorylation at S364 and/or S365 regulates its trafficking to the plasma membrane, contributing to increased GJIC, whereas phosphorylation of S368 results in Cx43 internalization and the downregulation of GJIC.^{40,41} Under pathological conditions, such as myocardial ischemia and wound healing, the phosphorylation of the residue S373, followed by simultaneous phosphorylation of S279, S282, and Y247, induces an acute increase in gap junction size, followed by rapid internalization of Cx43 and the downregulation of GJIC.³⁴

Notably, myocardial ischemia constitutes a paradigmatic example of the dramatic alteration of the dynamics of Cx43 phosphorylation, which affects channel conductance and localization. During ischemia, the phosphorylation of S325, S328, and/or S330, which are restricted to the intercalated disk, is lost.⁴² In addition, dephosphorylation of S365, which is called the GJIC gatekeeper, takes place, enabling subsequent phosphorylation of S368 by PKC, which negatively affects the electrical coupling between cardiomyocytes.^{43,44} Ischemia-induced phosphorylation of S373 also creates a so-called mode-1 binding domain for 14-3-3 proteins, which drives the internalization of Cx43.⁴⁵

Cx43 lateralization has been implicated in pathological cardiac conditions that are associated with a decrease in electrical coupling. The mechanisms that underlie Cx43's lateralization remain largely unknown. One possible explanation is that the activation of Src kinase and its interactions with Cx43 and ZO-1 may contribute to lateralization.^{46,47} Src activation leads to the separation of Cx43 from ZO-1, and gap junction plaque moves away from the intercalated disks to the lateral membranes. Another possibility is related to the acetylation of Cx43.⁴⁸ Colussi et al found that Cx43 is lateralized, acetylated, and co-immunoprecipitated with the acetylase P300/CBP-associated factor in a model of Duchenne cardiomyopathy (spontaneous *Dmd*^{mdx} mutant mice).⁴⁸

Cx43 dephosphorylation is a characteristic of ischemia, arrhythmia, and of a failing/aging myocardium.⁴⁹ When gap junctional coupling decreases, conduction becomes highly discontinuous, and a propensity toward reentrant arrhythmias increases. In addition, gap junctional uncoupling can increase the incidence of arrhythmic triggers and their propagation into an adjacent myocardium.⁵⁰

Macrophages are a type of white blood cell that engulfs and digests infectious agents, cellular debris, and foreign substances. The canonical function of circulating and resident macrophages is to provide innate immune surveillance of individual organs. This paradigm, however, was questioned regarding the origin of macrophages. A sub-

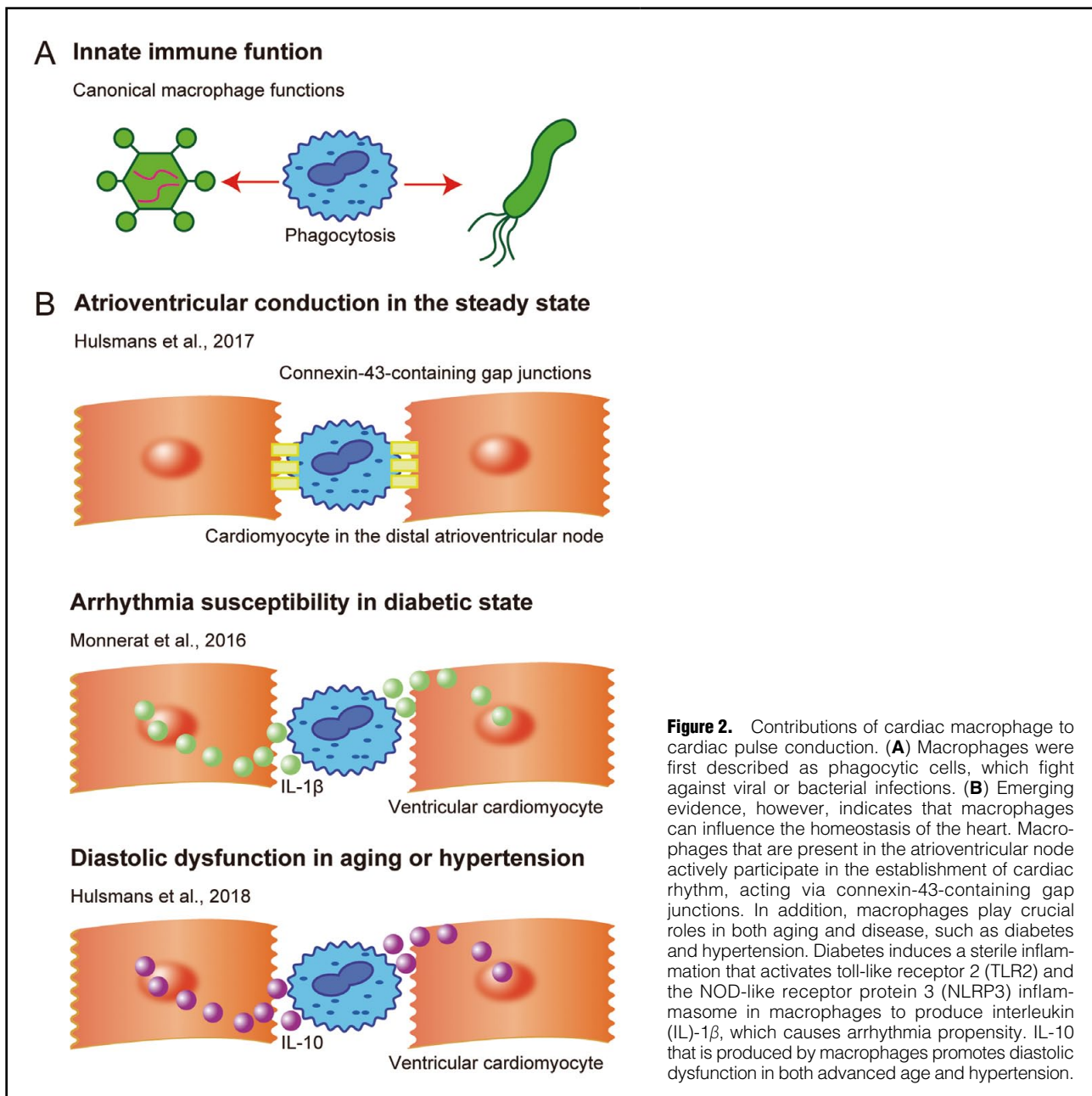


Figure 2. Contributions of cardiac macrophage to cardiac pulse conduction. **(A)** Macrophages were first described as phagocytic cells, which fight against viral or bacterial infections. **(B)** Emerging evidence, however, indicates that macrophages can influence the homeostasis of the heart. Macrophages that are present in the atrioventricular node actively participate in the establishment of cardiac rhythm, acting via connexin-43-containing gap junctions. In addition, macrophages play crucial roles in both aging and disease, such as diabetes and hypertension. Diabetes induces a sterile inflammation that activates toll-like receptor 2 (TLR2) and the NOD-like receptor protein 3 (NLRP3) inflammasome in macrophages to produce interleukin (IL)-1 β , which causes arrhythmia propensity. IL-10 that is produced by macrophages promotes diastolic dysfunction in both advanced age and hypertension.

population of macrophages that originates from embryonic tissue does not come through the bloodstream; they reside and proliferate in virtually all body tissues, including the brain, spleen, liver, lung, bone marrow, kidney, pancreas, and peritoneum. They are seeded before birth, can maintain themselves in adults by self-renewal,⁵¹ and can act specifically on each organ. For example, resident macrophages of adipose tissue contribute to the regulation of thermogenesis,⁵² of the spleen and liver for iron recycling,⁵³ and of the brain to participate in the process of synaptic maturation.⁵⁴ Such non-canonical roles emphasize the complex physiology of macrophages and their ability to perform specific tasks, depending on their microenvironment, in addition to their host defense ability.⁵⁵

Resident cardiac macrophages in mice account for approximately 5–10% of non-myocytes in the heart, and

the vast majority originate from embryonic yolk sac and fetal liver progenitors. They appear spindle-shaped and are intercalated between cardiomyocytes, fibroblasts, and endothelial cells.⁵⁶ Replenishment occurs at the rate of approximately once per month via proliferation. CCR2⁺ macrophages are replenished by blood monocyte recruitment and local proliferation, whereas CCR2⁻ macrophages are repopulated largely by local proliferation.⁵⁷

Recently, our view of cardiac conduction has been refined due to revelations regarding cardiac macrophages (**Figure 2**). Hulsmans et al found that cardiac macrophages facilitate electrical conduction through the distal AV node.⁵⁸ Cx43-containing gap junctions connect macrophages with conducting cardiomyocytes in the distal AV node. This coupling leads to cyclical depolarization of macrophages and modulates the electrical activity of cardiomyocytes.

Enhanced macrophage-cardiomyocyte interaction improves normal AV nodal conduction, whereas reduced interaction leads to aberrant AV node conduction.

Monnerat et al demonstrated that, in diabetic mice, toll-like receptor 2 (TLR2) and NOD-like receptor protein 3 (NLRP3) inflammasome are activated in cardiac macrophages, resulting in the secretion of interleukin (IL)-1 β .⁵⁹ IL-1 β -caused prolongation of the action potential duration induces a decrease in potassium current and an increase in calcium sparks in cardiomyocytes, which will cause arrhythmia propensity. Treatment with either an IL-1 receptor antagonist or inhibition of the NLRP3 inflammasome rescued the phenotype.

Cardiac macrophages also offer clues regarding the development of HFpEF. Hulsmans et al found that both in aged mice and hypertensive mice, cardiac macrophages produced IL-10, activated fibroblasts, and stimulated collagen deposition, leading to impaired myocardial relaxation and increased myocardial stiffness. Deletion of IL-10 in macrophages improved diastolic function.⁶⁰

Conclusions

Elucidation of the novel roles of cardiac macrophages has opened new avenues for possible therapeutic interventions. Targeting macrophage function would be a more effective means by which to tackle arrhythmias and diastolic dysfunction than would targeting cardiomyocytes or fibroblasts. Therefore, as a next step, we need to know whether macrophage dysfunction leads to AV block, arrhythmia propensity in diabetes patients, and diastolic dysfunction in humans. If macrophage function is linked to cardiac function in humans, reprogramming macrophages in situ with antibodies, such as CSF-1R, CD68, or scavenger receptors, could be a viable form of immunotherapy.

Disclosures

The authors declare no conflicts of interest.

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