JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 8, 2021

EDITORIAL COMMENT

When Sensing Goes Wrong

Role of Clec4e in Ischemic Heart Injury*

Murilo Delgobo, PhD,[a,b] Stefan Frantz, MD[a,b]

The immune system has been continually in the spotlight of cardiovascular research, standing at the crossroads of homeostatic myocardial maintenance and deleterious adverse remodeling (1). During an acute myocardial infarction (MI), for instance, the injured myocardium readily mobilizes local inflammatory and immune responses that are indispensable to bring about tissue repair. However, uncontrolled, myocardial inflammation can also cause further damage and fuel adverse remodeling (1). The exact molecular signals that trigger local immune-inflammatory responses in the injured myocardium remain an important unsolved problem in this field, though. MI is a sterile condition that markedly differs from the canonical immune activation during infections. Understanding how tissue damage (or danger) is sensed under such conditions could provide novel druggable receptors and pathways to fine-tune myocardial inflammation under disease conditions. In this issue of *JACC: Basic to Translational Science*, Veltman et al (2) shed some light in this field by showing that the C-type lectin domain family 4 member E (CLEC4), a pattern-recognition receptor normally expressed on immune cells and activated in bacterial infection, also sustains cardiac injury and adverse left ventricular (LV) remodeling in experimental models of myocardial ischemia/reperfusion (I/R) injury.

CLEC4E was first described as a receptor for the mycobacterial cell wall glycolipid (cord factor) (3). In addition to its role in infectious diseases, recent studies have shown that CLEC4E can recognize Sin3-associated protein 130, released during necrotic cell death, cholesterol crystals, and β-glucosylceramide released by damaged cells (3). Thus, cell damage could be sensed by CLEC4E and trigger an immune response. In the present study (2), the investigators built up on own previous observations indicating that *CLEC4E* transcripts are up-regulated in circulating cells from patients with acute MI to further investigate their roles in experimental models of myocardial I/R injury in pigs and mice, alongside further analyses of human biomaterial.

First, the investigators observed an early increase in myocardial expression levels of *CLEC4E* in pigs subjected to I/R injury. Moreover, the *CLEC4E* expression levels positively correlated with the infarct size, assessed by magnetic resonance imaging. Next, taking advantage of a transgenic mouse model, Veltman et al observed that global *Clec4e* deficiency is associated with reduced troponin release, infarct size, and neutrophil infiltration in mice subjected to myocardial I/R injury. Interestingly, *Clec4e* expression was detected in multiple cell types within the infarcted myocardium, including neutrophils (defined as myeloperoxidase, MPO^+ cells), smooth muscle cells (α-SMA^+), cardiomyocytes ($MLC2^+$), and macrophages ($CD68^+$). The absence of CLEC4E signaling not only improved early myocardial recovery, but also resulted in a better preserved LV function, analyzed by cardiac magnetic resonance imaging, 4 weeks after the I/R injury. Next, to gain mechanistic insights that could explain the observed phenotype of $Clec4^{-/-}$ mice, the investigators focused on the reduced migration of neutrophils to the injured heart in knockout mice. They observed an increased expression of G protein-coupled receptor kinase 2 (*Grk2*) in circulating

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the [a]Department of Internal Medicine I, University Hospital Würzburg, Würzburg, Germany; and the [b]Comprehensive Heart Failure Center, University Hospital Würzburg, Würzburg, Germany.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2021.07.003

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 8, 2021
AUGUST 2021:647–649

neutrophils from *Clec4e*$^{-/-}$ mice, but not wild-type mice, after I/R injury, a gene shown to mediate CXCR2 receptor desensitization and internalization. Impaired neutrophil migration to CXCL2 chemokine was indeed observed in neutrophils from *Clec4e*$^{-/-}$ mice, suggesting an intrinsic defect of neutrophil migration to this chemokine. As an unbiased approach, Veltman et al (2) also performed bulk RNA sequencing from LV tissue of wild-type and *Clec4e* knockout mice. The analyses revealed an enrichment for genes involved in extracellular matrix and collagen biosynthesis/assembly in *Clec4*$^{-/-}$ mice. Lastly, elevated *CLEC4E* expression was confirmed in the blood of patients with ST-segment elevated MI when compared with control patients with coronary artery disease. In MI patients, *CLEC4E* expression levels also correlated with troponin release and ejection fraction. Moreover, the investigators validated this finding in a separate cohort of 138 patients with acute MI, suggesting that *CLEC4E* expression may hold translational relevance.

The study of Veltman et al (2) advances our knowledge on molecular circuits that fuel myocardial inflammation upon ischemic injury. Several important questions remain to be addressed, though. A more detailed understanding of which immune cell subsets express CLEC4E in the heart, at baseline and post-injury conditions, remain elusive. In addition, a more comprehensive immunophenotyping of the leukocytes infiltrating the ischemic hearts in *Clec4e*$^{-/-}$ mice is required to fully dissect the impact of this pattern recognition receptor signaling in myocardial diseases. The use of global genetic ablation also leaves important questions unanswered. Besides neutrophils, MPO can be also expressed by Lyc6^{Hi} monocytes (4), meaning that other cell types might also account for the observed phenotypes. Recent studies have precisely characterized the heart immune compartment, allowing researchers to differentiate heart-resident from bone marrow-derived immune cells (5). This approach has proven to be extremely useful to distinguish cell types involved in physiological functions from others related to acute inflammatory response. CLEC4E has been shown to be expressed on distinct immune cell types present in the injured myocardium, and on smooth muscle cells in the viable remote myocardium. It is therefore likely that cardiac-resident cells expressing CLEC4E could be among the frontline responders to danger-associated molecular patters released by injured cardiomyocytes. The use of conditional genetic ablation and eventually the use of bone-marrow chimera models might help future studies to dissect cell type-dependent effects and discriminate the differential contribution of resident versus blood-borne cells to the phenotype observed in *Clec4e*$^{-/-}$ mice. Moreover, future studies might elucidate which cell types and effector functions are induced by CLEC4E activation and whether this receptor signaling could be pharmacologically targeted in patients with acute coronary syndrome. The observed *CLEC4E* expression at a single time point in acute coronary patients already hints that the experimental data might be translatable under clinical conditions. However, serial measurements might be necessary to clarify whether CLEC4E is simply a marker for tissue damage or also reflects the post-infarction inflammatory responses over a longer time period.

The study by Veltman et al (2) opens important avenues in pattern-recognition sensing during sterile myocardial injuries. In the case of CLEC4E receptor, the results clearly illustrate a novel and detrimental role in infarct size and LV function at early and late stages following I/R injury, with leukocyte migration and collagen deposition as possible mechanisms impacting function. The positive correlation between *Clec4e* and troponin levels in small and large animal models, and in MI patients strongly suggests that this pattern-recognition receptor holds potential to be further investigated as a biomarker in patients with acute coronary syndrome and in chronic heart failure. The study by Veltman et al (2) identifies a pattern-recognition receptor that plays a crucial role in initiating sterile inflammation in ischemic hearts. The quest for identifying which endogenous ligands engage to this receptor in the injured heart remains open. Future studies addressing those questions will strengthen our understating of innate immune sensing and may help identifying new druggable targets in cardiovascular diseases.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Stefan Frantz, Department of Internal Medicine I, University Hospital Würzburg, Oberdürrbacher Strasse 6, Würzburg, 97080, Germany. E-mail: Frantz_S@ukw.de.

REFERENCES

1. Swirski FK, Nahrendorf M. Cardioimmunology: the immune system in cardiac homeostasis and disease. *Nat Rev Immunol*. 2018;18:733–744.

2. Veltman D, Wu M, Pokreisz P, et al. Clec4e-receptor signalling in myocardial repair after ischemia-reperfusion injury. *J Am Coll Cardiol Basic Trans Science*. 2021;6(8):631–646.

3. Chiffoleau E. C-type lectin-like receptors as emerging orchestrators of sterile inflammation represent potential therapeutic targets. *Front Immunol*. 2018;9:227.

4. Swirski FK, Wildgruber M, Ueno T, et al. Myeloperoxidase-rich Ly-6C+ myeloid cells infiltrate allografts and contribute to an imaging signature of organ rejection in mice. *J Clin Invest*. 2010;120:2627–2634.

5. Epelman S, Lavine KJ, Beaudin AE, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*. 2014;40:91–104.

KEY WORDS CLEC4E, inflammation, ischemia-reperfusion injury, myocardial remodeling, magnetic resonance imaging