

EDITOR'S PAGE



Neutrophils in Myocardial Infarction



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Neutrophils are the most common leukocytes, making up more than one-half of all white blood cells in humans. Currently, neutrophils—and leukocytes in general—are rarely on the general cardiologist's radar but this is very likely to change. Here, we discuss recent basic science discoveries that motivate a cardiology spotlight on neutrophils. We argue that these cells' actions are at the center of a large unmet medical need, specifically arrhythmia, a condition that is managed today without drugs that address known and emerging underlying pathways. Much is happening at the intersection of cardiology and immunology, and *JACC: Basic to Translational Science* will pay particular attention to this area, which is aptly termed *cardioimmunology*.

Because neutrophils circulate for less than a day before they die, their production is a primary task of the bone marrow, where blood stem cells and

downstream myeloid progenitors produce vast quantities of neutrophils every day. Neutrophils excel at rapidly neutralizing infectious threats and sterile injuries. They are the first leukocytes in line to combat infection by swiftly deploying powerful tools, including reactive oxygen species that inflict chemical damage and neutrophil extracellular traps that capture and neutralize bacteria.¹ After phagocytosis, neutrophils employ proteases and reactive oxygen species to kill bacteria. All neutrophil defense mechanisms are available immediately and can forestall a threat until the host mounts more specific defenses, ie, antibodies produced by lymphocytes. However, the immediacy of neutrophil action comes at a price: none of the neutrophil defense mechanisms are specific (compared with the slower, highly specific adaptive immune response), and there is a considerable risk that neutrophil weaponry impacts host cells² rather than bacteria and fungi.

A recent *Science* publication³ explored the potential for neutrophil auto-aggression in resistin-like molecules (RELMs), until now lesser-known members of the neutrophil defense protein arsenal. RELMs are pore-forming proteins that kill bacteria. During infections, pore-forming proteins bind negatively charged lipids in the bacterial wall and insert themselves into the bacterial membrane to disrupt it.^{4,5} The resulting membrane defect allows free exchange of ions and water, consequently killing the bacteria. Kumowski et al³ investigated RELMs because resistin-like molecule γ (RELMy, gene name *Retnlg*) was among the top differentially expressed

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neutrophil genes after acute myocardial infarction (MI) in mice and because prior studies⁶ had documented the proarrhythmic action of neutrophils in mice and humans with MI. *Retnlg* knockout mice had 12-fold less ventricular tachycardia, as measured with implanted telemeters, in the first 24 hours after MI.³ Arrhythmia reduction was reproduced after neutrophil-specific deletion of the *Retnlg* gene. The profoundly decreased arrhythmia after *Retnlg* knockout led to subsequent studies into its mechanism of action. Patch clamp analysis of mouse cardiomyocytes exposed to RELM γ in a dish revealed delayed afterdepolarizations, which are smaller cell charge fluctuations that are commonly considered a powerful trigger of ventricular arrhythmia if they are ill-timed.⁷ Such afterdepolarizations could be caused by membrane defects similar to those that defense proteins produce in bacteria, a connection that motivated studies of membrane disruption by RELM γ in model systems and cells. Both mouse RELM γ and its human homolog resistin disrupted liposomes that featured phosphatidylserine in their outer membrane surface. Phosphatidylserine, a negatively charged phospholipid and a potential binding spot for RELMs, is absent from the outer layer of mammalian cell membranes but flips to the surface if cells are stressed. Fluorescent cell microscopy confirmed that RELM γ binds to phosphatidylserine on the cardiomyocyte surface and that the RELM γ binding site serves as an entry point for cell exclusion dyes, thereby indicating that RELM γ instigates cardiomyocyte membrane leakage. Adding a fluorescent calcium sensor increased intracellular calcium signal in the vicinity of RELM γ binding. Interestingly, time lapse imaging documented that the RELM γ binding site served as an origin of calcium waves traveling through—and likely depolarizing—the entire cardiomyocyte. Super-resolution microscopy, which visualized single fluorescently labeled RELM γ proteins, showed dozens of RELM γ molecules on distinct cardiomyocyte surface areas, an observation consistent with the so-called “carpet-like” membrane pore model. This carpet model implies that pore-forming proteins do not necessarily form superstructures; rather, the molecules embed in membranes in a less-ordered fashion.⁸ After peptides cover a membrane area like a carpet, the hydrophobic peptide tails are thought to insert into the bilayer and thus destroy the membrane. Imaging data indicated that RELM γ damages the cardiomyocyte membrane and that the resulting pores facilitate cell depolarization, likely because positively charged ions can freely enter the cell interior through RELM γ -formed pores. Normally, ion traffic across the cardiomyocyte membrane is

strictly regulated and carefully timed by dedicated ion channels and pumps that actively maintain appropriate gradients between the intracellular and extracellular space. However, after pores form, positively charged ions such as calcium may freely enter cardiomyocytes, destabilizing the resting membrane potential until depolarizations occur, and these irregular cellular charge fluctuations may give rise to ventricular tachycardia. Although the cells initially survived such membrane attacks, likely because they compensate with repair attempts, they eventually died. Investigations of various cell death mechanisms found that RELM γ inflicts cell death via necrosis—just as defense proteins do when attacking bacteria—rather than via any form of programmed cell death.

Because these aforementioned neutrophil actions represent an immune response gone wrong, it is worthwhile considering why it went wrong and where else this may matter. Three major events likely caused the immune cell misfire: 1) ischemia-induced danger signals attracted numerous neutrophils to the usually neutrophil-free myocardium; 2) stressed cardiomyocytes displayed phosphatidylserine on their outer membrane; and 3) ischemia created the acidic environment that pore-forming peptides need. Such conditions are typical for infection but also occur during sterile inflammation inside and outside the heart. When the authors investigated ischemic stroke in mice, RELM γ knockout mice had reduced ischemic brain damage, thereby demonstrating that RELMs may play a role in other inflammatory conditions that involve neutrophils.

Why are these basic science observations important? Because sudden cardiac death is among the most frequent causes of death worldwide, and modern medicine's tools to combat its root causes are insufficient. Although rapid defibrillation can save lives, it will not prevent arrhythmia recurrence. The work described here is one of the first descriptions of how immunity may influence conduction and arrhythmia. The clinical association of inflammatory events such as sepsis and surgery with atrial and ventricular arrhythmia supports a focus on immune cells, and such focus needs to go beyond neutrophils, which are likely absent in many clinical arrhythmic scenarios. Although Kumowski et al³ addressed acute MI, previous reports on neutrophil-derived myeloperoxidase in atrial fibrillation⁹ indicate that these cells and their actions may be more broadly important.

Fortunately, we are now learning more about neutrophils and other leukocytes, and these insights will shape translational efforts. Prior work¹⁰ has

revealed that, in mice, wholesale neutrophil depletion expands myocardial fibrosis and increases ventricular size and is thus detrimental for post-MI recovery. Mechanistically, neutrophils influence infarct healing by altering macrophage phenotypes and their functions, including their ability to remove cell debris via phagocytosis.¹⁰ From single cell transcriptomics^{11,12} we are learning that neutrophils comprise various cell subsets that produce RELM γ to different extents, although such subsets may mostly reflect neutrophil maturation and aging stages.¹³ It is clear that, in the clinic, arrhythmia cannot be prevented by depleting all neutrophils; rather, we should consider targeting neutrophil subsets or their specific products.

Like in MI, many conditions that are not typically considered part of the autoimmune disease spectrum may actually be driven by immune cell autoaggression. Immune cells are ubiquitously involved in supporting health and promoting disease. Studying leukocytes and their actions has revolutionized clinical care for cancer patients, many of whom now receive checkpoint inhibitor treatment that successfully enhances adaptive immune cell action against malignant cells. Broadly inhibiting immune functions is doomed to fail, either because target cells and

pathways have diverse functions in the specific condition or because neutralizing them compromises essential defense functions. We should also pause to consider the long history of failure to translate basic discoveries about pathways that promote ischemia-reperfusion injury. Learning from these lessons, we strive to better understand immunity in cardiovascular organs because precise drug actions are needed for effective therapeutic immunomodulation in arrhythmia and beyond.

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