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**Misbehaving Guests in the Right Ventricle**

**Macrophage–NLRP3 Activation in Pulmonary Hypertension**

Excessive inflammation has been linked to the development of right ventricle (RV) failure in pulmonary arterial hypertension (PAH) (1, 2). However, mechanisms of inflammation initiation and propagation during RV failure development are not entirely elucidated. The nucleotide-binding domain, leucine-rich containing family, and pyrin domain-containing protein 3 (NLRP3) inflammasome is a mediator of organ dysfunction in several conditions marked by inflammation or cellular stress (3, 4). On priming by damage-associated or pathogen-associated molecular patterns and activation by a variety of additional stimuli, NLRP3 employs ASC (apoptosis-associated speck-like protein) to form an NLRP3–ASC complex (Figure 1). This complex then recruits and activates caspase 1, which subsequently cleaves pro–IL-1β and pro–IL-18 to activate IL-1β and IL-18, respectively. Activated IL-1β and IL-18 are then released from the cell with the help of the pore-forming protein gasdermin D (also activated and cleaved by NLRP3–ASC–caspase 1) to induce pyroptosis, an inflammatory-type of lytic programmed cell death. Although NLRP3 activation and pyroptosis frequently occur during infections with intracellular pathogens, NLRP3 activation may also occur in the setting of sterile inflammation. For example, the NLRP3 inflammasome is activated in left heart failure and has been linked to the development of contractile dysfunction (5).

Emerging evidence suggests that the NLRP3 inflammasome is activated in the pulmonary vasculature in models of PAH (6). This is not surprising because triggers of the NLRP3 inflammasome, such as potassium efflux, calcium influx, and altered mitochondrial reactive oxygen species generation, are common in vascular cells in PAH (7). However, it remains unknown if NLRP3 inflammasome activation also occurs in the RV. In light of data on inflammasome activation in the left ventricle (LV), and given the observation that patients with severe PAH exhibit macrophage infiltrates in the RV (8), it is conceivable that NLRP3 may also be activated in the RV and contribute to RV maladaptation.

In this issue of the *Journal*, Al-Qazazi and colleagues (pp. 608–624) test the hypothesis that RV inflammation, driven by activation of the NLRP3 inflammasome in recruited macrophages, is a contributor to RV maladaptive remodeling in experimental pulmonary hypertension (9). The authors demonstrate that M1-polarized, monocyte-derived, CCR2+ macrophages are increased in RVs (but not LVs) of rats with monocrotaline- or sugen/hypoxia-induced pulmonary hypertension and highly express NLRP3. On the other hand, in a rat model of pulmonary artery banding without RV failure, NLRP3 signaling was not upregulated. Cultured monocytes from monocrotaline pulmonary hypertension rats exhibit NLRP3 activation and mediate mitochondrial damage in neonatal rat cardiomyocytes cocultured with these cells. The altered cardiomyocyte phenotype was prevented when the coculture systems were cotreated with the NLRP3 inhibitor MCC950. In vivo, MCC950 reduced RV NLRP3 activation and attenuated pulmonary vascular remodeling, hemodynamic alterations, and RV dysfunction. In RV tissues from patients with PAH with decompensated RV function, there was evidence of macrophage NLRP3 pathway upregulation compared with control subjects. Together, these data demonstrate...
Figure 1. Overview of the NLRP3 (nucleotide-binding domain, leucine-rich–containing family, and pyrin domain-containing protein 3) inflammasome signaling pathway. NLRP3 inflammasome signaling consists of three major steps. In the priming step, DAMPs, such as HMGB1 (high mobility group box protein 1) or IL-1β, as well as PAMPs, such as LPS, interact with cell membrane receptors such as TLR4 (Toll-like receptor 4), IL-1R (IL-1 receptor), or IL-6R to activate the transcription factor NF-κB (nuclear factor κB). NF-κB then translocates to the nucleus to increase transcription of the inflammasome components NLRP3, ASC (apoptosis-associated speck-like protein containing an caspase recruitment domain), and procaspase 1, as well as the effector precursors, pro–IL-1β and pro–IL-18. In the activation step, the NLRP3 inflammasome is activated by factors such as extracellular ATP, bacterial pore-forming toxins, or mitochondrial reactive oxygen species (ROS). Potassium efflux and calcium influx are also involved in this step. On activation, NLRP3 recruits and forms a complex with ASC as well as procaspase 1. In the final step, the assembled inflammasome platform cleaves pro–IL-1β and pro–IL-18 to activate IL-1β and IL-18, respectively. The NLRP3 inflammasome also activates the pore-forming protein GSDMD (gasdermin D), resulting in membrane pore formation, cytokine release, and, ultimately, pyroptosis. This pathway is best described in immune cells but has also been identified in nonimmune cells. DAMPs = danger-associated molecular patterns; PAMPs = pathogen-associated molecular patterns.

that NLRP3 activation in recruited macrophages contributes to RV dysfunction in experimental pulmonary hypertension and that NLRP3 inhibition attenuates pulmonary hypertension development and RV failure.

The study by Al-Qazazi and colleagues contains several strengths: 1) NLRP3 signaling was investigated in several pulmonary hypertension and RV remodeling models (including models of decompensated and compensated RV function); 2) RV tissues from patients with PAH were employed, and endpoint analysis was thorough and comprehensive. The aggregate of data presented suggests a paradigm in which CCR2+ macrophages are recruited to the pressure-overloaded RV, exhibit NLRP3 activation, and contribute to RV cardiomyocyte dysfunction and RV maladaptation. These data, therefore, suggest a similar paradigm as in patients with LV failure, in which CCR2+ macrophage accumulation is associated with maladaptive remodeling and systolic dysfunction (10).

Although innovative and intriguing, the study by Al-Qazazi and colleagues also raises several important questions. First, whereas NLRP3 inhibition with MCC950 attenuated pulmonary hypertension and RV failure phenotype, it remains unknown if improvements in RV structure and function were because of direct effects in the RV or were indirectly mediated because of lowering of RV afterload. Studies using pressure-volume loops to assess RV–pulmonary artery coupling or studies in a pulmonary artery banding model with RV failure development and maladaptive RV remodeling would be needed to answer this clinically important question. Second, whereas the studies in human RV tissues are exciting, more needs to be learned about the time course and variability of NLRP3 activation in the RV. For example, is NLRP3 activation seen only in end-stage tissues? And is RV inflammasome activation seen in all types of PAH/pulmonary hypertension, or is it specific to selective subtypes (e.g., patients with a higher degree of inflammation and immune activation)? Third, because the current study focused on male animals and because PAH is a sexually dimorphic disease in which women have been shown to exhibit better RV adaptation (11), this pathway may be less active in females. Lastly, the mechanism responsible for the recruitment of CCR2+ macrophages into the RV remains unknown. For example, this could be because of preexisting inflammation, mitochondrial reactive oxygen species generation, or other stimuli. Are there “stress signals” released from the RV? And is CCR2+ macrophage influx an attempt at tissue repair, or does it serve another purpose?

The observations made by Al-Qazazi and colleagues suggest that interventions targeting CCR2+ macrophages and/or NLRP3...
signaling may represent a novel approach to suppressing inflammation and adverse remodeling in PAH and RV failure. Because there is also evidence of NLRP3 activation in the pulmonary vasculature (6), NLRP3 inhibition may represent a novel treatment strategy to target both the lung as well as the RV, thus tackling the entire cardiopulmonary axis in PAH. Of note, several therapies targeting components of the NLRP3 signaling axis are currently in clinical trials for PAH or other diseases. For example, a pilot study with the recombinant IL-1 receptor antagonist anakinra in PAH demonstrated reduced levels of inflammatory plasma markers (12).

In the LVs, CCR2^− macrophages are more proinflammatory than CCR2^+ macrophages (10) and therefore represent an interesting therapeutic target. A similar paradigm may emerge in the RV. However, more work needs to be done to characterize initiators and dynamics of macrophage recruitment into the RV. Because mouse and human CCR2^− macrophages are functionally analogous (10), mechanistic studies investigating how mouse CCR2^− macrophages are activated to exert their proinflammatory and proremodeling effects will likely lead to further knowledge. Tools such as lineage tracing and single-cell RNA sequencing may provide important insights. Such studies likely will lead to a better understanding of the mechanisms of inflammatory cell recruitment and immune activation in the RV. Ultimately, this may lead to the development of novel strategies to attenuate the proinflammatory and proremodeling properties of these cells in patients with PAH or other types of pulmonary hypertension.

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