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# Inflammation, Inflammasome Activation and AF: Evidence for Causation and New Therapeutic Targets

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Markers of inflammation have been linked to atrial fibrillation (AF), but whether inflammation is causal or merely a marker of increased risk has long been debated. The association between AF and inflammation was suggested over 20 years ago by studies of postoperative AF occurring with high frequency (20–50%) after cardiac surgery. The incidence of postoperative AF peaks 2–3 days after surgery, temporally associated with activation of systemic inflammatory pathways, with early increases in plasma interleukin-1β (IL-1β), followed by IL-6 and C-reactive protein (CRP), a sensitive, but non-specific marker of systemic inflammation [1]. Elevation of CRP has also been associated with non-surgical AF. Potential mechanisms underlying this association may relate to the fact that CRP elevation is also associated with obesity, hypertension and heart failure, co-morbidities commonly associated with AF. Moreover, the limited impact of anti-inflammatory therapy on AF and conflicting reports of inflammatory marker associations with lone AF (AF in the absence of structural heart disease) have raised questions as to whether inflammatory processes are primary precipitants of AF or secondary to AF itself and/or its co-morbidities.

In this issue of Circulation, Yao and colleagues test the hypothesis that NLRP3 inflammasome activation has a causal role in the etiology of AF [2]. Inflammasomes are oligomeric protein signaling complexes that are composed of a sensor molecule, an adapter protein ASC and caspase I [3]. NLRP3, the object of study for AF by Yao, et al, can be triggered by multiple distinct classes of stimulants, including peptide aggregates, bacterial toxins, oxidized mitochondrial DNA, cellular potassium efflux and intracellular calcium elevation resulting from activation of IP3 receptors or ion channels from the transient receptor potential (TRP) family (Figure).[4–6] Interaction of the sensor molecules with a pyrin domain in ASC triggers assembly of multimers of ASC dimers, which then recruits and activates caspase 1 via a caspase activation and recruitment domain (CARD). Caspase 1 activation then proteolytically activates pro-inflammatory cytokines IL1β and IL18 and can cause an inflammatory type of cell death (pyroptosis), which can serve to remove foreign or damaged self-structures.

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That the triggers for NLRP3 inflammasome activation include peptide aggregates, oxidized mitochondrial DNA (released under conditions of mitochondrial stress) and increased cytosolic calcium levels is of particular relevance for AF. In differential gene expression studies of human left atrial tissues, we reported down-regulation of heat shock factor 1 (HSF1) and heat shock factor 2 (HSF2) targets in patients with paroxysmal AF compared to no AF, and in patients with persistent compared to paroxysmal AF, respectively [7]. In conjunction with studies reporting amyloid protein deposition in atrial tissues in aged hearts, dysregulation of the unfolded protein response may lead to peptide aggregates that activate NLRP3 and promote mitochondrial dysfunction [8]. AF susceptibility was also associated with down-regulation of genes associated with oxidoreductase activity and up-regulation of decarboxylase, aldolase, cyclase, and electron transport activity [7], supporting a role for metabolic stress and mitochondrial dysfunction in AF that may trigger NLRP3 inflammasome activation.

In an elegant set of experiments, Yao and colleagues used a robust mix of human tissue studies, an in vivo canine atrial tachycardia pacing model, and several carefully chosen genetically modified mouse models[2]. They report that, while NLRP3 levels in human right atrial tissue were unchanged in paroxysmal AF patients vs. controls, caspase-1 was activated, consistent with increased NLRP3 activity. In contrast, for persistent AF patients, levels of NLRP3, ASC and active caspase-1 (Casp1-p20) were all elevated. The canine atrial tachypacing (ATP) model resembled paroxysmal AF patients; no change in NLRP3 abundance was evident, but activated caspase-1 was elevated. By 2 months of age, CREM-TG mice (one of few models of spontaneous AF) showed increased atrial expression of NLRP3, ASC, active caspase-1, and increased atrial ectopy – prior to the onset of spontaneous AF. Atrial ectopy precedes and predicts AF in humans as well[9].

As the NLRP3 inflammasome also plays a significant role in innate immune cells, Yao and colleagues evaluated macrophage infiltration (CD68) in each of their systems[2]. CD68 protein levels were unchanged in human paroxysmal AF atria and in the canine ATP model, but increased in human persistent AF, suggesting that, while macrophages are not critical at the onset of AF, they may promote AF progression and persistence. NLRP3 protein was detectable in human, canine and mouse atrial myocytes. The authors developed a line of mice that expressed a cardiac myocyte specific NLRP3 gain of function knock-in (CM-KI). Like patients with paroxysmal AF, these mice had more frequent atrial ectopy and reproducible pacing-induced AF.

Using the CM-KI model, the authors established causal links between NLRP3 inflammasome activation and atrial arrhythmias by demonstrating that: 1) a selective inflammasome inhibitor (MCC950) that interrupts NLRP3 inflammasome complex assembly prevented AF inducibility and 2) cardiomyocyte-specific knock down of NLRP3 reduced AF inducibility and restored normal mRNA levels of *Ryr2, Kcna5, Girk1* and *Girk4*. Their studies also implicate NLRP3 inflammasome activation as a contributor to electrical and structural remodeling. NLRP3 activity may contribute to abbreviation of the atrial effective refractory period that is often observed in AF patients and experimental models, and to atrial hypertrophy and fibrosis that promotes AF persistence. Caution is warranted in extrapolating from the electrophysiologic changes observed in mice, given their

shorter refractory period and greater density of repolarizing $K^+$ currents. Nevertheless, these studies suggest that targeting NLRP3 inflammasome activation may have therapeutic benefit.

As inflammasome activation leads to IL1β activation, [5] a potential therapeutic candidate in this setting is canakinumab, a monoclonal antibody that blocks interaction of activated IL1β with its receptors (Figure), and the drug tested in the CANTOS trial [10]. In patients with a prior myocardial infarction and elevated CRP, canakinumab lowered plasma CRP levels and reduced cardiovascular events[11]. Event reduction paralleled changes in CRP level[12]. As AF was a pre-specified endpoint in the CANTOS trial, we await results from this sub-study with interest.

A recent study also showed that the NLRP3 inflammasome is a key signaling element in the systemic inflammatory response to a Western diet[13]. Obesity is an important risk factor for AF[14], and weight loss is associated with a reduction in CRP levels[15]. Thus, in addition to pharmacologic targeting of NLRP3, dietary/lifestyle changes may also help to reduce the burden of obesity and the NLRP3-associated inflammatory pathophysiology that promotes atrial ectopy and creates an AF substrate that increases its persistence.

In summary, the study by Yao, et al, provides novel and important insights into the significance and potentially causative role of NLRP3 inflammasome activation as a mediator of inflammatory signaling in atrial pathophysiology, and as a candidate target for therapeutic intervention[2]. This study used both gain-of-function studies to demonstrate the sufficiency of NLRP3 in the initiation of AF as well as loss-of-function studies to document the necessity of this pathway to AF initiation and progression. It also points the way toward more specific targeting of the inflammasome pathways for therapeutic intervention. Hopefully this specificity will yield more successful upstream therapies for AF than earlier efforts that used nonspecific anti-inflammatory agents. In summary, this study represents a significant advance. Further work in this intriguing area seems likely to be fruitful.

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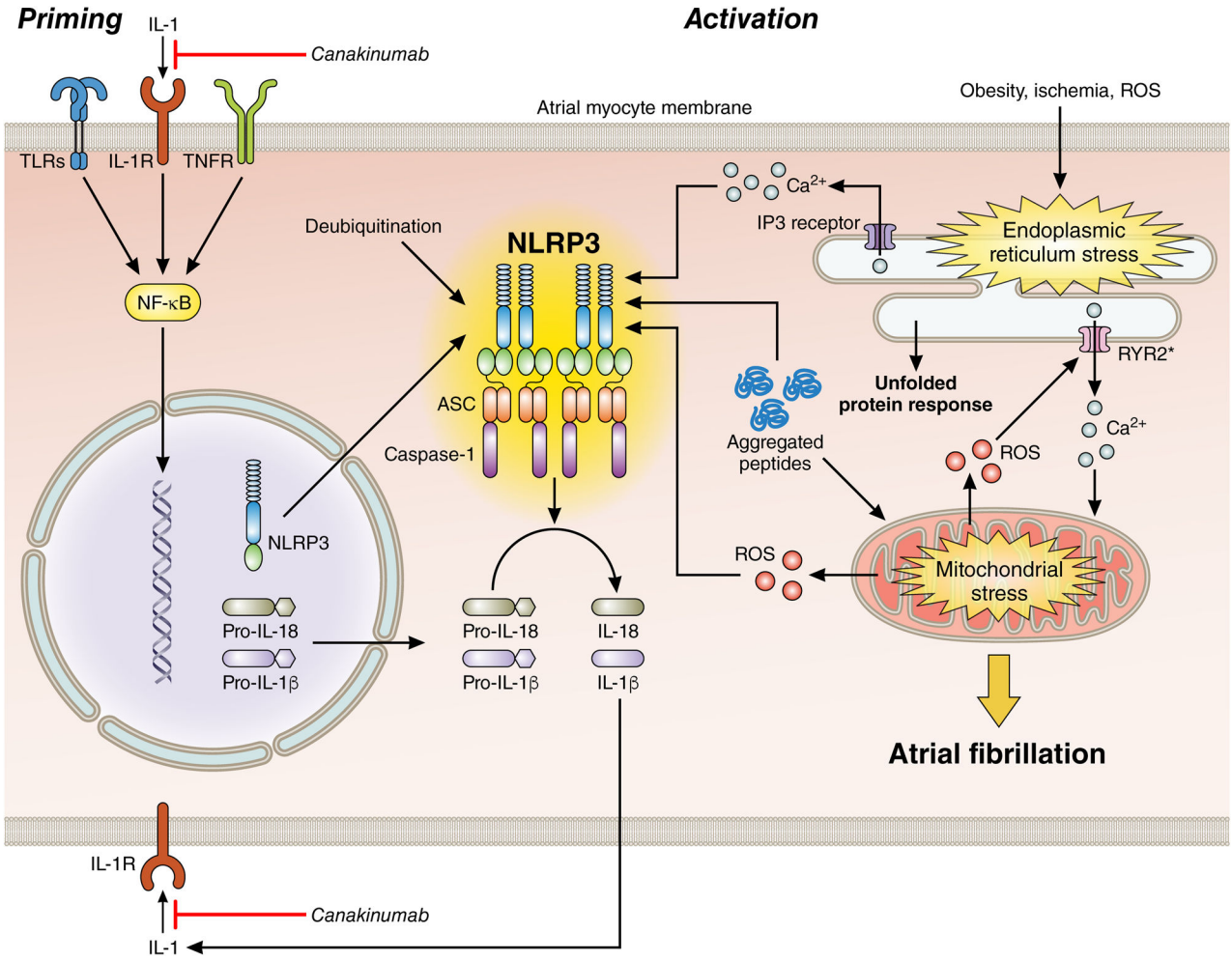


The NLRP3 receptor is activated in a 2 step process (priming and activation) by many different types of stimuli, ranging from activation of Toll like receptors (TLRs), the IL1 receptor (IL1-R), TNF-receptors (TNFR), and Gq-coupled that promote IP3 production. Endoplasmic reticulum stress associated with obesity, hypoxia and ischemia also activates NLRP3 receptors, resulting in increased IL-1β and IL-18 production. Protein and/or peptide aggregates elicit the unfolded protein response; this promotes autophagy and mitophagy, resulting in impaired mitochondrial function, and reactive oxygen species (ROS) production that increases spontaneous release of calcium from the sarcoplasmic reticulum, contributing to atrial ectopy that can initiate episodes of atrial fibrillation.