No place like inflammasome

By Lauren Martz, Staff Writer

Researchers at **Virginia Commonwealth University** have found that inhibiting inflammasome formation with antagonists of the membrane receptor P2X7 could help prevent heart failure following acute myocardial infarction.¹ The findings point to a repurposing opportunity for P2X7 antagonists that companies have in the clinic for inflammatory indications.

About one-third of patients with acute myocardial infarction (AMI) go on to develop heart failure.² Mechanistically, the lack of blood and nutrient supply to the heart tissues after an AMI triggers inflammation, which ultimately leads to cardiac remodeling and dysfunction.

The precise mechanism of cardiac inflammation following AMI was until recently unknown, and efforts to counter inflammation involved generic inhibition of inflammatory cytokines. Early last year, Japanese researchers first reported that inflammasome formation could be responsible for cardiovascular tissue inflammation following AMI.³

The inflammasome is a multiprotein complex formed following activation of P2X7 (purinergic receptor P2X ligand-gated ion channel 7; P2RX7). Inflammasome formation leads to the activation and release of inflammatory mediators including IL-1 β -converting enzyme (CASP1), IL-1 β and IL-18.

Virginia Commonwealth University (VCU) is running four clinical trials of IL-1 β inhibitors in patients with cardiovascular conditions. The inhibitors include Kineret anakinra from **Swedish Orphan Biovitrum AB** and Ilaris canakinumab from **Novartis AG**. However, inhibiting IL-1 β only blocks one component of the inflammatory response.

Now, Antonio Abbate, lead scientist on the trials, and colleagues at VCU have uncovered additional features of the inflammasome's role—and location—following AMI (see **Figure 1**, “Inflammasome activation pathway”). Based on this, the researchers concluded that antagonizing P2X7 could be more effective at preventing inflammatory damage and heart failure than blocking IL-1 β .

Abbate is assistant professor of medicine in the VCU Department of Internal Medicine and interim director of the Cardiac Intensive Care Unit at the VCU Pauley Heart Center.

In a mouse model of AMI, Abbate's team found that levels of Casp1 mRNA and other inflammasome components were upregulated. Notably, the components of inflammasome activation were found in leukocytes, endothelial cells and cardiomyocytes at the infarct border.

“What is newsworthy about this paper is that they have presented evidence that the cardiac myocytes can form inflammasomes. In the

context of myocardial infarction, people did not appreciate that the inflammasome formed in this location. The inflammasome produces IL-1 β , which we would expect to come from the immune cells infiltrating the myocardium. This is interesting because they are presenting new cell biology and new pathophysiology,” said Charles Serhan, director of the Center for Experimental Therapeutics and Reperfusion Injury at **Brigham and Women's Hospital** and professor of anesthesia and dental medicine at **Harvard Medical School**.

In cultured mouse cardiomyocytes, simulation of ischemia induced inflammasome formation, Casp1 activation and cell death compared with no treatment. In the cells, inhibition of P2x7 inhibited Casp1 activity and cell death, suggesting that preventing inflammasome formation in cardiomyocytes could help decrease inflammatory damage following AMI.

In a mouse coronary artery ligation model of AMI, small interfering RNA targeting P2x7 prevented increases in Casp1 activity and lowered cardiac remodeling compared with scrambled siRNA control. In the same model, intraperitoneal administration of a noncompetitive P2X7 antagonist for seven days, beginning five minutes after the procedure, reduced cell death at the ischemic border, cardiac remodeling and cardiac enlargement compared with vehicle control administration.

Results were published in the *Proceedings of the National Academy of Sciences*.

Taking it from the top

Because P2X7 is necessary for the formation of the inflammasome and the release of the downstream inflammatory molecules, targeting the receptor could have wider anti-inflammatory effects than targeting IL-1 β .

“Anti-IL-1 β therapy only blocks a single cytokine that, albeit very important, is by no means the only component of early tissue response to damage or infection. Thus, it would be very important to be able to inhibit inflammasome activity as a whole,” said Francesco Di Virgilio, professor of clinical pathology and chairman of the Department of Experimental and Diagnostic Medicine at the **University of Ferrara**.

“The problem here is that the inflammasome complex is intracellular [and] thus not easily accessible,” he added. The VCU team might have found a way around this problem because P2X7 is a plasma membrane-expressed molecule.

“Since low molecular weight P2X7 inhibitors with very promising drug-like properties are already available, I think that not much more is needed to develop this therapeutic approach, besides careful clinical experimentation,” Di Virgilio told *SciBX*. “Should I decide to test the effect of inflammasome inhibition on post-AMI remodeling, I would target P2X7.”

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**—Francesco Di Virgilio,
University of Ferrara**

Figure 1. Inflammasome activation pathway. Acute myocardial infarction (AMI) causes the release of danger signals including damage-associated molecular pattern molecules (DAMPs) and ATP in response to tissue damage, cell death and lack of blood and nutrient supply to the heart [a].

Extracellular ATP activates purinergic receptor P2X ligand-gated ion channel 7 (P2RX7; P2X7), causing the channel to open, resulting in K⁺ efflux from the cell [b(1)]. According to the findings from Mezzaroma *et al.*, this process occurs in cardiomyocytes. Extracellular K⁺ activates the membrane receptor pannexin 1 (PNX1), opening the channel and allowing transport of DAMPs into the cardiomyocyte [b(2)].

DAMPs serve as direct activating ligands for NLR family pyrin domain containing 3 (NLRP3; NALP3). Activation of NLRP3 induces PYD and CARD domain containing (PYCARD; ASC) recruitment and inflammasome formation [b(3)]. Inflammasome formation causes cleavage of procaspase-1 into IL-1 β -converting enzyme (CASP1), causing downstream activation of inflammatory cytokines including IL-1 β and IL-18 [b(4)].

Release of IL-1 β and other cytokines from cardiomyocytes ultimately induces immune cell recruitment to the heart, inflammation and cardiac remodeling [c].

Mezzaroma *et al.* show that antagonizing P2X7 prevents inflammasome formation and cardiac remodeling (i). IL-1 β inhibitors block immune cell recruitment downstream, blocking some cardiovascular inflammation and remodeling (ii).

antagonist used in combination with antiplatelet treatment. Blocking the inflammasome formation could further help reduce damage after AMI,” he said.

Abbate said the findings reported in the paper are not patented and are unavailable for licensing.

Martz, L. *SciBX* 5(1); doi:10.1038/scibx.2012.1

Published online Jan. 5, 2012

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COMPANIES AND INSTITUTIONS MENTIONED

Affectis Pharmaceuticals AG, Martinsried, Germany
Brigham and Women's Hospital, Boston, Mass.
Evotec AG (Xetra:EVT), Hamburg, Germany
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Harvard Medical School, Boston, Mass.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Swedish Orphan Biovitrum AB (SSE:SOBI), Stockholm, Sweden
University of Ferrara, Ferrara, Italy
Virginia Commonwealth University, Richmond, Va.

P2X7 antagonists in the clinic include **Evotec AG's** EVT 401, which is in Phase I testing to treat rheumatoid arthritis (RA), and **GlaxoSmithKline plc's** GSK1482160, which is in Phase I testing to treat pain.

Both companies declined to comment on the findings.

Luc St-Onge, CBO of **Affectis Pharmaceuticals AG**, wanted the VCU team to repeat its studies with a stronger P2X7 antagonist. “One problem is that in the paper, they used a research tool with potency for P2X7 that is not very high,” he said. “Also, the antagonist is nonselective for P2X7 over other purinergic receptors. The team would need to repeat the studies with an antagonist with potencies in the single-digit nanomolar range and with selectivity for the receptor, which would validate that the effects seen in the mouse models were in fact due to blocking activity against P2X7.”

Affectis is developing oral P2X7 antagonists and this year plans to submit an IND for AFC-5128 in neuropathic pain and multiple sclerosis (MS).

Serhan was interested in combining P2X7 inhibitors with other cardiovascular drugs. “I would like to see the effects of a small molecule