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IL-10 FOR CARDIAC AUTOPHAGY MODULATION: NEW DIRECTION IN THE PURSUIT OF PERFECTION

Anweshan Samanta, MBBS and Buddhadeb Dawn, MD

Division of Cardiovascular Diseases, Cardiovascular Research Institute, and the Midwest Stem Cell Therapy Center, University of Kansas Medical Center and Hospital, Kansas City, KS

Autophagy is a vital cellular homeostatic process that involves removal and recycling of aged and defective cellular components. During this complex process, interaction between proteins and organelles and specific receptors leads to internalization of the cargo into membrane-bound autophagosomes [1]. The autophagosome progressively elongates, matures, and eventually fuses with lysosome resulting in enzymatic degradation of its contents and recycling of certain cellular components, which also facilitates the production of ATP [1, 2]. Although autophagy is generally perceived as a protective mechanism against cell death, growing evidence has unraveled its links with disease causation in various organ systems [2].

In the heart, physiological autophagy occurs at a low rate and declines with age. The disruption of basal level of cardiac autophagy has been shown to cause heart failure [3], and autophagy promotion has been shown to prolong lifespan [4]. On the other hand, excessive autophagy can be detrimental due to loss of essential proteins and organelles, ultimately resulting in cell death and cardiac atrophy [5]. Furthermore, autophagy signaling shares several key regulatory molecules with apoptosis pathways, and autophagy itself may facilitate cell death under certain circumstances [6]. With this intertwined nature of molecular pathways that govern cell death and survival processes, the genesis of divergent outcomes of autophagy ranging from cellular protection to demise often remains poorly understood. However, neither unabated facilitation nor termination of autophagy appears to be a viable solution for therapy, and there arises the need to find a middle ground for an optimal level of autophagy, whereby adaption to stress is possible without unwanted degradation of functional proteins and organelles.

The role of autophagy in the spectrum of cardiac pathologies caused by pressure overload is particularly unclear. In humans, snapshot observations in heart samples from patients with aortic stenosis revealed increased myocyte autophagy in failing left ventricular (LV)

Address for Correspondence: Buddhadeb Dawn, MD, Division of Cardiovascular Diseases, University of Kansas Medical Center, 3901 Rainbow Blvd, 1001 Eaton, MS 3006, Kansas City, KS 66160, Tel: (913) 588-6015, Fax: (913) 588-6010, bdawn@kumc.edu.

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myocardium [7]. However, in animal models of pressure overload, autophagy has been reported to be both beneficial and maladaptive. In rats with supra-aortic constriction, the volume fraction as well as the numerical density of autophagic vacuoles decreased markedly during hypertrophic growth of myocytes, reflecting suppressed cytoplasmic degradation during compensatory cellular growth [8]. Consistent with these observations, induced deletion of autophagy-related 5 (*Atg5*) in the heart produced cardiac hypertrophy and failure in mice [3]. Interestingly, hearts with congenital cardiac-specific deficiency of *Atg5* were normal at baseline, but developed dilation and failure a week after transverse aortic constriction (TAC), suggesting a protective role of autophagy during myocardial adaptation to increased afterload. In contrast, rapid and sustained increase in autophagy accompanied by LV dysfunction and remodeling has also been reported [9]. These adverse consequences of TAC were ameliorated by heterozygous deletion of *Beclin 1*, which is necessary for autophagy, and aggravated by its overexpression, supporting a detrimental role of autophagy in pressure overload-induced heart failure [9].

In this current issue of the *Journal*, Kishore and colleagues [10] add a new facet to our understanding of signaling interplays among angiotensin II (Ang II) signaling, cardiac pressure overload, progression to heart failure, and cardiomyocyte autophagy. They describe, for the first time, the inhibition of pathological autophagy in the heart in response to chronic Ang II by Interleukin-10 (IL-10). Compared with wild-type mice, chronic Ang II infusion resulted in greater reduction in LV ejection fraction, worse remodeling, and increased mortality in IL-10 knockout (KO) mice. These findings were attributed to an increase in autophagy in IL-10 KO mice as evidenced by increased expression of LC3 (1/2) and *Beclin 1* and increased number of autophagosomes in myocytes. *In vitro*, IL-10 co-treatment attenuated the increase in *Beclin 1* and LC3 (1/2) protein levels in neonatal rat ventricular myocytes (NRVM) following Ang II exposure. Furthermore, Ang II inhibited phosphorylation of the prosurvival kinase Akt and 4E-BP1, a molecule downstream of mammalian target of rapamycin (mTOR) activation. Both of these effects of Ang II were blocked by IL-10, indicating that IL-10 prevents autophagy by blocking the inhibitory effects of Ang II on Akt. Mechanistically, the authors confirmed the involvement of the phosphoinositide 3-kinase (PI3K)/Akt/mTORC1 pathway in IL-10-induced benefits in a series of experiments using inhibitors. Importantly, IL-10 reduced the rate of autophagic flux after Ang II treatment and also inhibited autophagosome accumulation in the presence of chloroquine, indicating its ability to effectively modulate the rate of autophagy in myocytes. In addition, IL-10 was shown to promote the binding of Bcl-2 to *Beclin 1*, thereby limiting the availability of *Beclin 1* for autophagy.

These findings add to our growing understanding of potential cardiovascular benefits conferred by IL-10 [11–13]. First described as an inhibitor of Th1 cytokine synthesis, and produced by diverse cell types, IL-10 is increasingly recognized as a master regulator of the immune system. The unchecked Th1 response to enteric pathogens and the development of enteric colitis in 2–3-month old IL-10 KO mice underscores the broader role of IL-10 on whole-body immune homeostasis [14]. In the heart, IL-10 is predominantly expressed in noncardiomyocytes, and homozygous deletion of *IL-10* is associated with enhanced polymorphonuclear neutrophil infiltration and exacerbation of myocardial ischemia/reperfusion injury [11]. IL-10 KO mice also exhibit worse outcomes after cardiac

transplantation [15] and lipopolysaccharide injection [16], highlighting its multimodal cardioprotective properties.

Although the adverse outcomes with systemic absence of IL-10 have largely been attributed to deranged immune cell response, specific molecular signaling remains largely unknown. Still in the context of immune cells, IL-10 has been shown to inhibit starvation-induced autophagy in peritoneal macrophages using the class I PI3K/Akt/mTOR pathway [17]. The current results indicate that IL-10 is required for protection against chronic Ang II-induced cardiac dysfunction and remodeling also by inhibiting pathological autophagy [10]. Indeed, Ang II signaling through angiotensin receptor type 1 (AT1) has been shown to induce autophagy, while AT2 signaling curbed the same [18]. The current data indicate that IL-10 is upstream of Akt, and recruits the PI3K/Akt/mTORC1 cascade to prevent Ang II-induced autophagy in cardiomyocytes. This is a key observation, because PI3K/Akt axis is central to several other survival/death pathways in cardiomyocytes, including apoptosis. Further, Ang II is able to induce both autophagy and apoptosis in cardiomyocytes depending on dose [19], although the molecular determinants of these outcomes remain incompletely understood. Besides macroautophagy, Ang II is also known to activate mitochondrial autophagy by increasing mitochondrial reactive oxygen species [20], raising the possibility that IL-10 may also influence FOXO3 and Bnip3 pathways and impact mitophagy. Future studies may reveal whether the lack of IL-10 selectively perturbs autophagy alone, or a combination of cell death modes initiated by chronic Ang II exposure.

The interpretation of signaling should be made in light of the myriad cardiovascular effects of Ang II through AT1 and AT2 *in vivo* that extend well beyond macroautophagy [21]. Importantly, Ang II infusion may also elicit signaling that are broader than those resulting from pressure overload due by TAC. The aggregate outcomes of Ang II infusion in terms of hypertrophy, fibrosis, apoptosis, autophagy, and transition to ventricular failure *in vivo* perhaps depend on dose, duration, and activation of several transcription factors, including nuclear factor-kappaB [22, 23]. Interestingly, and further underscoring the complexity of Ang II signaling *in vivo*, Ang II has been shown to promote myocardial Akt phosphorylation under some experimental conditions *in vivo* [24]. Thus, the relevance of findings in NRVM *in vitro* needs to be further ascertained *in vivo* in models of human diseases.

Another signaling observation that merit discussion is the restoration of Bcl-2 and Beclin 1 association by IL-10. In noncardiac cells, IL-10-induced increase in Bcl-2 level has been shown to prevent cell death [25], and STAT3 activation has been shown to play a role in this process [26]. It is therefore likely that IL-10 utilizes STAT3 to increase Bcl-2 levels to correct the negative impact of Ang II on Bcl2-Beclin 1 interaction. However, the complexity of signaling here is also very substantive, because both Bcl-2 and Beclin 1 are known to modulate autophagy as well as apoptosis; IL-10 may activate other transcription factors beyond STAT3; and STAT3 may influence the transcription of other survival molecules besides Bcl-2. Interestingly, IL-10 was unable to reverse the inhibitory effects of Ang II on JNK 1/2 phosphorylation, raising the possibility that combination of IL-10 and JNK modulation may achieve finer control over autophagy and lead to greater benefits.

The current data identify new interactions among several highly complex signaling systems: IL-10 and immune regulation, Ang II and cardiovascular effects, pressure overload, apoptosis, and autophagy. Understandably, the outcomes of interference with one of these pathways may impact the other modules in cell-, tissue-, time-, dose-, and context-dependent manners. Nonetheless, these observations present heretofore unrecognized potential therapeutic opportunities. IL-10 or anti-IL-10 therapies have been considered for various immunological maladies and cancer [27]. And modulation of autophagy may impart significant benefits by restricting cardiac damage during stress or inflammation [1]. Indeed, rapamycin, metformin, resveratrol and chloramphenicol have been beneficial through enhancement of autophagy, while 3-methyladenine, trichostatin-A and granulocyte colony-stimulating factor have extended protection through autophagy reduction in various cardiac pathologies [1]. With its unique ability to impact the immune system, IL-10 may have much to add to this armamentarium. However, the effects of IL-10 on various cells should be considered carefully, because autophagy containment may be favorable in one tissue and deleterious in another. This is further complicated by the diversity of Ang II signaling, the occurrence of multiple types of autophagy, and a relative lack of definitive techniques to quantify autophagy levels in tissues. Considering the apparently contrasting roles of autophagy in the pressure-overloaded heart, optimization might just require the right molecules in the right cell at the right time. Daunting as this task may seem, achieving perfection has never been easy.

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