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Appetite for Arginine: Metabolic Control of Macrophage Hunger

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Engulfment of dying cells by phagocytes is essential to maintain tissue function and promote injury resolution and repair. This process, termed efferocytosis, requires persistent corpse engulfment and remains a poorly understood mechanism. Here, we preview findings from Yurdagul et al. (2020) that detail how continual efferocytosis is supported by metabolites derived from the dying cell itself.

Every second more than 1 million cells undergo apoptosis in an adult human (Ravichandran, 2010). To maintain homeostasis, these cells must be removed through a process called efferocytosis. Apoptotic cell (AC) removal involves several steps that include recognition of the dying cell through soluble factors and receptor-ligand interactions, corpse engulfment, and eventually degradation. Professional phagocytes, such as macrophages and dendritic cells, and other cell types like epithelial cells and fibroblasts efferocytose ACs. In most tissues, ACs outnumber phagocytes, meaning that for any given phagocyte, efferocytosis must be a continual process involving successive engulfment of multiple corpses. Recently, we have started to appreciate the key role that cellular metabolism plays during efferocytosis (Morioka et al., 2018; Perry et al., 2019; Wang et al., 2017). Amazingly, few studies have probed how AC-derived metabolites influence efferocyte biology (Viaud et al., 2018; Zhang et al., 2019). Metabolically, efferocytosis places huge demands on the phagocyte with virtually an entire extra cell's worth of metabolome being added in a short space of time. How these AC-derived metabolites are utilized will be important for phagocyte biology, and it is a question that Yurdagul et al. set out to address in this issue of *Cell Metabolism* (Yurdagul et al., 2020).

While the investigators hoped to understand how AC-derived factors are exploited by macrophages during efferocytosis, they focused on amino acids and acylcarnitines. Interestingly, mass spectrometry revealed that the amino acids arginine and ornithine were abundant in AC-containing macrophages compared to macrophages without ACs. To probe the importance of arginine metabolism during efferocytosis, they exposed arginase 1 (Arg1)-ablated IL-4-treated macrophages to ACs. Engulfment of a single AC was unperturbed in the absence of *Arg1*; however, uptake of a second AC was prevented, suggesting that arginine metabolism is important for continual efferocytosis.

To test the relevance of these findings *in vivo*, they assessed the role of Arg1 during atherosclerosis—when AC clearance from atherosclerotic lesions prevents the accumulation of secondary necrotic cells that exacerbate disease. A feature of atherosclerotic plaques is diminished efferocytosis due to impaired macrophage function driven by the lesion microenvironment. The authors showed reduced Arg1 levels in advanced versus early atherosclerotic lesions in mice and humans, and posited that arginine metabolism promotes efferocytosis in regressing lesions when Arg1 levels are higher.

Atherosclerosis can be modeled in mice by placing low-density lipoprotein receptor knockout mice ($Ldlr^{-/-}$) on a western diet (WD) for 16 weeks (progression model). Conversely, lesion regression can be induced by switching mice to a normal chow diet for 5 weeks after the 16-week WD period, while receiving in parallel a helper-dependent adenovirus containing the human LDLR gene to restore liver LDL levels and reduce plasma cholesterol. The authors assessed the role of macrophage Arg1 by transplanting bone marrow from wild-type (*Lyz2*-Cre^{-}) and *Arg1*$^{fl/fl}$ *Lyz2*-Cre^{+} mice into lethally irradiated $Ldlr^{-/-}$ mice and subsequently assigned mice to either the regressing or progressing models. Regressing lesions exhibited signs of enhanced efferocytosis and reduced plaque necrosis, both of which were lost in mice bearing *Arg1*-deficient macrophages. Multiple other clinical and histological parameters showed exacerbated disease in the regressive model in the absence of macrophage Arg1, collectively indicating that Arg1 is important during lesion regression.

To understand how arginine may be utilized to promote continual efferocytosis, Yurdagul et al. focused on the polyamine synthesis pathway. Polyamines comprise the metabolites putrescine, spermidine, and spermine and are linked to arginine metabolism via ornithine decarboxylase (ODC), which converts ornithine into putrescine (Figure 1). AC engulfment caused elevated levels of all three polyamines in macrophages. Silencing of *Odc1* recapitulated the effects of Arg1 ablation on efferocytosis. While macrophages with reduced ODC could successfully engulf one AC corpse, their ability to engulf a second was significantly impaired. This effect could be rescued with putrescine, but not spermidine or spermine, suggesting that an Arg1-ODC-putrescine pathway supports continual efferocytosis.

In an impressive *in vivo* experiment, the authors demonstrated that putrescine supplementation to $Ldlr^{-/-}$ mice on WD ameliorated atherosclerosis and promoted efferocytosis in lesional macrophages, highlighting the physiological importance of putrescine in resolving macrophages and AC clearance. Metabolic tracing of heavy-labeled arginine confirmed that AC-derived arginine is metabolized to

putrescine by macrophage Arg1 and ODC in a process dependent on the lysosomal arginine transporter *Pqlc2*. However, the relatively small contribution of AC-derived arginine to the total macrophage putrescine pool implies a sensitive mechanism at play regulating continual efferocytosis in this context.

Actin remodeling is a requisite event during efferocytosis to ensure successful corpse engulfment, a process dependent on the small GTPase Rac1. The authors showed that although silencing *Arg1* or *Odc1* did not perturb Rac1 induction during first AC engulfment, it was significantly impaired during second AC internalization. Rac1 activation requires the GTP-exchange factor Dbl (gene *Mcf2*), the expression of which is increased upon exposure to IL-4 and ACs. This *Mcf2* induction was not observed in the setting of reduced *Arg1* and *Odc1*, but was restored with putrescine. Importantly, silencing of Dbl decreased second AC internalization, but not first AC engulfment, and putrescine could no longer rescue second AC uptake in *Odc1*-silenced macrophages when Dbl was also knocked down. The authors demonstrated that IL-4 and ACs enhance the cytoplasmic localization of HuR, a protein that promotes mRNA stability and has binding sites in the 3′UTR of *Mcf2*. Putrescine increased the interaction of HuR with *Mcf2* mRNA, whereas spermidine and spermine had no such effect. Why putrescine is effective at facilitating HuR-*Mcf2* interactions in this setting, but spermidine and spermine are not, will be a future interest, especially given the increasing body of evidence supporting the importance of these metabolites in phagocyte function and repair.

Together, these findings put forward the concept that AC-derived arginine and ornithine are metabolized to putrescine in the efferocyte via Arg1 and ODC. In turn, AC-induced HuR stabilizes *Mcf2* mRNA in the presence of this putrescine, facilitating Rac1 activation that is required to support continual efferocytosis. This work is an important step in understanding how efferocytes process and utilize the huge bolus of metabolites from the incoming AC. It is likely that other AC-derived metabolites also shape efferocyte biology. The study previewed here incites other interesting questions: How does this play out in the tumor microenvironment where cancer cells display significantly altered metabolism, including changes in arginine and polyamine pathways? How might AC-derived metabolites contribute to the re-programming of the efferocyte? This is especially interesting in macrophages since AC uptake is immunologically silent and polyamines restrain inflammation in macrophages (Latour et al., 2019; Puleston et al., 2019). And how do different forms of cell death impact how metabolites from the dying cell are applied in the efferocyte? Ultimately, this study further opens the door to the prospect of therapeutic manipulation of injury and repair through the control of cellular metabolism.

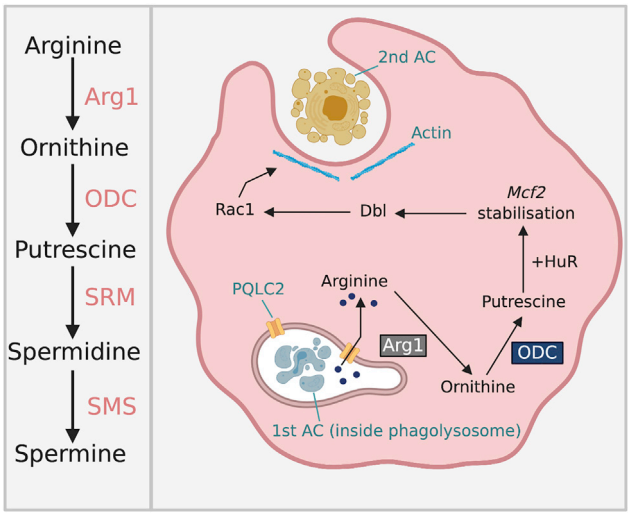

Figure 1. Apoptotic Cell-Derived Arginine Supports Continual Efferocytosis in Macrophages via the Polyamine Synthesis Pathway

Left: arginine acts as a precursor of polyamine synthesis through arginase 1 (Arg1), which converts arginine to ornithine. Ornithine decarboxylase (ODC), a rate-limiting enzyme for polyamine synthesis, then converts ornithine into the first polyamine, putrescine. Spermidine and spermine may then be synthesized through the action of spermidine synthase (SRM) and spermine synthase (SMS), respectively. Although missing from this schematic, spermidine and spermine also require decarboxylated s-adenosylmethionine for their production, provided by s-adenosylmethionine decarboxylase. Right: efferocytosis of an apoptotic cell (AC) leads to the release of AC-derived arginine into the macrophage cytoplasm through the lysosomal transporter PQLC2. Through Arg1 and ODC, arginine is converted to putrescine that facilitates *Mcf2* mRNA stabilization via the protein HuR, resulting in enhanced Dbl expression. Dbl can then activate the small GTPase Rac1, which mediates the actin remodeling necessary to promote internalization of successive AC corpses.

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DECLARATION OF INTERESTS

E.L.P. is a Scientific Advisory Board Member of ImmunoMet Therapeutics, Inc. and a founder of Rheos Medicines.

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