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RESEARCH HIGHLIGHT

LAP: the protector against autoimmunity

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The autophagy-related process LC3-Associated Phagocytosis, or LAP, is known to control the degradation of engulfed cells and microorganisms. Now Martinez *et al.* discover that LAP controls immune responses to dying cells and its inhibition leads to development of Systemic Lupus Erythematosus-like disease.

Sometimes a simple observation can change everything. A single membrane, for example, could change the way we think about autoimmunity. This is how the field of LAP, or LC3-Associated Phagocytosis, began. By counting membranes at phagosomes in immune cells, Doug Green and colleagues had discovered that autophagy proteins, which normally function to build double-membrane autophagosomes around intracellular substrates during autophagy, could instead modify pre-existing single-membrane phagosomes around engulfed microorganisms and apoptotic cells [1, 2]. Now Green and colleagues discover that LAP is a critical mediator of immune cell function in vivo, and in its absence, mice develop an autoimmune disorder resembling Systemic Lupus Erythematosus (SLE) [3].

During LAP, a small ubiquitin-like protein LC3 (microtubule-associated protein 1 light chain 3) is lipidated onto phagosome membranes to form LAPosomes (Figure 1). Lipidation is controlled by multiple autophagy-related proteins — ATG 3, 4, 5, 7, 10, 12, 16L — first identified by their functions in autophagosome biogenesis [4]. While these core autophagy proteins are required for LAP, other autophagy proteins are dispensable [4]. The upstream autophagy pre-initiation complex composed of the ULK1/2 kinase and adaptors ATG13 and FIP200, is required for autophagy but is not required for LAP [4, 5]. Also the lipid kinase VPS34, acting in a complex with Beclin1 and ATG14, is important for autophagy initiation, but ATG14 is not required for LAP, and instead a different adaptor, Rubicon, functions as part of a LAP-specific complex [6]. An additional LAP-specific role for NADPH oxidase-2 (NOX2) has also been defined [6, 7]. By leveraging mouse models of deficiency for core autophagy genes versus LAP-specific, or autophagy-specific genes, Martinez *et al.* were able to identify an important LAP function in immunity [3].

The authors found that the deletion of core autophagy or LAP-specific genes (*Atg5, Atg7, Beclin1* (*Becn*), *Rubicon* (*Rubcn*) and *Nox2*) from immune cells (macrophages, monocytes, and some neutrophils and dendritic cells), by lysozyme M (LysM)-Cre-mediated gene deletion, led to the development of autoimmune disease. Mice displayed reduced body weight, accumulation of circulating lymphocytes, monocytes, neutrophils, and activated $CD8^+$ T cells, increased proliferation in spleen, and developed autoantibodies (including anti-double-stranded DNA and nuclear antibodies) commonly associated with SLE. They also exhibited signs of kidney damage, with increased expression of IFN signature genes reported in SLE patients. By contrast, mice with immune cell deficiency in *Fip200* or *Ulk1*, which are required for autophagy but not LAP, appeared normal.

What could go wrong in LAP-deficient immune cells to cause SLE? The authors examined whether a failure to degrade engulfed apoptotic cells, a defect reported in LAP-deficient macrophages [2, 5], could be blamed. Indeed, while deficiency of *Atg7* or *Rubcn* had no observable effect on apoptotic cell engulfment, the degradation of engulfed cells was inhibited in multiple tissues (spleen, liver, kidney) compared to wild-type and *Fip200*-deficient animals. Moreover, $Rubcn^{-/-}$ mice challenged with repeated apoptotic cell injections over 8-weeks developed more rapid SLE-like hallmarks than controls, including circulating autoantibodies and kidney damage.

The authors further examined cytokine production in the context of LAP deficiency. When challenged with apoptotic cells in culture, LAP-proficient but autophagy-deficient macrophages (Cre^+ $Fip200^{f/f}$ and Cre^+ $Atg14^{f/f}$) responded normally by producing anti-inflammatory IL-10, and did not produce pro-inflammatory cytokines (IL-1β, IL-6 and CXCL-10). On the other hand, LAP-deficient macrophages (Cre^+ $Atg3^{f/f}$, Cre^+ $Atg7^{f/f}$, Cre^+ $Becn1^{f/f}$, Cre^+ $Rubcn^{f/f}$, Cre^+ $Nox2^{f/f}$) failed to generate IL-10 and secreted high levels of IL-1β, IL-6 and CXCL-10. Similarly *in vivo*, in response to apoptotic cell injections, pro-inflammatory cytokines were elevated in the serum of LAP-deficient (Cre^+ $Atg7^{f/f}$ or $Rubcn^{-/-}$), compared to LAP-proficient (wild-type and Cre^+ $Fip200^{f/f}$) mice, which displayed elevated levels of IL-10. The authors further found that spontaneous cytokine levels from unchallenged mice were altered in all LAP-deficient genotypes upon aging, with reduced IL-10, and elevated levels of a panel of pro-inflammatory cytokines (IL-1β, IL-6, IL-12p40, IP-10, CXCL1, MIP-1β, and MCP-1) that were absent from LAP-proficient mice.

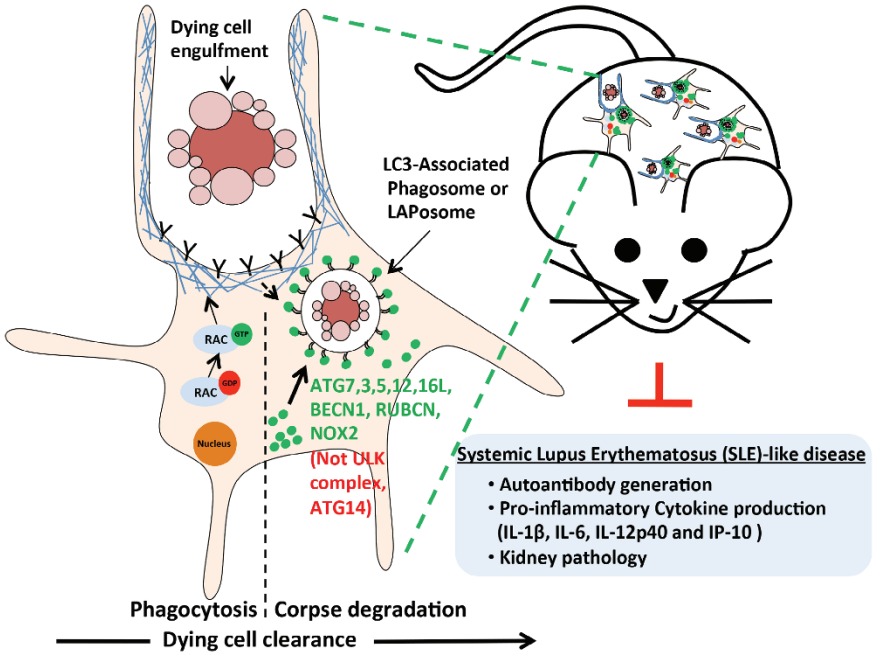

Figure 1 Dying cell clearance involves phagocytic engulfment (left side) and corpse degradation (right side). Degradation is regulated by autophagy proteins that lipidate LC3 (green circles) onto phagosomes, forming an LC3-Associated Phagosome or LAPosome. LAPosome formation requires LC3 lipidation machinery, as well as Rubicon and Nox2 (green text), but not the ULK complex or ATG14 (red text). The inhibition of LAPosome formation blocks corpse degradation and leads to Systemic Lupus Erythematosus (SLE)-like disease in mice (right, blue box).

These observations altogether support a model that pro-inflammatory cytokine secretion in response to apoptotic cell ingestion contributes to autoimmune disease in LAP-deficient mice.

The findings by Martinez and colleagues identify an important physiologic function for LAP acting as a protector against autoimmunity (Figure 1), which is a major step forward for this autophagy-related field. Mice with LAP-deficient immune cells develop autoimmune disease similarly to those rendered defective in apoptotic cell engulfment due to deletion of the phagocytosis receptor *Tim4* [8]. How a failure to digest engulfed cells leads to development of the hallmarks of SLE, including the production of autoantibodies, is an important question for further study. While LAP inhibition primarily affects corpse degradation acutely, whether persistent corpses could also have broader effects on phagocyte function in the longer term, as has been shown in *Drosophila* [9], may be interesting to consider.

Previously LAP was shown to be required for retinoid recycling by phagocytic retinal pigment epithelial cells, which contributes to maintaining vision in mice [10], and LAP-like LC3 lipidation also occurs on macropinosomes [5], and at the ruffled border in osteoclasts [11], suggesting that LAP may contribute significantly to physiology in multiple contexts. In their study, Martinez and colleagues leveraged previous work identifying numerous genes required for LAP versus autophagy to build, by correlation, a compelling model of LAP function *in vivo* [5-7]. While LAP is implicated convincingly by the authors' work, whether any other noncanonical forms of autophagy, potentially involving autophagosomal structures, could contribute to the observed effects is an important question for consideration. Finally, while LC3 lipidation onto phagosomes is a defining feature of the LAP field, the molecular mechanism of LC3 function at phagosomes remains elusive. LC3 is a member of three related families of proteins that participate in autophagy [12], and multiple members are found at LAPosomes [6]. While LC3 proteins are speculated to control lysosome fusion to phagosomes, their mechanism(s) of action will be important to identify.

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