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# AutophagosOMES: identification of autophagosomal cargo in CD4$^+$ T cells by proteomics

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**ABSTRACT**

Macroautophagy/autophagy, a cellular process that sequesters and breaks down cellular components in the lysosome/vacuole, is important in various events where cell composition undergoes changes. Broadly, autophagy is involved in T cell regulation including maintaining cell homeostasis. One process where a cell alters its composition is in the activation of helper T cells in the immune system. When helper (CD4$^+$) T cells are activated by antigens, they both grow and alter their cellular components to become effector T cells. Autophagy is the process that facilitates the breakdown of these cellular components and is therefore hypothesized to have a role in CD4$^+$ T cell activation. Previous research has concluded that CD4$^+$ T cell activation induces autophagy, providing an avenue for further research aimed at examining the ways in which this induced autophagy affects CD4$^+$ T cell proliferation and function. Toward this end, Zhou et al. researched the autophagosomal cargo present within CD4$^+$ T cells and the impact this cargo has on CD4$^+$ T cell proliferation.

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Macroautophagy (hereafter autophagy) is a eukaryotic catabolic process that plays essential roles in differentiation, activation, maintenance of cellular homeostasis and memory generation in lymphocytes [1–3]. Specifically, activation of CD4$^+$ T cells is accompanied by dramatic changes in their proteome, most likely facilitated by autophagy [4,5]. Therefore, a systematic identification of the autophagosomal contents in CD4$^+$ T cells will provide insights into changes in the proteome during activation and proliferation. Toward answering this problem, Zhou et al. purify autophagosomes from murine CD4$^+$ T cells and identify the cargo proteins via mass spectrometry [6].

Mouse models were constructed using two alleles, PTPRC$^a$/CD45.1 and PTPRC$^b$/CD45.2. In mice with the autophagy-related gene *Atg7* deleted in PTPRC$^b$, T-cell proliferation decreases compared to T cell proliferation with both wild-type PTPRC/CD45 and PTPRC$^a$/CD45.1. Zhou et al. used another mouse model with MAP1LC3B/LC3B, the homolog of yeast Atg8 in mice, labeled to detect the specific protein components in autophagy from T cells. Both models demonstrated that knocking out *Atg7* impairs the activation of T cells exposed to toxins and therefore leads to decreased CD4$^+$ T cell proliferation.

Zhou et al. employed an affinity labeling method using LC3B fused to APEX2 (LC3B-AP2), as this showed the highest reproducibility in a previously published study [7]. In the presence of biotin phenols and hydroxyl-peroxide, APEX2 can transform the former into highly reactive radicals that can covalently conjugate with nearby proteins. These proteins can then be identified by affinity purification and mass spectrometry-based profiling. Because LC3B is embedded in both the outer and inner autophagosomal membrane, the authors treated the sample with proteinase K to degrade cytosolic proteins bound to LC3B while those inside the autophagosome are protected. The authors first confirmed the colocalization of biotin with APEX2 through confocal imaging, observing significant overlap between biotin and APEX2, indicating that the proteins being biotinylated are proximal to APEX2 enzymes. They also confirmed the autophagosomal enrichment of LC3B-AP2 by performing confocal imaging with LAMP1, a lysosomal marker, observing partial colocalization. The authors then utilized a proteinase K protection assay confirming that the LC3B-AP2 labeling is identifying lumenal proteins within the autophagosome. Using mass spectrometry following proteinase K treatment the authors found several autophagic receptors including SQSTM1, NBR1 and TAX1BP1 lending support for the success of their methods in identifying autophagosomal proteins.

Next, the authors analyzed 112 proteinase K-protected and streptavidin-enriched proteins. From these, 7 candidate proteins are significantly upregulated following bafilomycin $A_1$ treatment, an inhibitor that prevents the lysosomal degradation of LC3 that is sequestered within autophagosomes. Upon further analysis, IL7R/IL-7Rα (a component of the heterodimeric interleukin 7 receptor) shows the highest fold change among all biological replicates. IL7R is constantly internalized and recycled through membrane-enveloped organelles. To ensure that IL7R is part of the autophagosomal cargo, the authors performed immunostaining of IL7R with LC3 or WIPI2 (early autophagosomal markers) and observed colocalization that increases upon bafilomycin $A_1$ treatment. Furthermore, the authors performed flow cytometry for IL7R expression levels from the spleen of *atg7*$^{-/-}$ mice to validate whether the lack of autophagy impairs IL7R degradation. IL7R is upregulated both at the surface and whole-cell level in naive *atg7*$^{-/-}$ CD4$^+$ T cells (undifferentiated T cells that have yet to be exposed to an antigen for differentiation),

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suggesting that ILR7 is degraded by autophagy. However, no similar significant effects are observed in effector T cells (differentiated helper T cells).

After determining that IL7R levels are higher in autophagy knockout mice, Zhou et al. tried to link increased IL7R to decreased IL2R assembly. This link explains the role of autophagy in modulating $CD4^+$ T cell behavior because IL2R is part of a signaling pathway that leads to $CD4^+$ T cell proliferation. Zhou et al. observed that IL2R levels are the same in the wild-type and *atg7*$^{-/-}$ cells, but IL2 signaling in *atg7*$^{-/-}$ cells leads to lower IL2RA/IL2Rα levels. These lower IL2RA levels then decrease the flux through the IL2 signaling pathway as measured by a decrease in the phosphorylation of the downstream effector STAT5. IL7 and IL2 receptors form by the combination of IL2RG/gamma chain with IL7R or IL2RA-IL2RB, respectively. Zhou et al. found that the colocalization of IL2RG with IL2RA is decreased in the *atg7*$^{-/-}$ knockout cells. IL7R can compete with IL2RA for binding to limiting levels of IL2RG. Thus, autophagy can play a role in maintaining an adequate pool of free IL2RG by restricting the level of IL7R, allowing the free IL2RG to combine with IL2RA more frequently in $CD4^+$ cells. This hypothesis would explain the higher levels of IL2R and higher $CD4^+$ T cell proliferation in cells that are autophagy competent.

Through the results of this study the authors determined that the autophagic degradation of IL7R is crucial for T cell proliferation. In cells where autophagy is inhibited, IL7R competitively inhibits IL2R signaling through sequestration of the limited amount of IL2RG, interfering with subsequent downstream signaling that is crucial for proliferation of $CD4^+$ T cells. Thus, autophagy plays a key role in the transition from IL7R-mediated homeostasis to IL2R-mediated expansion in effector T cells. It is also possible that autophagy regulation is directly involved in $CD4^+$ T cell differentiation. IL2 signaling is antagonistic to differentiation into $CD4^+$ Th17 cells through suppressing expression of IL6ST/IL-6Rβ. Thus, the decreased IL2 signaling in autophagy-deficient cells may be influential in differentiating cells into $CD4^+$ Th17 cells. This study also supports the validity of using the LC3B-AP2 mouse model in overcoming the obstacles of traditional methods toward identifying autophagosomal constituents. However, the authors note that proteins embedded either in large complexes or with very few electron-rich amino acid residues exposed at the surface have a lower chance of being tagged by biotin-phenol radicals. Overall, this paper bolsters the observation that autophagy is involved in shaping the proteomic landscape of the $CD4^+$ T cell at different points in its life cycle [6].

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