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AUTOPHAGIC PUNCTUM

PIK3C3/VPS34 helps school T cells in the thymus

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ABSTRACT

The development of a broad repertoire of T cells in the immune system requires interaction of T cell receptors expressed by immature T cells with peptide/major histocompatibility complexes (MHCs) displayed by specialized epithelial cells in the thymus, in a process called T cell positive selection. Thymic epithelial cells (TECs) display unique antigen processing machinery which shapes the collection of self-peptides that drive positive selection. In our recent studies, we explored the contribution of the lipid kinase PIK3C3/VPS34 to the generation of positively selecting peptides in TECs. We found that TEC-specific PIK3C3/VPS34 facilitates the positive selection of CD4 but not CD8 T lineage cells, in a mechanism independent of its role in canonical macroautophagy/autophagy. Instead, we propose that PIK3C3/VPS34 alters vesicle trafficking in TECs that modulates lysosomal protease activity which, in turn, controls the generation of MHC class II-presented peptides optimized for positive selection of CD4 T cells.

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T cells of the immune system are critical for fighting infections and cancer. They express T cell receptors (TCRs) that sense pathogen-derived peptides bound with specialized self-proteins, called major histocompatibility complex (MHC) molecules, at the surface of pathogen-exposed host cells. Two main subsets of T cells can be distinguished: CD4-expressing T cells with helper functions recognize peptides bound by MHC class II (MHCII) molecules, whereas CD8-expressing T cells with cytotoxic functions recognize peptides bound by MHC class I (MHCI) molecules. Each T cell expresses a unique TCR, which permits the host to respond to virtually an unlimited variety of potential pathogens and transformed cells. The generation of a vast repertoire of TCRs is achieved during T cell development in the thymus by somatic recombination of gene segments encoding the highly variable TCRs. However, this recombination process also generates TCRs that are unable to sense their cognate antigen when presented by the host's limited set of MHC products. Hence, developing T cells must undergo extensive screening to enforce the production of a diverse and functional TCR repertoire in a process called thymic positive selection.

The thymus is a specialized lymphoid organ located directly above the heart that supports the differentiation and education of immature T cells, called thymocytes. Following commitment to the T cell lineage, thymocytes rearrange their TCR gene segments, start expressing TCRs at their surface, induce expression of both CD4 and CD8, and are then screened for reactivity with self-peptide/MHC complexes displayed at the surface of cortical thymic epithelial cells (cTECs). When a TCR interacts weakly with self-peptide /MHC it receives survival signals and further differentiates to become a mature T cell expressing only CD4 or CD8, depending on its interaction with one of the host's MHCII or MHCI products, respectively. Importantly, the nature of the self-peptides produced by and displayed at the surface of cTECs in the context of MHC products plays a critical role in the outcome of thymic positive selection. Among all MHC-expressing cells, cTECs display unique antigen-processing qualities that remain incompletely understood. Macroautophagy/autophagy has been implicated in the processing of self-antigens expressed in cTECs via MHCII molecules. Unlike most cell types that require stress signals to induce autophagy, cTECs are highly active in autophagy under homeostatic conditions. In these cells, autophagosomes constantly fuse with late endosomal MHCII-containing compartments, thus allowing cytosolic antigens access to the MHCII antigen loading machinery. Based on these findings, investigators proposed that autophagy contributes significantly to the collection of self-peptides displayed on MHCII molecules in cTECs with the potential to modulate CD4 T cell positive selection.

In our recent study [1], we focused on the class III phosphatidylinositol 3-kinase PIK3C3/VPS34 (phosphatidylinositol 3-kinase catalytic subunit type 3), which plays key roles in canonical autophagy as well as endocytosis and vesicle trafficking, cellular processes that all influence antigen processing. To assess the contribution of PIK3C3/VPS34 to the antigen-presenting functions of TECs, we generated and analyzed mice with a TEC-specific gene ablation of *Pik3c3/Vps34* (PIK3C3/VPS34^{TEC} mice). Neonatal PIK3C3/VPS34^{TEC} mice display normal thymic cellularity that progressively and severely decreases until adulthood when thymopoiesis is nearly completely lost. This results in significant T cell lymphopenia of the peripheral lymphoid organs in adult PIK3C3/

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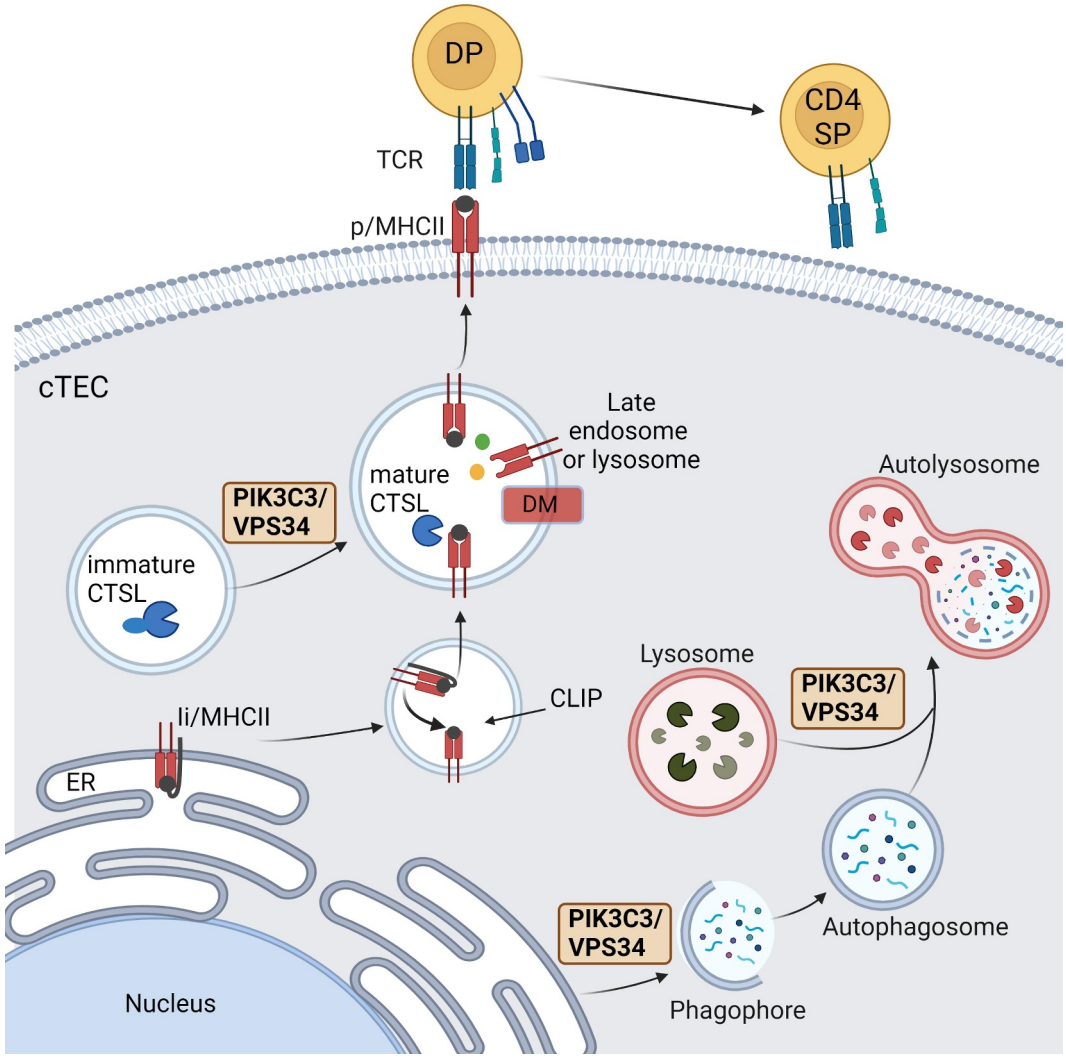

Figure 1. PIK3C3/VPS34 controls the generation of self-peptide/MHC class II complexes (p/MHCII) for positive selection of CD4 single-positive thymocytes (CD4 SP). In cortical thymic epithelial cells (cTECs) MHCII is synthesized in the endoplasmic reticulum (ER) and loaded with the chaperone invariant chain (Ii/MHCII) which is subsequently trimmed to the shorter class II-associated invariant chain peptide (CLIP) during endocytic trafficking. With the help of the peptide exchange factor H2-DM, CLIP then dissociates from MHCII and is replaced with self-peptide that is processed by lysosomal proteases such as CTSL. PIK3C3/VPS34-mediated vesicle trafficking promotes the maturation of CTSL necessary for generating self-peptide ligands. p/MHCII then traffics to the cell surface to promote the positive selection of CD4- and CD8-expressing double-positive (DP) thymocytes to the CD4 T cell lineage. While PIK3C3/VPS34 is required for homeostatic autophagy in cTECs, canonical autophagy is likely not the primary mechanism by which PIK3C3/VPS34 promotes thymic positive selection of CD4 T cells. This figure was made with Biorender.com.

VPS34^{TEC} mice. These findings indicate that PIK3C3/VPS34 is critically required for TEC homeostasis.

To assess the role PIK3C3/VPS34 plays in thymic selection, we restricted our analysis to neonates to avoid confounding factors associated with thymic hypotrophy observed in adult PIK3C3/VPS34^{TEC} mice. We found a significant decrease (roughly 50%) in the prevalence of positively selected CD4 but not CD8 thymocytes. Careful phenotypic analysis revealed that the alterations in CD4 thymocyte development are not due to defects in maturation steps that occur after positive selection. To directly test for defects in positive selection of specific T cell clones, we bred six separate TCR transgenic lines (two MHCI- and four MHCII-restricted lines) to the PIK3C3/VPS34^{TEC} mice. Strikingly, we found a near complete block in the positive selection of all MHCII-restricted lines but no significant alterations in the selection of the MHCI-restricted lines. We next asked whether PIK3C3/VPS34 in TECs influences the clonal properties of the positively selected polyclonal TCR repertoire by performing TCR sequencing of sorted CD4 thymocytes. Surprisingly, the clonal diversity is equally maintained between PIK3C3/VPS34^{TEC} and littermate controls, yet we found evidence of significantly decreased clonal sharing. These findings strongly support a role for PIK3C3/VPS34 in controlling the thymic positive selection of CD4 but not CD8 T cells.

We next sought to determine if PIK3C3/VPS34 controls the quality of self-peptide/MHCII complexes displayed on cTECs. To this end, we stained cTECs with a monoclonal antibody that reacts to MHCII while bound with a specific peptide, CLIP (class II-associated invariant chain peptide), derived from an accessory protein that facilitates MHCII antigen presentation. Our results show a significant increase in the presentation of the CLIP peptide in PIK3C3/VPS34-deficient cTECs, yet total levels of MHCII surface expression are unaffected. To determine the cellular mechanism involved,

we assessed the contribution of autophagy. Utilizing an autophagy reporter mouse strain, we confirmed that cTECs from PIK3C3/VPS34^{TEC} mice exhibit defective autophagy under homeostatic conditions. Next, we analyzed thymic selection in mice carrying a TEC-specific deficiency of the critical autophagy factor ATG5 (autophagy related 5), which revealed unperturbed thymic selection of the polyclonal CD4 T cell repertoire as well as a transgenic MHCII-restricted TCR line. Thus, while PIK3C3/VPS34 is necessary for autophagy in cTECs, autophagy does not appear to be the primary mechanism by which PIK3C3/VPS34 controls CD4 T cell selection. We next explored lysosomal proteases that influence MHCII-restricted antigen processing in cTECs, because PIK3C3/VPS34 has been implicated in controlling vesicle trafficking necessary for maturation of CTSD (cathepsin D). We focused on CTSL, the most critical protease in cTECs for generating the positively selecting MHCII-bound peptides, and found significantly decreased levels of mature CTSL in a PIK3C3/VPS34-deficient cTEC-derived mouse cell line. Thus, our findings point toward alterations in CTSL activity in cTECs as a potential cause of defective CD4 T cell selection in PIK3C3/VPS34^{TEC} animals.

In conclusion, our study shows that PIK3C3/VPS34 controls cellular processes in cTECs that are required for the generation of self-peptide/MHCII complexes optimized for the positive selection of CD4 T cells. We propose that the thymic positive selection defect in PIK3C3/VPS34^{TEC} mice is caused by impaired protease activity associated with altered vesicle trafficking, rather than canonical autophagy (Figure 1). This work provides new insights into the unique antigen processing pathways in cTECs with practical implications for the development of therapeutic strategies to reschool thymocytes in patients with defective thymic function.

Disclosure statement

L. Van Kaer is a member of the scientific advisory board of Isu Abxis Co., Ltd. (South Korea). The other authors have declared that no conflict of interest exists.

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Reference

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