

RESEARCH HIGHLIGHT

Projection of an immunological self shadow to developing T cells via macroautophagy

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The adaptive immune system, which establishes specific memory for pathogens and foreign antigens that we encounter, is orchestrated by T cells. In contrast to antibody producing B cells, T cells recognize their antigen not in its native form, but as small proteolytic fragments presented by major histocompatibility (MHC) molecules on the cell surface. Cytotoxic CD8⁺ T cells monitor octameric or nonameric peptides presented by MHC class I, and helper CD4⁺ T cells detect longer peptides (≥ 9 amino acids) presented on MHC class II molecules. These peptides are usually produced by different proteolytic machineries within cells. While MHC class I ligands originate from proteasomal degradation, MHC class II ligands are generated by lysosomal hydrolysis [1]. For phagocytic cells, source proteins of MHC class II presented peptides enter lysosomes via endocytosis. However, cells with limited endocytic activity probably rely on other pathways to deliver substrates to lysosomal degradation, followed by loading onto MHC class II molecules.

Thymic epithelial cells (TECs) are among these cells with low endocytic activity. Both cortical and mature medullary TECs (cTECs and mTECs, respectively) express MHC class II molecules and are essential for T cell selection and tolerance induction in the thymus. Since the proteolytic machineries that give rise to MHC class I and II

ligands cannot distinguish between self and foreign proteins, a mixture of self and foreign peptides are presented on MHC molecules during infections. In order to avoid priming of autoreactive T cell responses, several tolerance mechanisms have to neutralize autoreactive T cell specificities prior to pathogen encounter [2]. One of these is central tolerance, during which autoreactive developing T cell specificities are deleted. Central tolerance induction occurs as negative T cell selection in the thymic medulla by mTECs and dendritic cells (DCs). In order to tolerize developing T cells against a wide variety of self-proteins, mTECs express even peripheral tissue specific antigens via low level transcription initiated by the autoimmune regulator (Aire) transcription factor. This promiscuous expression was coined projecting an immunological self shadow in the thymus. In addition, the thymic cortex trains developing T cells to recognize MHC presented peptides (MHC restriction) in a process called positive selection on primarily cTECs. Therefore, self-peptide presentation on MHC class II molecules in the healthy host is essential to shape the helper CD4⁺ T cell repertoire.

But how is the immunological self shadow projected to developing T cells? How do the source proteins of these self-peptides gain access to lysosomal degradation for MHC class II presentation on TECs with limited endocytic

activity? Insight into these questions is provided in a recent publication by Ludger Klein, Jelena Nedjic and colleagues in *Nature* [3]. They found that self-peptide presentation on MHC class II molecules of TECs for positive and negative CD4⁺ T cell selection depends in part on macroautophagy. Autophagy delivers cytoplasmic constituents to lysosomes [4]. At least three separate autophagic pathways exist: chaperone-mediated, micro- and macroautophagy. During macroautophagy, more than 30 autophagy related (Atg) proteins assist in the formation of a double membrane coated vesicle, called autophagosome, and its fusion with lysosomes. Autophagosomes build around cell organelles and protein aggregates, that are destined for degradation, but the mechanism, by which macroautophagy substrates are marked for destruction, remains unclear. Macroautophagy and MHC class II loading interface both with lysosomal degradation in late endosomes, and therefore macroautophagy substrates can be presented on MHC class II molecules *in vitro*. One viral antigen, several model antigens and self-proteins have been demonstrated to gain access to MHC class II presentation via macroautophagy [5-7]. Suggesting a more general role for MHC class II antigen processing via macroautophagy, autophagosomes fused frequently with IFN- γ induced MHC class II containing compartments in a variety of human

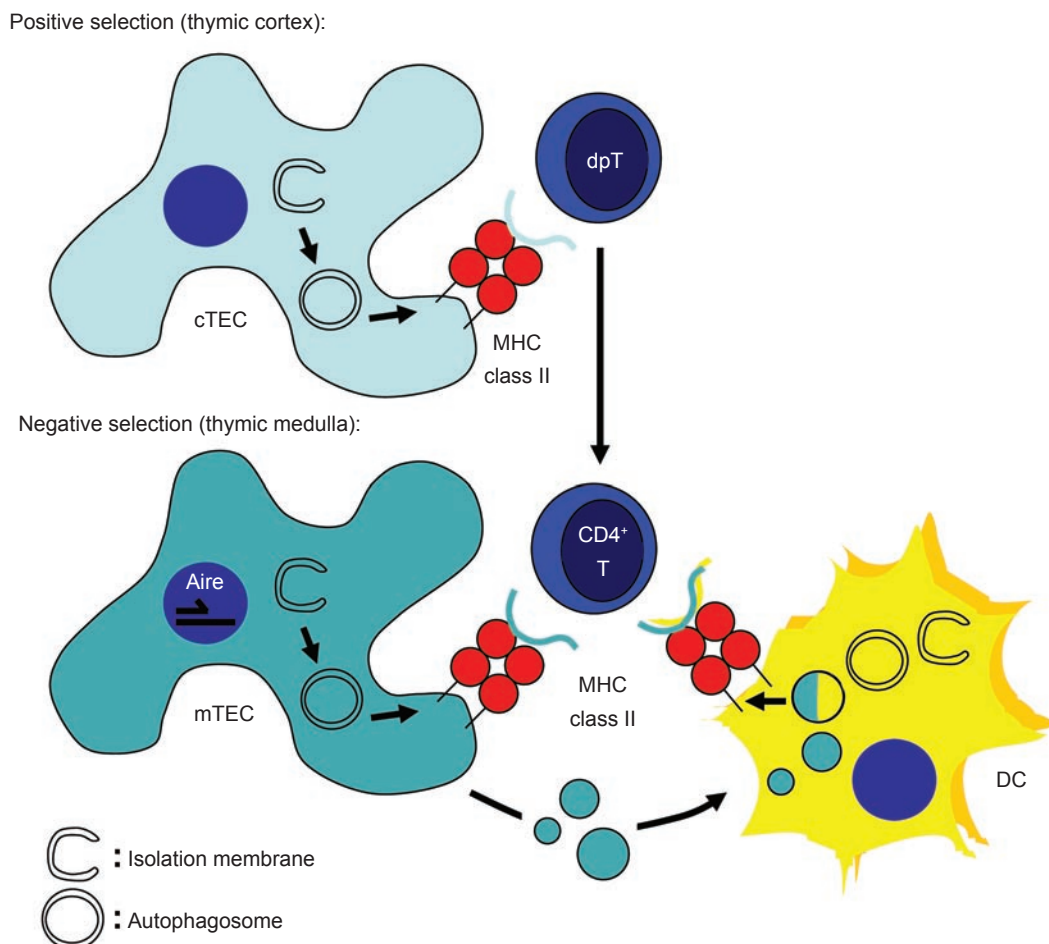


Figure 1 Positive and negative T cell selection in the thymus depends in part on macroautophagy. Cortical and medullary thymic epithelial cells (cTECs and mTECs), as well as thymic dendritic cells (DCs), constitutively perform macroautophagy. Loss of macroautophagy in the thymus compromises positive selection of some T cell specificities on MHC class II molecules (MHC class II) from double-positive (dpT) to single-positive CD4⁺ T cells (CD4⁺ T) by cTECs. Macroautophagy deficient thymi have also diminished negative selection of CD4⁺ T cells by DCs and/or mTECs with promiscuous self-antigen expression due to Aire transcription.

epithelial cell lines and immature DCs [8]. Moreover, antigens, targeted for uptake into autophagosomes, were more efficiently presented to human CD4⁺ T cell clones than their unmodified counterparts [8]. These studies documented efficient and ubiquitous antigen delivery for MHC class II presentation via macroautophagy *in vitro*, but the *in vivo* relevance of this pathway was unknown to date.

Klein, Nedjic and colleagues now demonstrate that the high constitutive macroautophagy level in TECs, which was observed before [9], is of functional relevance [3]. In elegant

experiments they address its role in positive and negative selection by engrafting macroautophagy deficient fetal thymi under the kidney capsule of T cell receptor (TCR) transgenic or athymic nude mice. Then they compare the fate of TCR transgenic developing T cells in macroautophagy deficient and sufficient thymic grafts (positive selection). In addition, they analyze the polyclonal T cell repertoire of nude mice, transplanted with Atg5 positive and negative thymi, for its ability to induce autoimmunity (negative selection). Interestingly, of five transgenic TCR specificities two were insufficiently

selected by Atg5 negative thymi, resulting in lower thymic cellularity and decreased frequency of mature CD4⁺ single positive T cells. Confirming the functionality of Atg5 negative cTECs, three MHC class II and also three MHC class I restricted TCR specificities were normally selected. Furthermore, the repertoire of MHC class II presented peptides on cTECs was altered, because an MHC class II α chain derived peptide was better presented on MHC class II molecules of Atg5 negative cTECs than on wild-type cTECs, probably due to decreased competition by peptides from macroautophagy substrates. These find-

ings suggested that macroautophagy is required for the efficient presentation of some self-peptides for positive T cell selection in the mouse thymus.

In the same study, the influence of macroautophagy deficiency on negative selection of T cells was also investigated. Nude mice transplanted with Atg5 negative thymi developed inflammation in colon, liver, lung, uterus and Harderian glands, while wild-type thymi transplanted mice showed no signs of autoimmunity. These autoimmune responses could be transferred with T cells primed in nude mice that had been transplanted with Atg5 negative thymi. Therefore, macroautophagy is required to process self-proteins for negative T cell selection by MHC class II molecules in the thymic medulla. This leads to efficient projection of an immunological self shadow to developing T cells.

This elegant study on the requirement of antigen processing onto MHC class II molecules via macroautophagy for positive and negative T cell selection in the thymus is the first demonstration that this alternate MHC class II loading pathway is relevant *in vivo*. But like most findings that open new fields, it raises also a number of questions, which should be addressed in the future. In my

opinion the most interesting ones are: How are the proteins, constituting the immunological self shadow, selected into autophagosomes? Is macroautophagy deficiency connected to human autoimmune diseases? Along these lines, mutations in the two macroautophagy associated genes Atg16L1 and immunity-related GTPase family M (IRGM) have been genetically linked to Crohn's disease, an inflammatory disease of the gut [10]. However, the mechanisms by which these mutants promote the disease process are unknown so far. In addition to these tolerance and autoimmunity related questions, the more general one, to which extent macroautophagy participates in adaptive immunity *in vivo*, is also still unanswered. With the more detailed understanding of physiological processes that regulate macroautophagy and of the molecular machinery of this process, the tools are available to address these questions in the near future.

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