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Review

Autophagy-mediated antigen processing in CD4$^+$ T cell tolerance and immunity

Ludger Klein [a,*], Christian Münz [b,1], Jan D. Lünemann [b,*]

[a] Institute for Immunology, Ludwig-Maximilians-University, Goethestr. 31, 80336 Munich, Germany
[b] Institute of Experimental Immunology, University Hospital Zurich, Winterthurer Strasse 190, 8057 Zurich, Switzerland

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ABSTRACT

Macroautophagy, a homeostatic process that shuttles cytoplasmic constituents into endosomal and lysosomal compartments, has recently been shown to deliver antigens for presentation on major histocompatibility complex (MHC) class II. Autophagy-mediated antigen processing in thymic epithelial cells has been suggested to be involved in the generation of a self-MHC restricted and self-tolerant CD4$^+$ T cell repertoire. Furthermore, there is accumulating evidence that the up-regulation of autophagy by pattern-recognition receptor signaling represents an innate defense mechanism against intracellular pathogens. Thus, through linking pathogen breakdown with the presentation of pathogen-derived autophagy substrates on MHC class II, autophagy serves a dual function at the interface of the innate and the adaptive immune response.

1. Introduction

Autophagy is a homeostatic process by which cells recycle nutrients and degrade cytoplasmic constituents such as defective organelles and macromolecular aggregates for lysosomal degradation. There are at least three distinct pathways of autophagy: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. Microautophagy is characterized by the uptake of cytoplasmic components at the lysosomal membrane via budding into the lysosome, through a poorly defined mechanism. Substrates for CMA carry signal peptides for sorting into lysosomes, similar to other protein transport mechanisms across membranes, and are directly imported into lysosomes through the LAMP-2a transporter [1,2], assisted by cytosolic and lysosomal HSC70 chaperones. Macroautophagy is the major route for lysosomal degradation of cytoplasmic constituents. During macroautophagy, cytosolic constituents including organelles are enclosed in a double-membrane vesicle, called autophagosome [3,4], which then fuses with late endosomal/lysosomal organelles for degradation of the inner autophagosomal membrane and its cargo. The resulting breakdown products of macromolecules are subsequently released back into the cytosol through permeases in the lysosomal membrane, where they can be reused for anabolic or catabolic reactions [5].

Studies on the molecular mechanisms of macroautophagy and its importance in protein metabolism [5] have set the stage to analyze its role in multiple biological processes including innate and adaptive immune responses. In keeping with its cellular clearance function, macroautophagy participates in limiting pathogen replication in host cells. In addition, macroautophagy delivers viral, parasitic, and bacterial antigens to late endosomal compartments, where macroautophagy substrates are then degraded by lysosomal hydrolases. The fusion vesicles between autophagosomes and late endosomes, the so-called amphisomes, display a multivesicular and multilamellar morphology reminiscent of major histocompatibility complex (MHC) class II containing compartments (MIICs) [6]. Indeed, studies in cell culture systems, including antigen presentation assays, co-localization studies and sequencing of MHC class II bound peptides, demonstrated that substrates of autophagy can be loaded onto MHC class II for CD4$^+$ T cell recognition. Animal models to monitor or genetically disrupt macroautophagy now provide the basis for elucidating the immunological relevance of autophagy in vivo.

As this review focuses on the role of autophagy in mediating CD4$^+$ T cell responses and in regulating CD4$^+$ T cell immunity through processing and presentation of intracellular antigens on

Abbreviations: MHC, major histocompatibility complex; DC, dendritic cells; APC, antigen presenting cell; TCR, T cell receptor; EBV, Epstein Barr virus; TEC, thymic epithelial cell; PRR, pattern-recognition receptors

* Corresponding authors. Fax: +49 89 5160 2236 (L. Klein); +41 44 635 6883 (J. Lünemann).
E-mail addresses: ludger.klein@med.lmu.de (L. Klein), jan.luenemann@usz.ch (J.D. Lünemann).
[1] Fax: +41 44 635 6883.

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MHC class II molecules, we will first outline the basic principles of antigen processing.

2. Antigen processing and presentation

T and B cells both express highly diverse receptors for antigen whose enormous variability is established through somatic rearrangement of gene fragments during early development of the respective lineage. Despite this similarity in the genetic makeup of the T and B cell receptor, there is a fundamental difference in how these two classes of lymphocytes recognize antigen: B cells directly recognize antigens through interaction of their receptor with free antigens or epitopes on the surface of supramolecular structures (e.g. cells, bacteria, viruses), whereby these epitopes can be made up of carbohydrates, lipids, proteins and even anorganic compounds. By contrast, T cells primarily recognize relatively small peptides that are generated by proteolytic breakdown of protein substrates. These peptide snippets are not recognized in free form, but have to be embedded in MHC molecules on the surface of cells.

MHC molecules come in two flavors, MHC class I and MHC class II. Both classes of molecules share as a characteristic feature of their tertiary structure a so-called peptide-binding groove, which consists of two alpha helices outlining the rims of the peptide-binding moiety and a beta sheet that forms the bottom of the groove. Despite the overall very similar structure of MHC class I and II molecules, the details of their tissue distribution, physiological function and how the peptides they present to T cells are generated, are remarkably distinct. For instance, MHC class I is expressed on essentially all tissues, whereas constitutive expression of MHC class II is restricted to so-called professional antigen presenting cells (APCs) of hematopoietic origin and epithelial cells of the thymus. Only upon exposure to interferon-γ (IFNγ), tissues other than these will up-regulate MHC class II expression. Furthermore, whereas peptide/MHC class I (pMHCI) complexes represent the ligands for the T cell receptor (TCR) of $CD8^+$ cytotoxic T cells, pMHCII complexes are recognized by $CD4^+$ helper T cells. This dichotomy in the recognition of pMHCI or II ligands by either cytotoxic or helper T cells, respectively, reflects the process of positive selection during T cell development in the thymus. This rather wasteful process ensures that immature T cells commit to the $CD8^+$ or $CD4^+$ lineage according to the principal capacity of their randomly rearranged TCR for MHC class I or II, respectively. As a result, cytotoxic T cells express the CD8 co-receptor, whereas helper T cells express the CD4 co-receptor, which interact with non-polymorphic regions of MHC class I or II, respectively.

The basic principles of how peptides embedded in MHC class I or II are generated have been well-established over the last two decades (Fig. 1) [7]. Thus, MHC class II bound epitopes are primarily generated through the proteolytic processing of proteins that reach endosomal/lysosomal compartments subsequent to having been taken up from the extracellular space. By contrast, MHC class I bound peptides are mostly derived from proteasomal substrates, that is, mis-folded cytoplasmic proteins that have been earmarked for degradation by ubiquitination.

At first glance, the paradigm that MHC class I or MHC class II bound peptides originate from topologically distinct sources represents an elegant way how the immune system copes with the challenge that the eradication of intra- or extracellular pathogens necessitates fundamentally different effector mechanisms. Thus, the control of viral infections requires that those cells that have been infected are eliminated by MHC class I-restricted $CD8^+$ cytotoxic T cells. By contrast, the clearance of extracellular pathogens such as most bacteria or helminths is largely dependent on antibody responses. In this case, MHC class II-restricted $CD4^+$ helper T cells fulfill a critical pacemaker function for humoral immune responses in the form of cytokines that orchestrate the efficient production of antibodies by B cells.

A closer look, however, renders an absolutely strict topological demarcation of the origin of MHC class I and MHC class II bound peptides epitopes rather unlikely. For example, the initiation of certain antiviral cytotoxic $CD8^+$ T cell responses is dependent on the MHC class I-restricted presentation of viral epitopes by dendritic cells (DCs), the major type of so-called "professional antigen presenting cells". Paradoxically, however, many viruses do not infect DCs, and yet elicit strong cytotoxic T cell responses. This observation underscores the physiological relevance of an "unconventional" MHC I loading pathway that involves the shuttling of exogenous material, in this case viral proteins, into the MHC class I loading pathway of DCs, a process termed cross-presentation [8]. Vice versa, early evidence for exceptions from the "rule" that MHC class II molecules are exclusively occupied by peptides of extracellular origin was provided by the sequencing of peptides eluted from MHC class II, revealing that around 20% of the identified epitopes originated from intracellular sources such as mitochondrial proteins, cytoskeletal proteins, metabolic enzymes, chaperones and nuclear proteins [9].

How do epitopes derived from cytoplasmic proteins gain access to MHC class II molecules? The candidate pathways implicated in so-called "endogenous MHC class II loading" can be grouped into two groups, depending on whether the intersection with the conventional exogenous MHC class II loading pathway occurs upstream or downstream of the proteolytic degradation of the substrate. The first category involves proteasomal antigen processing and subsequent TAP-mediated import of degradation products into the ER and thus may be best described as "spill-over" from the MHC class I pathway into MHC class II loading compartments [10]. The second category encompasses a rather heterogeneous group of mechanisms that deliver cytoplasmic material for lysosomal degradation, including chaperone-mediated autophagy and macroautophagy (reviewed in [11]). We will primarily focus on macroautophagy in this review, because this pathway was frequently suggested to deliver cytoplasmic material for MHC class II loading.

3. Autophagy substrates are loaded onto MHC class II molecules

More than a decade ago, the lab of Gitta Stockinger reported that treatment of macrophages and B cells with the macroautophagy inhibitor 3-methyl adenine (3-MA) prevented the presentation of an endogenously synthesized protein on MHC class II [12]. On this basis, these authors suggested that macroautophagy may shuttle cytosolic proteins into MHC class II loading compartments. In a subsequent study, Mautner and co-workers found that transfection of a renal carcinoma cell line with a model antigen resulted in the endogenous processing for MHC class II restricted presentation to $CD4^+$ T cells, and again, this process could be inhibited by 3-MA [13]. A caveat of both studies was that they involved the ectopic over-expression of the respective model antigens. The first study to document a role for macroautophagy in loading of a physiologically expressed cytoplasmic antigen dealt with the recognition of the nuclear antigen 1 (EBNA1) of Epstein Barr virus (EBV) by $CD4^+$ T cells. It was found that EBNA1 gains access to MHC class II in EBV transformed B cells [14], and that EBNA1 was detectable in autophagosomes when lysosomal proteolysis was inhibited [14]. Inhibition of macroautophagy by 3-MA or RNA interference directed against Atg12 strongly diminished recognition of these cell lines by EBNA 1 specific $CD4^+$ T cells [14]. In further support of a role for macroautophagy in endogenous MHC class II loading, recognition of tumor antigen derived epitopes on MHC class II after RNA transfection of DCs was reduced by 3-MA [15]. In support of

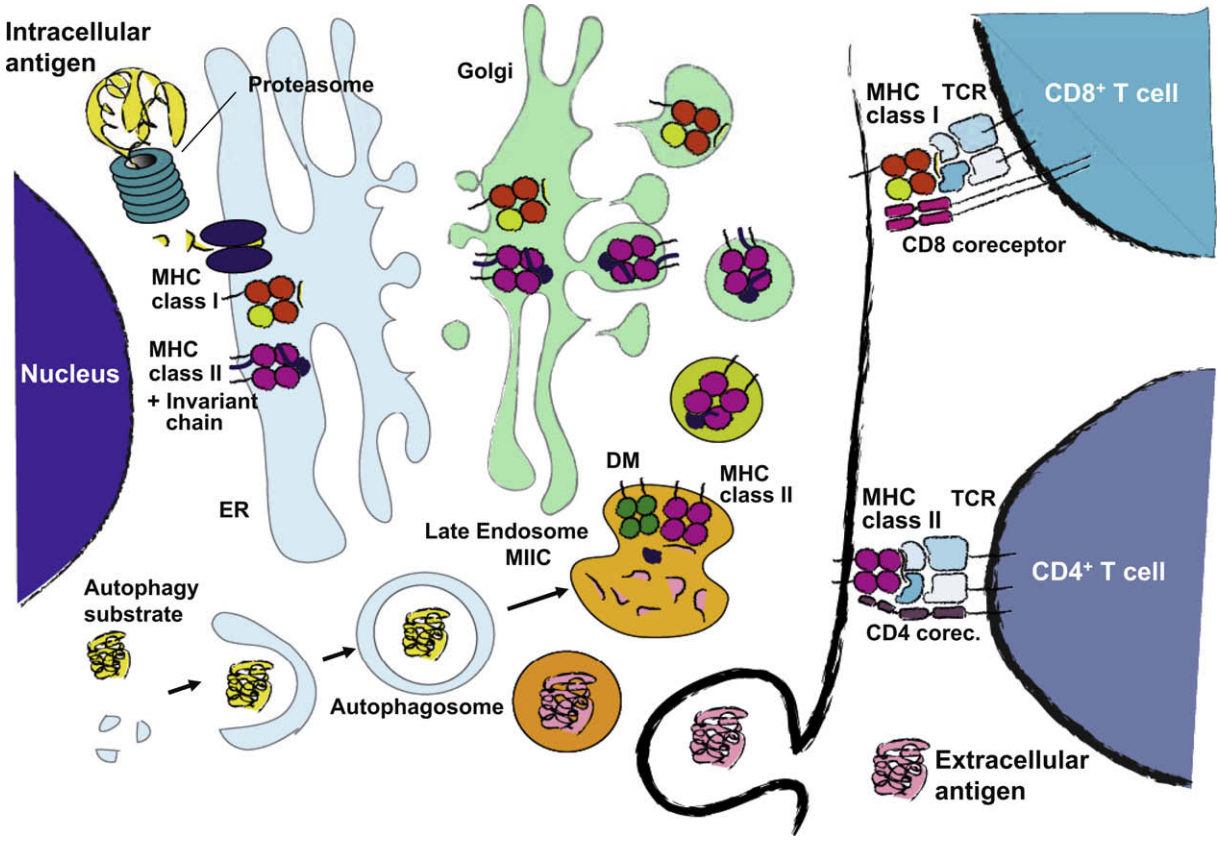

Fig. 1. Macroautophagy in MHC class II antigen processing. Antigens for MHC class I presentation are derived from proteins that are degraded in the cytosol by the proteasome. Subsequent to their import into the endoplasmic reticulum (ER), peptides of 8–9 amino acids in length are loaded into the peptide-binding groove of MHC class I molecules. Peptide-loaded MHC class I complexes are then exported through the Golgi apparatus to the cell surface for recognition by $CD8^+$ T cells. MHC class II molecules bind peptides generated in the endosomal–lysosomal systems and display them on the cell surface for recognition of $CD4^+$ T cells. Extracellular antigens are taken up via endocytosis/phagocytosis into endosomal compartments and are degraded by lysosomal proteases before they gain access to late endosomal MHC class II-loading compartments (MIICs). The invariant chain (Ii) (shown in purple) protects the peptide-binding groove of MHC class II molecules from premature loading within the ER. In MIICs, lysosomal proteases degrade the Ii, and the remaining peptide (CLIP for class II-associated Ii peptide) is exchanged for antigenic peptides. MHC class II molecules occupied by high-affinity peptides are then transported to the cell surface for $CD4^+$ T cell immune surveillance. Besides this well-established "classical" exogenous MHC class II loading pathway, there is now compelling evidence that a substantial proportion of MHC class II ligands are derived from intracellular proteins and that autophagic pathways contribute to the delivery of these to the MIICs. During macroautophagy, which besides the proteasomal pathway represents a second major route of degradation of intracellular constituents, cytoplasmic and nuclear antigens are enclosed in a double-membrane vesicle which then fuses with lysosomes and late endosomes to form multivesicular and multilamellar compartments, called amphisomes. It is at this stage that an intersection with the "classical" MHC class II loading pathway is thought to occur.

a broader relevance of endogenous MHC class II loading, it could be demonstrated that macroautophagy is constitutively (i.e. even under nutrient rich conditions) active at a relatively low but detectable level in a variety of MHC class II positive APCs such as DCs, B cells and monocytes. Co-localization studies indicated that in these cell types, autophagosomes indeed frequently fuse with MHC class II-loading compartments, and deliberate experimental delivery of a viral antigen to autophagosomes by targeting to the autophagosomal membrane through fusion with Atg8/LC3, a ubiquitin-like protein that gets covalently attached to the autophagic membrane during macroautophagy, resulted in robust recognition by specific $CD4^+$ T cells [16]. The most direct evidence for delivery of autophagy substrates to the MHC class II pathway was obtained by eluting and sequencing MHC II bound epitopes before and after switching to nutrient deprived conditions, indicating that starvation induced macroautophagy resulted in a higher prevalence of cytoplasm- or organelle-derived peptides [17].

4. Autophagy in positive and negative selection of the $CD4^+$ T cell repertoire

The first in vivo situation that was identified to involve macroautophagy-mediated antigen processing for MHC class II presentation is T cell selection. The specificity of the TCR on the surface of individual thymocytes results from random somatic rearrangement of gene segments at an early intrathymic developmental stage. Inevitably, the stochastic nature of this process leads to the emergence of T cells that carry receptors that are either "useless" or "harmful": the former because they may not confer the capacity to interact with self-MHC molecules at all, and the latter because they may possess a specificity for self-antigens and hence be potentially dangerous. In order to remove such specificities from the T cell pool, the nascent T cell repertoire is subject to positive and negative selection [18]. For the sake of conceptual clarity, it may in the context of this discussion be helpful to think of these two processes as being spatially and temporally segregated (although this is an issue of long-standing controversy). Thus, immature thymocytes first "test" the capacity of their TCR to engage pMHC class I and pMHC class II complexes presented by cortical thymic epithelial cells (cTECs), and T cell survival ("positive selection") at this stage is contingent upon low affinity interactions. Furthermore, it is also on the basis of interactions with pMHC ligands on cTECs that thymocytes commit to the $CD4^+$ or $CD8^+$ lineage according to the capacity of their TCR to interact with MHC class II or MHC class I, respectively. Remarkably, about 80–90% of thymocytes fail to fulfill the criteria for positive selection

and die "by default". Subsequent to positive selection, thymocytes translocate to medullary regions of the thymus where their TCRs are tested for overt self-reactivity with MHC ligands on different subsets of DCs as well as medullary thymic epithelial cells (mTECs). Here, T cells that interact strongly with pMHC complexes on APCs in the medulla are subject to negative selection (also known as "clonal deletion").

The essential or at least significant role of cTECs and mTECs for positive selection or tolerance induction, respectively, provides an obvious explanation for why these two developmentally related thymic stromal cell subsets are the only non-hematopoietic cell types that constitutively express MHC class II molecules. Remarkably, both cTECs and mTECs were found to be extremely inefficient in delivering epitopes derived from exogenous antigens onto MHC class II, a feature that clearly distinguishes them from thymic DCs [19]. On this basis, it was suggested that they may predominantly present endogenously derived peptides on MHC class II [20]), and the discovery in LC3-GFP transgenic, macroautophagy reporter mice that TECs, and foremost cTECs, display an unusually high rate of starvation independent, constitutive macroautophagy provided an intriguing piece of evidence in favor of this idea [21]. More recently, it has been reported that in both cortical and medullary TEC lines, LC3 was colocalized with markers for MHC class II loading compartments [22]. These results strongly suggest that cytoplasmic self-antigens expressed in the thymus gain access to MHC class II loading via macroautophagy.

The hypothesis that macroautophagy in cTECs contributes to the generation of pMHC class II ligands for positive selection was addressed using the macroautophagy deficient Atg5$^{-/-}$ mouse mutant [23]. The neonatal lethality of Atg5$^{-/-}$ mice, at least in part caused by impaired bridging of a metabolic crisis related to the transition from trans-placental to milk-derived nutrient supplies, precluded a straight-forward assessment of T cell development in this system. To circumvent this complication, embryonic thymi were transplanted under the kidney capsule of wild-type recipients, a well-established procedure to study the effects of a particular genetic lesion specifically in TECs (in these grafts, hematopoietic cells, i.e. T cells and DCs, turn-over and are eventually replaced by host-type cells, whereas both cTECs and mTECs are replenished from a precursor pool of donor origin). It turned out that positive selection of a number of MHC class II-restricted TCR specificities was affected in the absence of macroautophagy in thymic epithelium [24]. Of note, depending on the TCR tested, the alterations in the efficacy of positive selection could be either of detrimental or beneficial nature, indicating that the general capacity of cTECs to support positive selection was not impaired. Thus, the most likely explanation for the effects of abrogated epithelial macroautophagy on positive selection is that certain peptides that normally foster the positive selection of particular TCR specificities may not or only inefficiently be presented, while other, "macroautophagy-independent" ligands may be over-represented under these circumstances.

A similar experimental set-up was also used to address whether the polyclonal T cell repertoire generated in the absence of macroautophagy in TECs was self-tolerant. Here, athymic nude mice (that due to a mutation in the Foxn1 transcription factor lack fully differentiated TECs and consequently also a T cell system) were grafted with Atg5$^{-/-}$ or wild-type embryonic thymi. In such chimeras, the graft is colonized by hematopoietic precursors and within about four weeks, the peripheral lymphoid organs of these animals are seeded with a T cell repertoire that has been "educated" by MHC ligands on macroautophagy deficient TECs. Quite dramatically, many chimeras carrying an Atg5$^{-/-}$ graft exhibited a substantial weight loss starting from about five weeks after grafting. Histological evidence and secondary transfers of peripheral T cells from diseased animals confirmed that these symptoms were caused by immune-mediated tissue damage.

In sum, the function of constitutive macroautophagy in TECs may therefore be two-fold. First, macroautophagy may generate endogenously derived MHC class II-bound peptides on cTECs for positive selection of CD4$^+$ T cells. Second, it is conceivable that macroautophagy similarly shuttles cytoplasmic or organelle-derived self-antigens into the MHC class II-loading pathway of mTECs, in this case for tolerance induction within the nascent CD4$^+$ T cell repertoire. This latter scenario might be of particular significance considering that mTECs broaden the scope of self-antigens available for central tolerance induction by ectopically expressing a wide array of otherwise strictly tissue-specific self-antigens [25].

5. Harnessing autophagy to enhance CD4$^+$ T cell immunity

In addition to its role in thymic T cell selection, MHC class II loading after macroautophagy probably also contributes to the priming of CD4$^+$ T cell responses during infection and after vaccination. This scenario is particularly relevant for intracellular pathogens that are restricted by macroautophagy and whose breakdown products could give rise to MHC class II ligands after degradation in lysosomes. Thus, bacteria and parasites that either escape from endosomes and replicate in the cytosol or condition the phagosome to serve as their replication niche – by preventing fusion with lysosomes – have been found to be delivered for lysosomal degradation via macroautophagy. Among these pathogens are group A *Streptococci*, *Listeria monocytogenes* and *Shigella flexnerii*, which replicate in the cytosol after breaching the endosomal membrane and may subsequently be engulfed by autophagosomes [26–28].

The role of macroautophagy as an innate defense mechanism against cytosolic bacteria seems to have forced successful pathogens to evolve evasion strategies. Thus, *Listeria* decreases engulfment into autophagosomes by surrounding itself with coats of cellular components. It induces an actin coat by ActA, independent of the contribution of this protein to bacterial mobility [28], or by hiding in incompletely listeriolysin O-permeabilized endosomes [29]. Similarly, the VirA protein of *Shigella*, another protein involved in bacterial actin-based mobility, is recognized by the molecular macroautophagy machinery and can lead to selective engulfment of these bacteria by autophagosomes [27], *Shigella* blocks VirA recognition through expression of the IcsB protein, resulting in bacterial evasion from macroautophagy.

Pathogens that condition their phagosomes after endocytic uptake, like *Mycobacterium tuberculosis* or *Toxoplasma gondii*, can be targeted for lysosomal degradation via stimulation of macroautophagy [30–34]. As a cellular defense mechanism, these conditioned phagosomes can be delivered to lysosomes via fusion with or engulfment by autophagosomes. This macroautophagic clearance of pathogen-conditioned phagosomes requires immunity-related p47 GTPases such as immunity-related GTPase M (IRGM) and a6 (Irga6) [31,33,35–37]. Their recruitment to the bacterial phagosome enhances fusion with lysosomes and subsequent clearance of the pathogens. Interestingly, this innate restriction of intracellular pathogens is co-opted by the immune system for CD4$^+$ T cell priming. Thus, MHC class II presentation of the antigen Ag85B of *M. tuberculosis* was found to be enhanced after stimulation of macroautophagy through blockade of the mTOR pathway with rapamycin [38]. Rapamycin treated DCs induced 2–6-fold higher CD4$^+$ T cell responses against Ag85B after vaccination through adoptive transfer into mice. Moreover, a combination of Ag85B-overexpressing mycobacteria and induction of macroautophagy elicited protective CD4$^+$ T cell responses which controlled pathogen titers in the lung at 1 log lower set-points. These findings suggest that intracellular pathogen degradation, which on the one hand

serves the purpose of restricting pathogen growth as an innate immune mechanism, at the same time also leads to the presentation of pathogen-derived antigens on MHC class II molecules for the induction of protective $CD4^+$ T cell responses. Such an enhanced MHC class II presentation after macroautophagy induction harbors obvious potential to increase the efficacy of vaccination strategies.

6. Regulation of macroautophagy by immune signals

The ability of the host to successfully fight off invading pathogens often relies on a tightly controlled interplay of innate and adaptive immune mechanisms. The latter are largely controlled by a group of germline-encoded receptors known as pattern-recognition receptors (PRRs). These molecules include Toll-like receptors, nucleotide-binding and oligomerization domain (NOD)-like receptors, retinoic-acid-inducible gene I (RIG-I)-like helicases and a subset of C-type lectin receptors, which endow cells of the innate immune system with the ability to recognize a large number of molecular patterns expressed by bacteria, viruses or fungi. Generally, the signaling pathways that are triggered by PRR ligation through pathogen associated molecular patterns (PAMPs) lead to cellular responses that range from increased antigen-presenting capacity, for instance by the expression of co-stimulatory molecules, to the production of soluble mediators such as type I interferons, pro-inflammatory cytokines and chemokines, which orchestrate the immune response against the invading pathogen.

Recent data have revealed an intricate, mutual interplay between PRRs and macroautophagy, whereby macroautophagy on the one hand facilitates the recognition of cognate ligands by PRRs by fostering their physical interaction, and on the other hand serves as an immune effector mechanism downstream of PRR stimulation. For example, intracellular replication intermediates of vesicular stomatitis virus (VSV), recognized by PRRs, are delivered to endosomally located TLR7 by macroautophagy, which results in robust type I interferon-dependent innate immune responses by plasmacytoid DCs (pDCs) [39]. As an effector mechanism, PRR signaling can induce or augment macroautophagy [40,41]. In this context, intracellular PRRs rather than receptors that survey the extracellular environment may be key candidates for linking pathogen sensing with macroautophagy induction. For instance, insect cells rely on intracellular peptidoglycan-sensing molecules to trigger macroautophagy following *L. monocytogenes* infection [42]. This autophagic response appears to be required for the control of *L. monocytogenes* infection and survival of the host. Likewise, the intracellular PRRs Nod1 and Nod2, which sense peptidoglycan moieties released from bacterial cell walls, can act as nucleation sites for macroautophagy initiation following bacterial infection in mammalian cells [43]. Nod1 and Nod2 recruited the macroautophagy protein ATG16L1 to the plasma membrane at the bacterial entry site, and the physiological relevance of this finding is bolstered by the observation that function-altering Nod2 mutations lead to impaired wrapping of invading bacteria by autophagosomes [43]. Thus, macroautophagy does not only target PAMPs to endosomal PRRs for immune recognition, but is also actively controlled by PRR stimulation, suggesting that PRR signaling pathways could be exploited for the elimination of intracellular pathogens through induction of macroautophagy.

Besides PRR signaling, cytokines such as interferons (IFNs) and members of the tumor necrosis factor (TNF) family also are capable of modulating macroautophagy. Restriction of HSV-1 infection by macroautophagy in vitro and in vivo was found to be dependent on type I IFN signaling [44]. IFN-gamma has been reported to enhance *M. tuberculosis* and *Ricksettia conorii* degradation by macroautophagy in infected cells [30,31]. Interestingly, whereas IFN-gamma induces macroautophagy and mycobacterial clearance – through a mechanism that involves immunity-related GTPases (IRGs) – [45], Th2 signature cytokines such as interleukin (IL)-4 and IL-13 appear to inhibit IFN-gamma induced autophagic delivery of mycobacteria into degradative compartments [46]. While the complex regulation of macroautophagy by cytokines is only incompletely understood, it deserves mentioning that mouse tissues are probably more susceptible to IFN-gamma-mediated macroautophagy induction because their IRGs are IFN-gamma inducible (in contrast to those of humans), indicating that immune signals that stimulate macroautophagy can differ between rodents and man.

Concerning TNF-family pathways, TNF-alpha was found to upregulate macroautophagy in cells lacking NF-*k*B activation [47] and TNF-related apoptosis-inducing ligand (TRAIL) was described to induce macroautophagy in human epithelial cells [48]. Consistent with this, inactivation of Fas-associated death domain (FADD), the signaling adapter protein of the TRAIL receptor, decreases macroautophagy induction by TRAIL [49]. As a third TNF-family member, CD40L has been demonstrated to induce macroautophagy-mediated fusion of *T. gondii*-containing phagosomes with lysosomes through CD40 signaling on mouse and human macrophages [32]. Together, these findings suggest that the immune-mediated regulation of macroautophagy via cytokines or membrane-bound molecules could represent a feedback mechanism by which activated T cells augment macroautophagy under inflammatory conditions.

7. Conclusions and outlook

A growing body of evidence indicates that the immune system employs macroautophagy for the immune surveillance of cytoplasmic compartments and the eventual clearance of intracellular pathogens. The "non-classical" presentation of autophagosomal substrates on MHC class II, supposedly enhanced under inflammatory conditions, represents another example of the intricate cross-talk between the innate and the adaptive arms of the immune system. As a mechanism involved in the steady-state turnover of cellular components, macroautophagy does not only shuttle pathogen-derived foreign antigens, but presumably also self-antigens into the MHC class II pathway. The necessity to prevent the immune system from mounting an autoreactive response against these intracellular self-antigens may have driven the evolution of a mechanistically as yet poorly understood unusually high rate of starvation- and inflammation-independent macroautophagy in TECs. This phenomenon was shown to contribute to $CD4^+$ T cell selection and is essential for the generation of a self-tolerant T cell repertoire. Future work will establish whether macroautophagy, in addition to its function in thymic T cell selection, is similarly involved in the maintenance of peripheral tolerance within the $CD4^+$ T cell compartment.

Recent studies have also suggested a role for macroautophagy in regulating intracellular antigen processing for MHC class I presentation and in packaging antigens for optimal cross-presentation on MHC class I molecules [50,51]. While these additional pathways require further investigation to understand the underlying mechanisms, autophagy-mediated antigen delivery to both MHC class II and MHC class I molecules should be explored for its potential to increase the efficacy of vaccination strategies, to enhance adaptive immune responses during chronic infections or to limit inflammatory tissue damage in T cell-driven autoimmune diseases. Future studies might provide a more precise understanding of how and by which mechanisms autophagy regulates T cell immunity and tolerance during health and disease in vivo and how this pathway could be harnessed for vaccination and immunotherapy.

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