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The labyrinth unfolds: architectural rearrangements of the endolysosomal system in antigen-presenting cells

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Antigen-presenting cells (APCs) capture and present pathogens to T cells, thus arousing adaptive immune responses geared at the elimination of these invaders. In APCs, pathogens acquired from the extracellular space intersect with MHC class II (MHC-II) molecules in the endolysosomal system, where processing and loading of antigenic peptides occur. The resulting complexes can then be directed to the cell surface for recognition by T cells. To achieve this, the endosomal pathway of APCs must undergo dramatic rearrangements upon pathogen encounter. In this review we discuss recent strides in our understanding of how APCs modulate the organization and function of their endolysosomes to best suit different stages of antigen acquisition, processing and presentation cascade.

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Introduction

The endolysosomal system functions as a trading platform through which the cell and its environment exchange information and goods in order to maintain homeostasis and communicate impending predicaments. To enable reception and utilization of a wide variety of materials and signals, this system comprises many different flavors of vesicular membrane carriers, whose interactions with one another determine downstream cellular outcomes. Following internalization from the extracellular space, endosomes undergo progressive maturation, characterized by acidification and sorting of ubiquitinated cargoes into intraluminal vesicles (ILVs). The resulting multivesicular bodies (MVBs) can subsequently fuse with lysosomes to deliver the contents of their ILVs for degradation [1], while materials not intended for degradation recycle from the limiting endosomal membrane back to the cell surface. Cytoplasmic cargoes encapsulated within autophagosomes also feed into this endocytic tract for access to the proteolytic compartment, thus allowing cross-talk between intracellular and extracellular domains [2]. This diverse system of vesicles must work in concert to fulfill its roles as the cell's sensory platform and digestive organelle. To ensure controlled uptake and progression of cargoes through the system, cells have evolved strategies to organize and manipulate their endolysosomal repertoire in space and time. In this review, we discuss the nature of these strategies and their relationship(s) to endocytic function at the host–pathogen interface.

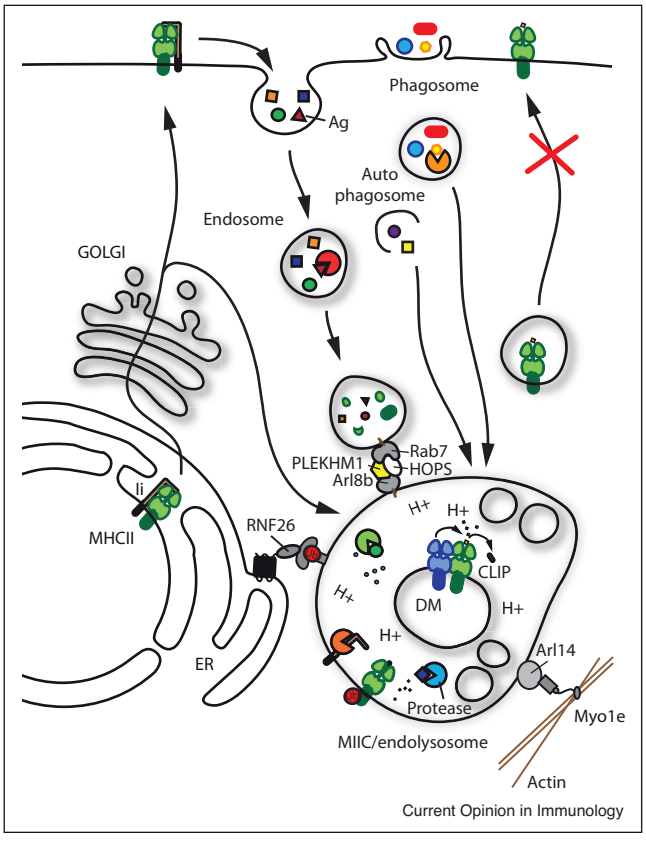
As the main port of cellular entry, the endocytic pathway is routinely hijacked by a wide variety of pathogens during infection [3]. Accordingly, the endolysosomal system has adapted to serve as the front line of the immune response. This is perhaps best exemplified by professional APCs—immune cells tasked with detection and presentation of antigens. APCs, including dendritic cells (DCs), macrophages, B cells and thymic epithelial cells rely profoundly on the endocytic pathway to fulfill their physiological function, as it allows them to take up, process and display antigens in a controlled manner. Following internalization, pathogenic constituents are transported to late endosomal compartments, where they are processed into short peptides and subsequently loaded onto MHC-II molecules for export to the plasma membrane. Once peptide-MHC-II complexes (p-MHC-II) reach the cell surface, they can activate CD4 T cells, stimulating differentiation into T helper cell subsets essential for proper activation of cytotoxic T cells and differentiation of B cells [4]. Consequently, disturbances in the workings of the endolysosomal system, caused for instance by pathogens [5], metabolites or drugs, can directly impair antigen processing and presentation, resulting in hampered immunity. While we understand, to some extent, the general mechanisms controlling endosome behavior and function, our knowledge of endosomal perturbations in the context of antigen presentation is still quite poor. Below we review our current understanding of endolysosomal architecture, motility, and membrane dynamics in APCs, with emphasis on the imperative changes occurring during maturation of these cells upon pathogen encounter. We also debate how deregulation of MHC-II trafficking and autophagy in

antigen-presenting cells can contribute to the development of auto-immunity.

'Class II' goes to the MIIC

Following their biosynthesis and association with the invariant chain chaperone (Ii) in the endoplasmic reticulum (ER), MHC-II dimers traffic via the *trans*-Golgi network to the so-called MHC-II compartment (MIIC) known to host antigen processing and peptide loading (Figure 1) [6]. Although MIICs can vary in structure and maturation state, they generally comprise late endosomes or lysosomes characterized by intraluminal membranes, acidic pH, and mild proteolytic activity—all attributes that favor antigen processing and preservation of resulting epitope integrity [4]. When antigens acquired through endocytosis, phagocytosis, macropinocytosis (exogenous), or autophagy (endogenous) enter the endocytic tract, they are directed to the MIIC for degradation [7,8]. Antigenic peptides produced during this process can then be directly loaded onto MHC-II residing in the same compartment. To facilitate peptide loading, MHC-II-associated Ii must undergo proteolysis [9], leaving only the contiguous internal segment of Ii (CLIP) within the MHC-II peptide-binding groove [10]. The endosomal chaperone HLA-DM then mediates removal of CLIP, creating an opportunity for antigen-derived peptides to bind [11].

Figure 1



Schematic overview of MHC-II antigen presentation in APCs before maturation.
In immature APCs, MHC-II in complex with the invariant chain Ii exits the biosynthetic tract via the TGN and is targeted to the endosomal pathway either directly or via the plasma membrane. Once MHC-II/Ii complex reaches the acidic and proteolytic MHC-II compartment (MIIC), much of Ii is trimmed away and MHC-II/CLIP awaits antigens. After sorting into ILVs, MHC-II interacts with HLA-DM for peptide loading. As APCs sample their environment and collect antigens (Ag) through various pathways (exogenous: endocytosis, phagocytosis; endogenous: autophagy), these are routed to the MIIC for processing, loading, and presentation. This requires retrograde vesicle transport toward the microtubule minus-end where antigen processing compartments are retained by ER-associated RNF26. Fusion with these endolysosomes is then orchestrated by the small GTPases Rab7 and Arl8b in conjunction with their effectors RILP, PLEKHM1 and HOPS. Additionally, Arl14/Myo1e complex inhibits export of p-MHC-II complexes to the plasma membrane.

Under steady state conditions, most MHC-II dimers, along with HLA-DM, are sequestered onto ILVs of the MIIC [12]. Intraluminal sorting of MHC-II, as well as vesicle budding and scission, is orchestrated by the endosomal sorting complexes required for transport (ESCRT) [13] and likely requires MHC-II ubiquitination by ligases of the membrane-associated RING-CH (MARCH) family [14], although the impact of this modification on antigen presentation remains debated [15]. Before incorporation into ILVs, ubiquitin may be removed and recycled from sorted MHC-II upon action of deubiquitinating enzymes [16]. Eventually, MHC-II reaches intraluminal membranes, whose composition greatly differs from that of the limiting membrane. ILVs are highly enriched in cholesterol and tetraspanin proteins (CD63, CD81 and others) [17], and sequestration of MHC-II in the lumen of the MIIC may, therefore, have functional consequences. Indeed, FRET studies have indicated that MHC-II and HLA-DM interact on ILV membranes, rather than on the limiting membrane of the MIIC [18], implying that MHC-II peptide loading may comprehensively occur on internal vesicles. While intraluminal tetraspanin networks have been suggested to stabilize DM-MHC-II interactions and facilitate peptide loading on ILVs [19], cholesterol likely plays a substantial role in MHC-II intracellular traffic [20].

In immature APCs, MHC-II-positive ILVs are eventually degraded in lysosomes or released from the cell as exosomes [21]. Following encounter with a pathogen, however, MIICs transition from ILV-rich antigen-producing and loading factories to tubular organelles, poised for transport of mature MHC-II complexes to the plasma membrane for recognition by T cell receptors [22]. How the MIIC is organized and remodeled to best serve the host during different stages of the infection process—and how these same attributes can be utilized by the pathogen to gain advantage over the host—is discussed below.

Before the invasion—organization of the endolysosomal system in immature APCs

In numerous APCs, including macrophages and immature monocyte-derived DCs (moDC), the endolysosomal system features a bilateral architecture, comprised of a rather immobile perinuclear (PN) vesicle cluster, or 'cloud', and a highly dynamic peripheral pool of endosomes and lysosomes [23••]. The fate of individual vesicles in either region of the cell relies on small GTPases, which determine directionality of transport between the PN cloud and the periphery and orchestrate maturation and cargo exchange [24]. Through interactions with various effector proteins, GTPases of the Rab, Arf, and Arl families couple vesicles to microtubule motors for directional transport and mediate recruitment of remodeling factors required for membrane fusion and fission. At least two GTPases, Rab7 and Arl8b, associated with late endosomes and lysosomes, oversee traffic flow along the antigen processing and presentation cascade (Figure 1) [25,26]. Rab7, in complex with its effector Rab-interacting lysosomal protein (RILP) and the microtubule-based dynein motor, transports maturing endosomes including those containing antigens toward the PN cloud, where degradation occurs. Rab7 then collaborates with Arl8b through a shared effector Pleckstrin homology domain containing protein family member 1 (PLEKHM1) and its associated homotypic fusion and protein sorting (HOPS) complex to promote fusion between late endosomes and lysosomes [27•], thereby ensuring cargo degradation and acquisition of antigenic peptides by MHC-II.

Spatiotemporal regulation of endolysosomal organization and behavior is not only autonomously determined, as described above, but is also influenced by other organelles, most notably the endoplasmic reticulum (ER). For instance, endosomes entering the PN cloud can be anchored and retained through interactions with the perinuclear ER-associated ubiquitin ligase Ring finger protein 26 (RNF26), and this ER docking facilitates access of incoming vesicles to late compartments (Figure 1) [23••]. Furthermore, fission of endosomes is also curated by the ER [28,29]. As these processes are crucial for faithful sorting and timely delivery of internalized materials for proteolysis, it stands to reason that their underlying mechanisms would also influence efficiency of antigen processing and peptide loading, although formal investigation thereof remains to be performed (Table 1).

Table 1

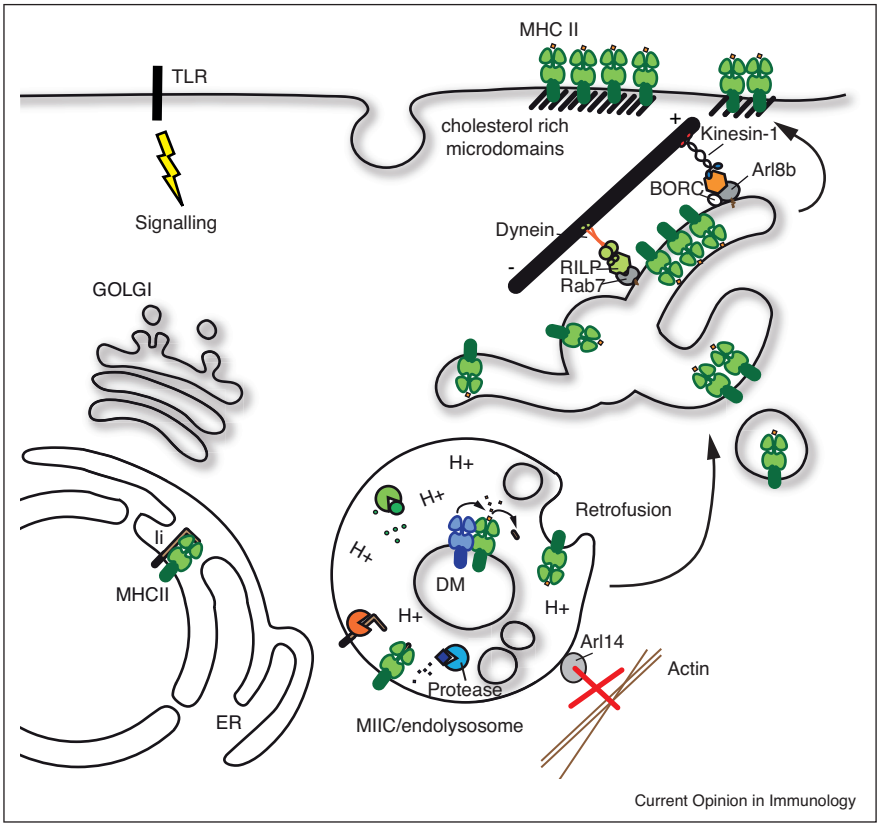
Open issues on endosomal modulation in the context of antigen presentation

Issue
Endosomal modulations in autoimmune diseases
The regulation of autophagy in TECs
Pathogen-mediated alterations of the endocytic network in APCs
The molecular mechanism of retrofusion
Functional differences between ILVs and the limiting membrane of the MIIC
ER-mediated control of endosomal processes in professional APCs
The molecular pathway leading to endosomal tubulation and transport upon maturation

The fight is on—architectural changes in the endolysosomal system upon APC maturation

In the early steps of pathogenic invasion, APCs become activated upon binding of innate immune receptors, such as Toll-like receptors (TLRs), to pathogenic ligands and integration of inflammatory mediators produced by other cell types. As a result, APCs embark on a cell-wide maturation program, which transiently increases MHC-II biosynthesis, inhibits antigen acquisition through non-specific phagocytosis and macropinocytosis [4], and demands dramatic reorganization of the MIIC architecture [4]. Following activation, MIICs transition from true MVBs to tubular endolysosomes devoid of internal membranes in order to allow efficient trafficking of peptide-loaded MHC-II (p-MHC-II) to the plasma membrane for presentation [30,31]. How MHC-II is transferred from ILVs to the limiting membrane of the MIIC is unclear. It has been speculated that, following APC activation, pre-existing multi-vesicular MIICs would fuse with lysosomes, leading to degradation of pre-loaded MHC-II residing on internal membranes. Meanwhile, newly synthesized MHC-II would traffic to these late compartments but avoid being sorted into ILVs due to suppressed ubiquitination [32], remaining instead on the limiting membrane for peptide loading and transport. However, this supposition is challenged by the observation that most p-MHC-II complexes are delivered to the plasma membrane before MHC-II ubiquitination and sorting are downregulated [30,33]. So how then does peptide-loaded MHC-II arrive at the limiting MIIC membrane to enter tubules for transport? It has been proposed that instead of suffering degradation, ILVs harboring p-MHC-II fuse back with the limiting membrane of the MIIC through a process termed 'retrofusion' (Figure 2) [34–36]. The existence of such a path for ILVs carrying p-MHC-II complexes would explain a number of observations. Firstly, the notion that surface p-MHC-II originates from cholesterol-laden ILVs is notably consistent with the finding that MHC-II arrives at the plasma membrane in cholesterol-rich microclusters [37]. More importantly, since APC stimulation occurs concurrently with the acquisition and processing of pathogenic antigens, the abundant luminal pool of p-MHC-II constituted before maturation likely encompasses a myriad of pathogen-specific epitopes, and its elimination would be counter-productive to antigen presentation. Retrofusion would thus offer a path for rescue of these epitopes from lysosomal degradation, allowing them to be presented. In the end, the two models for MHC-II transfer probably coexist, and p-MHC-II complexes from all possible sources contribute to antigen presentation in order to achieve optimal immune stimulation.

Figure 2



Schematic overview of MHC-II antigen presentation in maturing APCs.
Upon recognition of pathogens by receptors, such as TLRs, APCs undergo a process of maturation, during which global rearrangements of the endolysosomal system take place. In the MIIC, retrofusion of ILVs and restriction of intraluminal sorting may allow p-MHC-II complexes to reach the limiting membrane of the endolysosome. BORC-mediated recruitment of Arl8b to the MIIC leads to engagement of kinesin-1 motor that drives elongation of MHC-II-positive membranes into tubules toward the microtubule plus-end. Concomitantly, Rab7-RILP-dynein transport complex provides an opposing force necessary to induce membrane fission. Once the resulting MHC-II carriers reach the periphery, they fuse with the plasma membrane to expose p-MHC-II complexes for T cell recognition.

Given its small physical scale and dynamic nature, the process of retrofusion has not yet been directly observed (Table 1). However, viruses and toxins have been described to hijack this pathway, following endosomal entry, in order to reach the cytosol of the host cell and achieve infection [38–40]. Similarly, it has been speculated that retrofusion may allow access of exosome encapsulated material to the cytosol of target cells [41].

‘Ins’ and ‘outs’ of MHC-II transport to the cell surface

Once p-MHC-II complexes have reached the limiting membrane of the MIIC, they are sorted into tubules destined for transport to the cell periphery. Subsequent fusion of p-MHC-II positive tubules with the plasma membrane implants these antigen epitopes for T cell recognition. While retrograde transport to the perinuclear region is governed by dynein, as described above, anterograde transport toward the plasma membrane relies on kinesin-1 motor activity [42,43]. Intriguingly, both anterograde and retrograde transport appear to be essential for MHC-II delivery to the cell surface. In macrophages, lysosomal tubulation requires both Rab7 and Arl8b, and lipopolysaccharide (LPS) stimulation triggers this process in a mammalian target of rapamycin (mTOR)-dependent manner [44,45]. It is thought that Arl8b-mediated kinesin-1 activity drives lysosomal elongation into tubules, and together with the counter force provided by the Rab7-dynein complex results in tubule fission and liberation of p-MHC-II carriers (Figure 2) [46]. Recent work reveals that TLR-dependent engagement of the mTOR axis induces membrane accumulation of Arl8b [45], but the precise molecular underpinnings thereof remain unexplored (Table 1). The multisubunit BLOC-one-related complex (BORC) known to stimulate Arl8b recruitment and influence positioning of lysosomes in the cell periphery is likely involved [47–49], but the existence of additional regulatory layers cannot be

excluded. Rab7 can also mediate endolysosomal transport to the cell periphery via its effector FYVE and coiled-coil (CC) domain-containing protein (FYCO1) [50]. Whether this trafficking route participates in delivery of p-MHC-II complexes to the plasma membrane of APCs is not known.

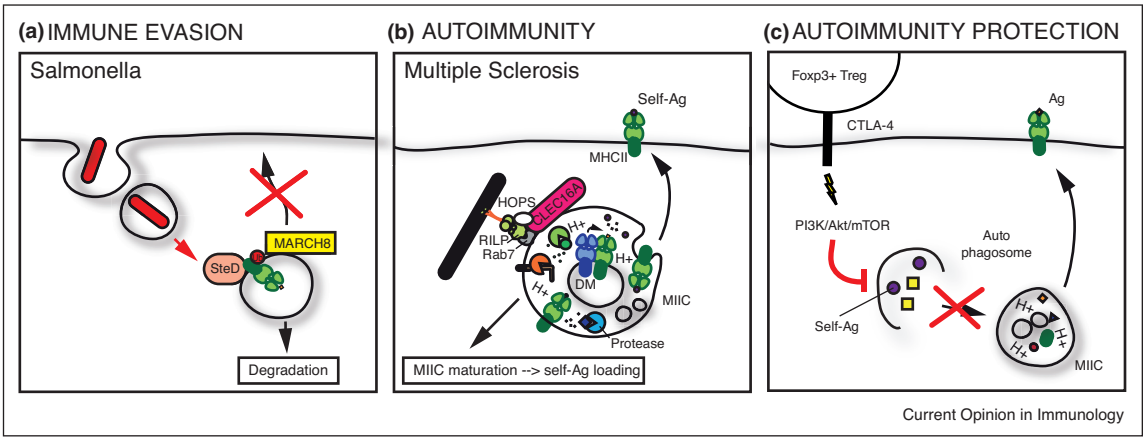
Collectively, diverse transport systems are needed to properly control traffic of MHC-II-containing vesicles to the plasma membrane during APC maturation. Interestingly, although the same machineries are also present in immature APCs, in the absence of appropriate triggers, MIICs do not migrate to the cell surface, suggesting the existence of inhibitory mechanisms. Indeed, in a multidimensional depletion screen the small GTPase Arl14/Arf7 was identified as a negative regulator of MHC-II export in moDCs [51]. Association of Arl14 with Arf7 effector protein (ARF7EP) causes recruitment of motor myosin 1E for anchorage of MIICs to the actin cytoskeleton (Figure 1). By contrast, inactivation of this system in immature DCs sends MHC-II molecules to the cell surface, recapitulating a mature DC phenotype in the absence of activation (Figure 2).

Once the export of peptide-loaded MHC-II complexes is achieved, their stable residence at the plasma membrane is protected to ensure longevity of antigen presentation [52]. If endocytosed, owing to lack of ubiquitination, p-MHC-IIs recycle back to the cell surface, instead of following the degradation route [53]. Hence, MHC-II ubiquitination must be tightly controlled to best serve APC function throughout the immune response. For example, in germinal centers, B cells are selected based on their ability to capture and present antigens to T helper cells. In order to favor their selection and proliferation, these cells modulate MARCH1-mediated ubiquitination of MHC-II dimers to promote presentation of their most recently acquired antigens [54•]. Timely tuning of MHC-II turnover, therefore, contributes to the rise of high-quality antibody responses. In contrast, when triggered by pathogens, aberrant ubiquitination and surface depletion of mature MHC-II dimers can have dramatic consequences on T cell activation. For instance, the gram-negative bacteria *Salmonella* enhances MARCH8-dependant ubiquitination of endocytosed p-MHC-IIs, thereby hampering their recycling and dampening antigen presentation to T cells (Figure 3) [55••]. Taken together, these studies illustrate the power of modulating ubiquitination status of MHC-II and suggest avenues for intervention through transient inhibition of the enzymatic activities involved.

Antigen presentation and tolerance: how alterations in MHC-II traffic and autophagy set off autoimmunity

Because APCs play a pivotal role in the rise of immune defenses, tight control over their function is essential for immune tolerance. Deregulation of MIIC distribution can lead to excessive antigen presentation and result in autoimmune disorders. For instance, the C-type lectin CLEC16A was identified as a factor responsible for such deregulations in a genome-wide screen combined with multiple sclerosis data sets [56]. Increased expression of CLEC16A causes abnormal MIIC biogenesis, which

Figure 3



Alterations in the MHC-II endocytic pathway can promote immune evasion and lead to autoimmunity.
(a) *Salmonella* bacteria evade host antigen presentation by manipulating the endocytic route of MHC-II. *Salmonella*'s effector SteD positively regulates MARCH8-mediated ubiquitination of endocytosed p-MHC-II complexes, preventing their recycling to the cell surface. **(b,c)** Malfunctions in MHC-II and/or antigen trafficking pathway can result in aberrant antigen presentation and lead to autoimmunity. (b) In multiple sclerosis, the C type lectin CLEC16A enhances maturation of MHC-II positive endosomes, likely through interacting with RILP and the HOPS complex. Altered MIIC biogenesis allows for loading and presentation of self-antigens and may trigger an autoimmune response. (c) The autophagy pathway delivers auto-antigens to the MIIC for loading and presentation by MHC-II. In order to prevent autoimmunity, regulatory T cells disrupt the autophagy pathway in DCs through CTLA-4 engagement and subsequent activation of the PI3K/Akt/mTOR pathway.

could affect CD4 T cell activation and result in autoimmune response (Figure 3). Since it associates to RILP and the HOPS complex, CLECL16A may affect transport and fusion of MHC-II-carrying endosomes with lysosomes and thus alter MHC-II peptide loading and presentation of autoantigens. Hence, conservation of self-tolerance not only requires proper MHC-II trafficking, but also depends on the delivery of self-antigens to MHC-II carriers. This can be achieved by macroautophagy, when cytosolic substrates reach the endocytic network in autophagosomal membranes. In DCs, fusion of autophagosomes with MIICs leads to processing and loading of cytosolic self-antigens onto MHC-II for presentation to effector T cells. Interestingly, Foxp3+ Treg cells disrupt autophagy in DCs (draining lymph nodes, splenic, and bone marrow-derived DCs) through CTLA-4 engagement and consecutive activation of the PI3K/Akt/mTOR pathway (Figure 3) [57•]. The resulting deficiency reduces CD4 T cell auto-reactivity and lowers the risk of autoimmunity.

In contrast, autophagy in thymic epithelial cells (TEC) drives presentation of self-antigens to thymocytes and has thus been implicated in T cell selection [58,59]. CLEC16A was recently found to influence thymic selection via autophagy, and its depletion could counter the development of autoimmunity [60••]. Precisely how CLEC16A regulates TEC autophagy is unknown, but it may involve modulation of mTOR activity [61], as well as implicate RILP and HOPS. The latter option is substantiated by the finding that transport and fusion of autophagosomes with late endosomes and lysosomes are timed by several Rab7 effectors, including RILP, PLEKHM1 and the cholesterol sensor ORP1L [62]. Specifically, these processes are sensitive to the presence of cholesterol in endolysosomal membranes, as cholesterol depletion forces Rab7-bound ORP1L to mediate contacts with the ER. As a consequence, retrograde transport and maturation of autophagosomes are inhibited. It is, therefore, plausible that CLEC16A intervention at this juncture may alter decisive steps in autophagy to influence immune tolerance.

Conclusions and perspectives

The endosomal pathways of cells entrusted with responsibilities of antigen processing and presentation are more diverse and dynamic than originally anticipated. The architecture and dynamics of the endolysosomal system in APCs are controlled by various factors, which ultimately determine healthy MHC-II responses. New insights on this complex network of vesicular organelles discussed in this review expand our perception of MHC-II function, and its numerous forms of modulation, and edify our understanding of the MHC-II compartment to illustrate how endosomal regulators contribute to scrupulous immune responses and tolerance. Deciphering how the endolysosomal architecture is modified in the course of infections and autoimmune diseases (Table 1) will help define therapeutic targets and ultimately to design appropriate clinical strategies. The first examples are now at hand.

Conflict of interest statement

Nothing declared.

Acknowledgements

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- •• of outstanding interest

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