Chapter 3

Routes to manipulate MHC Class II Antigen Presentation

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MHC class II molecules (MHC-II) present antigenic fragments acquired in the endocytic route to the immune system for recognition and activation of CD4+ T cells. This ignites a series of immune responses. MHC-II strongly correlates to most autoimmune diseases. Understanding the biology of MHC-II is therefore expected to translate into novel means of autoimmunity control or immune response improvement. Although the basic cell biology of MHC-II antigen presentation is well understood, many novel aspects have been uncovered in recent years including means of antigen delivery, preparation for MHC-II loading, transport processes and vaccination strategies. We will discuss past, present and future of these insights into the biology of MHC-II.

Introduction

Like all glycoproteins, MHC-II α- and β-chains are synthesized and assembled in the ER. Here they associate with Invariant Chain (Ii), which prevents premature binding of peptides to the MHC-II peptide-binding-groove and promotes exit from the ER and transport through the Golgi. The Ii contains a dileucine-based motif recognized by Adaptor Protein 2 (AP2) or AP3 complexes [1-3]. This motif is required for sorting at the Trans-Golgi-Network (TGN) and plasma membrane (PM) towards the MHC-II containing compartments (MIIC) [4-6] (pathway components discussed in this chapter are summarized in Figure 1). In the MIIC, residing proteases degrade antigens and Ii with the exception of a small fragment (called CLIP), protected by its embedding in the peptide-binding-groove of MHC-II. The CLIP fragment is exchanged for new (antigenic) peptides catalyzed by a unique and dedicated chaperone DM (H2-M in mice, HLA-DM in humans). DM is a MHC-II look-alike that interacts with MHC-II, stabilizing it in a state devoid of peptides, which would otherwise be prone to aggregation and degradation [7]. The peptide exchange reaction is stimulated by acidic pH and occurs in subdomains of the MIIC [8].

Probably, the MIIC does not have a 'Quality Control System' like the ER that allows exit of properly folded and assembled proteins only. The expression of MHC-II/CLIP complexes at the PM in the absence of DM illustrates this point. The MIIC moves to the PM for surface deposition of MHC-II, most likely after a certain intracellular residency time. In addition, MHC-II-bearing exosomes might be released conveying immunological information beyond the initial antigen presenting cell (APC).

The biology of antigen presentation by MHC-II has been studied for over 20 years by many groups and has yielded a fairly consistent view on the molecular basis underlying the successful acquisition of peptide fragments in the endocytic pathway for presentation at the PM. In general terms, assembly of MHC-II, their preparation for peptide loading, the generation of peptide fragments and transport processes are understood at almost atomic resolution. Yet many new findings complicate the initially simple biology. We will describe the state-of-art understanding based on recent insights. We will follow the general route of MHC-II from its birth in the endoplasmic reticulum (ER), through the endosomal pathway to the PM, exosomes and to their degradation. Along this path, we will not only describe new biological findings but also their application as new tools to manipulate MHC-II antigen presentation.

Delivery of Antigens to MIIC

Uptake of exogenous antigens can occur via several routes, reviewed by [9]. Each immune cell type has found its own one. B cells are poorly phagocytic, but they can take up IgM-coated Salmonella in a B cell receptor (BCR)-mediated pathway for antigen presentation in context of MHC-II [10]. The effectiveness of antigen presentation in dendritic cells (DC) depends on the cells' origin, maturation-stimulus and route of antigen uptake. These observations aid the understanding of immune response initiation and designing of vaccination strategies using DC and activated monocytes [11].

Recently it has been shown that DC continue to accumulate antigens via DEC205-mediated endocytosis and FcγR-mediated phagocytosis even after maturation, the latter involving the PIP5K isoforms α and γ [12]. Freshly synthesized or recycled MHC-II is loaded with the newly acquired antigens [13]. Endocytosis of DEC205 antibody-coupled peptides results in efficient uptake and delivery to the MIIC. This strategy has been applied to DC of melanoma patients resulting in effective peptide presentation. The stimulatory capacity of those DC was maintained after cryopreservation, which makes it an interesting approach for cancer immune therapy [14]. Antigen acquisition targeting Ig, Fc, complement or lectin receptors appears to be a feasible strategy to improve MHC-II antigen presentation and immune response outcome.

How cytosolic antigens reach MHC-II and why, although abundant, they only represent a minor fraction of all presented peptides (MHC ligand database; **http://www.syfpeithi.de**) [15], was unclear for a long time. Autophagy is a cell biological solution for the entry of cytosolic material into the lysosome. Membranes form and encapsulate parts of the cytosol forming autophagosomes, a process requiring ATG5 [16]. As reviewed by Nedjic and

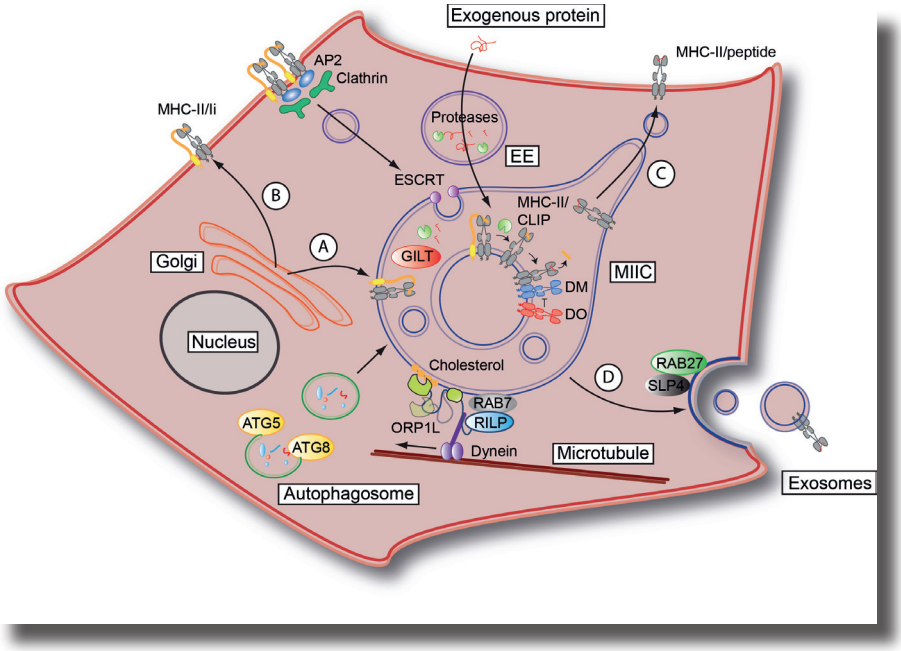

Figure 1 | The Transport Route of MHC Class II

After Ii binding in the ER, MHC-II is transported to the MIIC either directly (A) or via the PM (B) due to a dileucin motif in the associated Ii. In the MIIC, the Ii is degraded and the remaining CLIP fragment is exchanged for a new peptide in a process chaperoned by DM. After loading MHC-II is transported to the PM (C) or secreted on exosomes (D). MHC-II, MHC class II molecule; Ii, Invariant chain; AP2, Adaptor Protein 2; GILT, Gamma-interferon-inducible lysosomal thiol reductase precursor; DM, HLA-DM; DO, HLA-DO; ESCRT, Endosomal Sorting Complex Required for Transport; ATG, Autophagy related; ORP1L, oxysterol-binding protein; RILP, Rab7-interacting lysosomal protein; SLP4, Synaptotagmin-like protein 4; EE, early endosome; MIIC, MHC-II containing compartment.

colleagues, cortical thymic epithelial cells contain many autophagosomes [17] generating an array of different peptides, thereby shaping the self-tolerant T cell repertoire, as became clear from studies in ATG5-/- thymi [18]. ATG5-/- DCs are defective in phagosome-to-lysosome fusion, thereby inhibiting processing and presentation of extracellular microbial antigens [19]. Particular proteins are more selectively targeted to the autophagosome when coupled to ATG8/LC3, which in turn enhances their MHC-II presentation rate [20].

Antigen Processing in the MIIC

Antigens have to be unfolded for efficient degradation and peptide formation. One recently identified endosomal protein (called GILT) oxidizes disulfide linkages found in many extracellular proteins [21] and is essential for the presentation of a series of antigens [22]. GILT activity can strongly enhance antigen presentation of a melanoma antigen [23]. Acidic pH and chaperones might participate in further unfolding before substrates are processed by resident proteases. Many of these are cysteine proteases of the cathepsin family and are involved in inflammation, autoimmunity and cancer (reviewed by [24] and [25]). Nowadays, various cathepsin inhibitors are tested in primary immune cells as tools for immune response modulation in autoimmune diseases (Table 1).

Manipulating Peptide Loading of MHC-II in the MIIC

DM can stably associate with a unique co-chaperone DO (H2-O in mice, HLA-DO in humans), which is fairly selectively expressed in immature B cells and certain DC types. DO is also an MHC-II look-alike and functions as a pH sensor that alters the pH optimum of DM-mediated peptide loading of MHC-II to more acidic conditions and thereby changes the peptide repertoire presented by MHC-II [26]. In fact, presentation of many peptides is prevented by DO yielding more MHC-II/CLIP expression at the PM. Consequently, T cell help for B cells is reduced when DO is expressed [27]. DO may skew the DM support of MHC-II peptide loading to late and more

acidic endosomes, which are preferentially accessed by antigens taken up by BCR-mediated endocytosis. It is believed that this is preventing autoimmune responses by controlling the activation of B cells present peptides corresponding to antigens taken up by the BCR [26]. Indeed, NOD mice (susceptible to develop Type 1 Diabetes) overexpressing DO present an altered self-peptide repertoire which prevents activation of diabetogenic T cells and hence diabetes onset. DO possibly shapes the overall MHC-II self-peptide repertoire to improve T cell tolerance [28].
Small molecules affecting peptide loading of MHC-II can be of interest for autoimmunity (inhibitors) or vaccines (accelerators). Amines like chloroquine are known to neutralize MIIC and inhibit peptide loading [29]. Protease inhibitors affect the degradation of Ii or antigen (Table 1). More recently, a family of compounds was described to accelerate MHC-II peptide loading *in vitro*, without the help of DM, and promote peptide binding in APC *in vivo* [30]. Altered Peptide Ligands (APLs) are built to be more protease resistant, to reach the MIIC more easily and to be presented more efficiently (for review [31]). Manipulating MHC-II function becomes a realistic option to direct immune responses.

A topological Problem: Retrofusion or Exosomes?

MIIC consist of a limiting (outer) membrane and luminal vesicles (LV). It is still under debate whether LV are a stable structure or dynamically form and disappear again through retrofusion with the limiting membrane. The subdomains of MIIC differ. The LV contain tetraspanin molecules, cholesterol and the lipid LBPA, while the limiting membrane concentrates molecules like the GTPase RAB7, LAMP and the cholesterol transporter ABCA1. MHC-II and DM are detected on both membranes. Studies have shown that the LV are their preferred site of interaction [8]. MHC-II/DM complexes might be stabilized through association with the tetraspanin web (made of CD63, CD82 and others) [32, 33]. The interaction of MHC-II and DM on LV has interesting consequences for pathogens in phagosomes, such as *Salmonella*. Phagosomes do not have LV and MHC-II located at their limiting membrane fails to acquire peptides due to lack of DM support, therefore allowing immune escape of intracellular bacteria [8].
The LV can be secreted to the extracellular environment as exosomes. Mass spectrometry analysis of protein and lipid content of purified B cell exosomes verifies their origin from LV of MIIC (albeit at a considerably higher resolution) [34, 35]. The turn-over of MIIC takes only hours, whereas the half-life of MHC-II (8-48 hours) [36] or CD63 (2 days) [37] is considerably longer implying that the proteins have to be recovered from the LV not to be lost through exosome secretion. To our opinion, exosomes are the result of inefficient retrofusion of the LV to the limiting membrane of MIIC before fusion with the PM. How the process of retrofusion occurs is unclear. Alternatively LV might be stable nanocomplexes that survive necrosis, like proteasome and ribosome, which can be easily detected in tissue culture medium and body fluids [38]. Nonetheless, exosomes can have interesting functions.

Function and Application of MHC-II-bearing Exosomes

Exosomes are shed by almost all cell types and differ in content accordingly (reviewed in [39]). Exosomes contain cytosol. How the cytosolic contents are selected, is unknown. Exosomes resemble their cell of origin's topology and expose proteins like MHC-I and -II, CD1, tetraspanins, costimulatory molecules and adhesion molecules, such as ICAM-1. In fact, MHC-II-bearing exosomes can be considered 'nano immune cells' and have been found to exert both immune stimulatory and regulatory functions in intercellular communication (Figure 2). When shed by DC, exosomes can present antigen in context of MHC-II to activated T cells or T cell lines directly [40] or indirectly to naïve T cells when recaptured by recipient APCs [41]. Such recapture requires binding to host membranes mediated by LFA-1 and its ligand

Table 1 | Recently developed Cathepsin Inhibitors and their Effects

Inhibitor	Target	Effect
ZRLR	Cathepsin B	Enhances presentation of Tetanus Toxin-C (TTC) fragment to T-cells [70].
CatG Inhibitor	Cathepsin G	Reduces processing of TTC and Hemagglutenin (HA) peptides and their presentation to CD4+ T-cells [71].
Suc-VPF	Cathepsin G	Reduces processing of TTC and Hemagglutenin (HA) peptides and their presentation to CD4+ T-cells [71].
Compound 47	Cathepsin S	Inhibits processing of invariant chain [72, 73].
CAA0225	Cathepsin L	Involved in degradation of autophagosomal membrane markers [74].

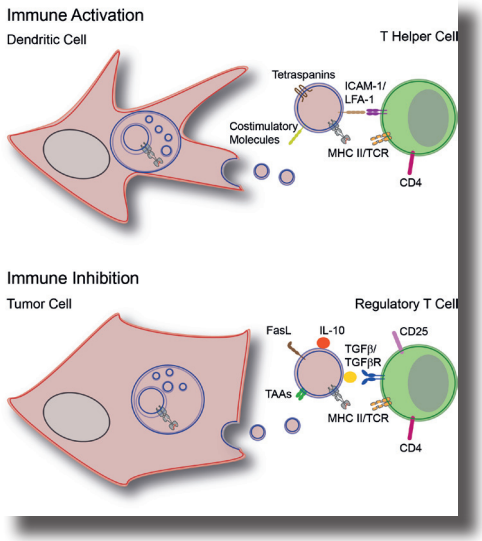

Figure 2 | Exosome Functions
Exosomes derived from dendritic cells can exert immune activating functions. Naïve T cells can be primed in an antigen-specific manner. Exosomes derived from tumor cells can exhibit immune regulatory functions by activating regulatory T cells. ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; TCR, T cell receptor; IL-10, interleukin-10; TGFβ, tumor growth factor β; TAA, tumor associated antigen

ICAM-1 on the exosome [42, 43]. Tumors are known to shed exosomes. Szajnik *et al.* have recently defined a new escape mechanism in cancer based on the activation of regulatory T cells (Tregs) in tumor patients [44]. Whether exosomes enter the recipient cells' MIIC for retrofusion, fuse with the PM or act as 'nano immune cells' is a fascinating subject and still unclear.

The immunogenetic potential of DC-derived exosomes has been demonstrated for vaccines against *Leishmania major* [45] and as cancer therapies *in vivo* in the past (reviewed in [46]). An alternative to the isolation of exosomes from patient-derived DCs might be the generation of artificial exosomes [47]. The advantage of exosome-based vaccines over other vaccination strategies remains to be proven in side by side comparative studies.

And out we go… MHC-II Transport to the Plasma Membrane

MHC-II is transported from the MIIC to the PM along microtubules in at least two different ways; 1. MIIC can move to the PM followed by fusion of the limiting membrane with the PM [48] 2. Tubules extend from MIIC towards the PM [49, 50], and vesicles may bud off to fuse with the PM [51]. This has been detected mainly in activated DC and not in other cell types. Close inspection of both routes shows that MIIC move in a bidirectional and stop-and-go manner by the activities of the dynein and kinesin motor proteins. Cholesterol, shown to influence MHC-II PM expression [52], controls the RAB7 effector ORP1L that controls RILP and the dynein motor. High cholesterol prevents the release of the dynein motor, inhibiting MIIC delivery to the PM [53]. How movement to the PM by kinesin motors is controlled, is unclear. Finally, MIIC have to fuse to the PM which probably requires the activity of RAB27A and its effector SLP4. This is directly correlated to the secretion of exosomes in HeLa cells [54]. Sorting of MHC-II to exosomes was shown to be ubiquitination-independent. Forced ubiquitination of MHC-II induces a decrease of MHC-II at the PM, but no enrichment on exosomes [55]. In the study of Buschow *et al.* it was shown that coculturing of DC with cognate T cells induced DC activation and exosome secretion independent of ubiquitination [56]. Although parts of MHC-II transport control are understood in detail, regulation of most other steps remains obscure and may be uncovered in the coming years.

Finally at the PM; Internalization and Recycling

It has been known for some time that the half-life of MHC-II at the PM is cell type dependent. When MHC-II associated to Ii arrives at the PM, they are internalized because of the dileucine motif in the Ii [57]. MHC-II, devoid of Ii, does not contain such internalization motifs and remains at the PM. Still, MHC-II has a longer lifespan on B cells than on monocytes, which is even further reduced following IL-10 exposure [36]. IL-10 upregulates a ubiquitin ligase called MARCH1, which modifies MHC-II and reduces its half-life [58]. The downregulation of MARCH1 expression in mature DC compared to immature DC corresponds to an increase in MHC-II half-life at the PM of the former [59] (Figure 3). Viruses and bacteria can use the ubiquitination machinery to manipulate MHC expression [60].

Salmonella typhimurium inhibits PM expression of MHC-II in DCs by ubiquitinating the HLA-DRβ chain using bacterial type III secretion system effectors rather than MARCH1 to directly modify HLA-DRβ [61-63]. HIV-Nef affects MHC-II expression and peptide loading by a different mechanism. The Nef protein triggers MHC-II internalization in a cholesterol dependent and clathrin- and dynamin-independent manner [64], but the exact details are unknown.

Upon internalization MHC-II has two options: 1. return to the PM prior to or after peptide exchange or 2. become degraded. In recycling endosomes, MHC-II can be loaded with new peptides in a DM-dependent [65] or independent manner [66] and transported back to the PM. This process involves at least ARF6, RAB35 and EHD1 [67]. Uptake of antigens in early endosomal compartments (that are relatively poor in proteases [68]) will allow presentation of other peptides than those generated in MIIC, hence broadening the peptide repertoire for the good or bad, which is unpredictable. Where MHC-II ends-up, when recycling fails, remains unclear. The reductase GILT (breaking disulfide bonds in the Ig domains of MHC-II) and cathepsins, such as cathepsin G, which degrades the MHC-II β-chain *in vitro* [69], might be involved. How the life of MHC-II is exactly terminated, however, remains an open question. Clearly, MHC-II is a cannibal presenting fragments of its deceased brothers or sisters. In fact, such MHC-II derived peptides are major constituents of the MHC-II peptide repertoire.

Conclusion

The cell biology of MHC-II has been studied for over two decades starting with the work of Unanue et al., who demonstrated inhibition of antigen presentation by chloroquine [29]. Since then many steps have been solved at the cell biological and atomic level. MHC-II antigen presentation comprises of processes like transport via various endosomal compartments, synthesis, ubiquitination and degradation. The routes of MHC-II involve many targets for manipulation to affect MHC-II expression and peptide loading, which is relevant for disease states like autoimmunity, infection and cancer.

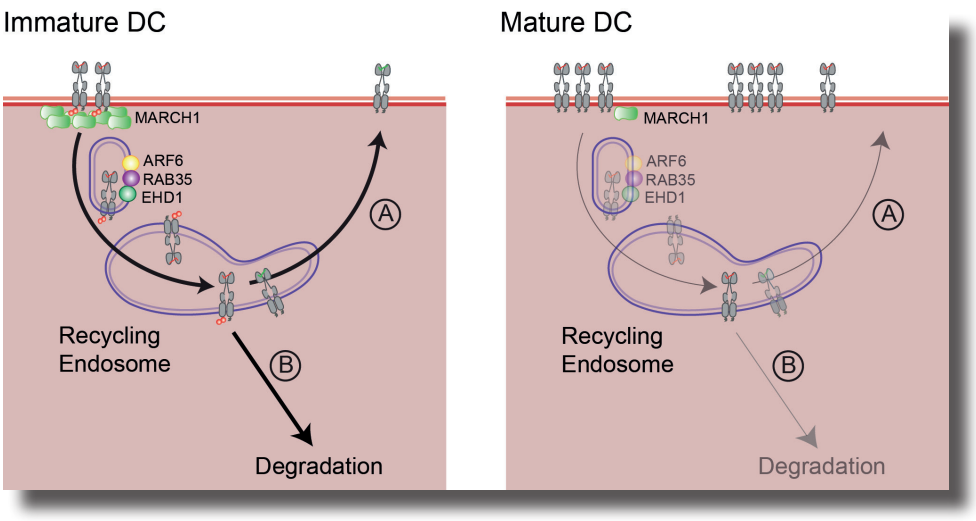

Figure 3 | MHC Class II Internalization, Recycling and Degradation
At the PM of immature DC, MHC-II can be ubiquitinated by MARCH1 and is transported via ARF6/RAB35/EHD1-positive tubular structures towards Recycling Endosomes (RE). After internalization, MHC-II may return to the PM (A) or proceed to degradation (B). In mature DC, MARCH1 expression is downregulated, resulting in decreased internalization, a long cell surface half-life and elongated presentation of antigen. PM, plasma membrane

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- • of special interest
- •• of outstanding interest

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