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Review

How to target MHC class II into the MIIC compartment☆

Malgorzata A. Garstka*, Jacques Neefjes

Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands

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ABSTRACT

Major histocompatibility complex (MHC) class II molecules (MHC II) present exogenous antigens to CD4+ T cells to modulate immune responses. To contact these antigens, MHC II is delivered to the late endosomal MHC class II compartment (MIIC). This compartment has a complex architecture and consists of internal membranes or vesicles surrounded by a limiting membrane. These subdomains have different protein and lipid content. Also MHC II peptide loading is spatially organized in MIIC as it interacts with DM on intralumenal vesicles (ILVs) to bind antigen. How this is controlled is only understood in a sketchy manner. This may involve ubiquitin modification of MHC II, possibly by E3 ligases of the March family. But other proteins are likely involved as well including E3 ligases, deubiquitylating enzymes (DUBs), adaptor, scaffold, motor and vesicular coat proteins. Our lab performed a genome-wide siRNA screen to define novel proteins and pathways involved in MHC II antigen presentation. The data set is used to select candidate proteins involved in targeting MHC II into MIIC. This process involves ubiquitin modifications and various new molecules not considered as yet in this complex pathway. These molecules may be targeted by drugs to manipulate MHC II responses in auto-immunity, transplantation and other disease states.

1. Introduction

MHC II control the specificity of the adaptive immune system. They are usually expressed on antigen presenting cells (such as B cells, monocytes, macrophages and dendritic cells) and display antigenic peptides to $CD4^+$ T cells. MHC II meet their antigenic peptides in late endosomal compartments called MIIC before display at the cell surface. The cell biology of MHC II antigen presentation is complex and involves many steps: MHC class II folding, transport, to and sorting in MIIC, peptide binding, transport of MHC II-peptide complex to the plasma membrane, and when not needed anymore, MHC II endocytosis, and lysosomal degradation. Not surprisingly, this complex cell biology is studied by many labs over the last two decades and still shows many new and surprising features, as discussed below.

MHC II assembles in the ER with a third chain, the invariant chain or Ii. Ii has multiple functions. Ii prevents premature peptide binding in the ER, and contains sequences for targeting MHC II to the MIIC (Neefjes et al., 2011). MIIC is a late endosomal compartment where MHC II encounter antigenic peptides generated by resident proteases. In order to bind peptide antigen, Ii has to be removed by similar proteases as involved in antigenic peptide generation. Only a small Ii fragment (called CLIP) escapes full degradation by surviving in the class II peptide-binding grove. The (unique because late endosomal) chaperone DM then supports the exchange of CLIP for antigenic (and other self) peptide fragments (Neefjes et al., 2011).

MHC II is ultimately delivered to the plasma membrane for antigen presentation. At the end of its natural life, MHC II is probably removed by endocytosis for degradation in lysosomes. A fraction may escape, exchange peptides and recycle back to the plasma membrane for–again- antigen presentation (Wilson and Villadangos, 2005). The details of this process are poorly understood.

2. Current status

MIIC compartments constitute a key hub for MHC II antigen presentation as here the endocytic, exocytic and degradation pathways intersect. MIIC is a late endosomal compartment containing class II, HLA-DM and cathepsins, which is the minimal requirement for MHC II peptide loading. In this late endosomal compartment, regions of the limiting membrane invaginate to form intralumenal vesicles surrounded by a limiting membrane, a structure also known as multivesicular body (MVB). Electron microscopy shows that MHC II as well as HLA-DM localize at the limiting membrane and concentrate in intralumenal vesicles (Sanderson et al., 1994). FRET studies have indicated that MHC II and HLA-DM mainly interact at the intraluminal vesicles (ILVs), which suggests that the

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* Corresponding author at: Cell Biology II, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands. Tel.: +31 20 512 2033; fax: +31 20 5122029.
E-mail address: m.garstka@nki.nl (M.A. Garstka).

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vesicles have to fuse back at some point for embedding MHC II in the plasma membrane. MHC II in *Salmonella* containing phagosomes (that lack these ILVs) are indeed poor in acquiring antigenic peptides (Zwart et al., 2005). The processes of sorting and 'retrofusion' of ILVs and MHC II are poorly understood.

So, what do we know about the targeting of MHC II to the MIIC? Ii is known for a long time to be important for targeting of the immature MHC II complex to MIIC. Ii contains two di-leucine motifs in its cytosolic tail that interact with AP1 and AP2, which are clathrin adaptors that localize to the Golgi and plasma membrane, respectively, to haul class II–Ii complexes into the late endosomal MIIC (Neefjes et al., 2011).

More mature MHC II lack Ii and should use different mechanisms of transport. This probably involves the small GTPases Arf6 and Rab35 (Walseng et al., 2008).

Ubiquitin modification of MHC II and other proteins can be expected to drive internalization and endosomal sorting (Shin et al., 2006; van Niel et al., 2006). The MHC II αI and β chains can be ubiquitylated by a transmembrane spanning RING E3 ligase called March 1 (Lapaque et al., 2009). March 1 localizes to the plasma membrane, early and late endosomes (Neefjes et al., 2011). As MHC II is present at the same sites, ubiquitination of MHC II can occur there (Lapaque et al., 2009). MHC II ubiquitylation decreases during DC maturation which correlates with decreased March 1 mRNA levels as well (Neefjes et al., 2011). This effect may be accelerated by the co-stimulatory molecule CD83 that is highly expressed in mature DCs and that inhibits the interaction between MHC II and March 1 and thus MHC II ubiquitylation (Neefjes et al., 2011). Collectively, the stability of MHC II will be enhanced in mature DC, which has been observed before (Pierre et al., 1997). March 1 knock out mice display increased levels of MHC II at the plasma membrane in immature DCs even before activation (Walseng et al., 2010).

Ubiquitylation is required for sorting plasma membrane proteins within multivesicular/lamellar bodies such as MIIC (Wright et al., 2011) and MHC II may not be an exception (Hor et al., 2009; van Niel et al., 2006). Is MHC II ubiquitylation also required for endocytosis? This is more controversial as MHC II follows normal endocytosis in March 1 knock out mice (Walseng et al., 2010). One option is that MHC ubiquitination is compensated for by other E3 ligases, such as March 8 and March 9 (Hor et al., 2009; Ohmura-Hoshino et al., 2006). In immature dendritic cells MHC II beta chain engineered to not accept ubiquitin (by mutating single lysine in the cytoplasmic tail for arginine) remained at the plasma membrane, while a ubiquitin fused directly to the MHC II beta chain showed decreased surface expression (Ma et al., 2012). Again, McGehee et al. (2011) reported a mouse with engineered MHC II molecules unable to accept uniquitin and could not observed any effect on MHC II distribution and other processes dependent of MHC II such as antibody production. Various experiments reported effects of ubiquitin modifications on endosomal behavior of various proteins, including MHC II. However, contradicting reports simply indicate that this issue is not fully set and requires further study.

One system involving ubiquitin modification for function is studied in detail: the epidermal growth factor receptor (EGFR). EGFR is ubiquitylated and delivered in an ESCRT (endosomal complex required for transport)-dependent way to multivesicular bodies and incorporated into intralumenal vesicles where signaling is terminated and degradation initiated. ESCRT components work in an concerted manner with ESCRT-I accumulating ubiquitylated cargo at the endosomal membrane, ESCRT-I and -II forming the intralumenal vesicles and sorting content and ESCRT-III performing the actual scission of these vesicles. Although this is the most common pathway for multivesicular body formation, alternative pathway – less well characterized – do exist as well (Babst, 2011).

To identify factors involved in ubiquitin-dependent delivery of MHC II to the MIIC, we employed our genome-wide RNAi screen to define proteins and pathways involved in MHC II antigen presentation. In our flow cytometry-based analysis we determined MHC II surface expression and peptide loading and in the microscopy-based follow up the intracellular distribution of MHC II was visualized (Paul et al., 2011). Silencing proteins involved in targeting MHC II to the MIIC should affect MHC II at the plasma membrane and alter the intracellular localization of MHC II, especially in late endosomes (marked with CD63). Of note, an siRNA screen is never saturating and at best the sketches of pathways can be extracted for such work. We identified two ESCRT components – Vps28 and Vps36, as well as novel proteins. One of these, Toll-interacting protein (Tollip), fulfilled the criteria for a novel protein in the ESCRT pathway (Paul et al., 2011). These data place the ESCRT pathway as important in MHC II antigen presentation. The new protein Tollip could cooperate with these ESCRT complexes, but could also be involved in more MHC II specific pathways related to multivesicular body formation. Indeed, Tollip is relatively selectively expressed in immune tissues and its mRNA levels decreased during DC maturation. This corresponded to the increase in MHC II surface level as also observed when Tollip is silenced with siRNA (Paul et al., 2011).

Tollip was first identified in a yeast two-hybrid screen as a binding partner of interleukin-1 receptor (IL-1RI). Tollip can bind ubiquitin, and Tollip-IL1RI interaction requires ubiquitylation of the receptor. In the absence of Tollip, the IL-1RI receptor accumulates at MVB and is not efficiently degraded. Tollip also interacts with Toll-like receptors 2 and 4 (Capelluto, 2012). This suggests some form of Tollip specificity toward immune-related molecules and makes it an attractive candidate in the control of specific ubiquitin-dependent MHC II trafficking.

Ubiquitylation could be an endosomal sorting signal required for the regulation of MHC II intracellular transport. Immature dendritic cells contain a large fraction of MHC II in endocytic MIIC unlike mature dendritic cells. This correlated with the levels of ubiquitinated MHC II that total to around 10% of total MHC II in immature dendritic cells (Shin et al., 2006). Ubiquitination is followed by deubiquitination by DUBs and the relevant ones in the MHC II system are only partially defined. MHC II de-ubiquitylation can occur at MVB by USP8 or AMSH, or by unidentified MHC II-specific DUBs.

MHC II interacts with HLA-DM on the intralumenal vesicles of multivesicular bodies (or MIIC) for peptide loading (Zwart et al., 2005). Once bound with the antigenic peptide, class II redistributes to the plasma membrane. In mature DCs MHC II localizes to the plasma membrane and the internal vesicles only contain small amount of MHC II. This suggests that during DC maturation, multivesicular bodies reorganize and the internal vesicles fuse back to the limiting membrane of MIIC before fusion to the plasma membrane. Alternatively MHC II could be secreted as exosomes but then should have a very short half-life which is not observed for immune cells (Neefjes et al., 2011). The molecular mechanism of retrofusion, if any, is unknown, but it could involve proteins implicated in the formation of multivesicular bodies, such as Tollip.

3. Perspectives

Although the biology of MHC class II antigen presentation has been studied extensively and many factors have been defined, many issues remain unclear. These include:

1. The nature of the MIIC: is this a specific immune-endosome or general late endosome?
2. The biogenesis of MIIC: is this analogous to other multivesicular bodies;