Summary and Discussion

antigen presentation
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For a cell to communicate information about its internal health and status to the immune system, a sophisticated cellular machinery has originated for distinct antigenic subclasses; peptides and lipids from intra- and extracellular origins. CD1 presents lipids from both exogenous and endogenous origins, while for peptides two distinct molecular pathways have developed, namely MHC class I (for endogenous and viral peptides) and MHC class II (for peptides from extracellular origins) (reviewed in Chapter 9). While MHC class I is expressed in all nucleated cells, MHC class II is only expressed in specialized antigen-presenting cells. MHC class II molecules, including HLA-DR, assemble in the ER, where it associates with its specific chaperone, Invariant chain (Ii). Together with Ii, HLA-DR is transported to late endosomal/lysosomal compartments, where it meets peptides taken up through the endocytic route and processed by lysosomal peptidases. These MHC class II bearing late endosomal/lysosomal compartments, or MIIC, share a typical morphology; a limiting membrane surrounded structure with multiple internal vesicles. In these MIIC, the remainder of Ii, Class II-associated invariant chain peptide (CLIP), is exchanged for an antigenic peptide. This process is facilitated by another chaperone, HLA-DM. After the peptide is loaded on HLA-DR, the complex can be transported to and presented on the plasma membrane. Here, the complex is recognized by CD4+ T cells.

To gain insights into the process of antigen loading, we monitored the interaction between HLA-DR and HLA-DM in living cells (Chapter 10). Cell lines were constructed containing HLA-DR/CFP, HLA-DM/YFP and Ii, in which we measured Fluorescence Resonance Energy Transfer (FRET) in the MIIC. FRET is the radiationless energy transfer from a donor, in this case CFP, to a suitable acceptor, in this case YFP. Since this biophysical phenomenon only takes place when the fluorophores are in close proximity, a detection of FRET signal is indicative for a physical interaction. Interestingly, we found interaction between HLA-DR and HLA-DM only to occur on the internal vesicles of the MIIC and not on the limiting membrane. Since HLA-DR/HLA-DM interactions are essential for antigen loading, we defined a specific antigen-loading micro environment within this lysosomal structure. This micro environment within the MIIC is specifically disrupted by pathogens, such as Salmonella typhimurium. The bacterium actively prevents the formation of internal vesicles in its own phagosomal/lysosomal structure and thereby actively enables a local immune escape. Whether the antigen-loading subdomain can be generalized among different HLA-DR isoforms and if the effects we described in HEK293 cells can also be observed in specialized antigen-presenting cells, are still open questions.

In antigen loading and presentation, vesicular movement is essential. The vesicles containing cargo from extracellular origins need to encounter and fuse with lysosomal compartments in order to degrade the cargo. Likewise, after MHC class II molecules are transported to this late endosomal/lysosomal structure and the antigen is loaded, the antigen-loaded MHC class II needs to be transported to the plasma membrane in order to present its antigenic information. One of the proteins directly involved in these transport processes is Rab7. Rab7 is a member of the Rab GTPase superfamily, and is essential for minus-end microtubule organizing centre (MTOC)–directed transport. For MTOC-directed transport and perinuclear vesicle localization, Rab7 needs to associate to Rab7-interacting lysosomal protein (RILP) and oxysterol-binding protein-related protein 1L (ORP1L) (Chapter 11). Both RILP and ORP1L are effectors of Rab7 and can simultaneously bind to GTP-loaded Rab7. RILP can directly interact with the dynactin projecting arm p150Glued, which is required for dynein motor recruitment to these vesicular structures.

ORP1L function is, however, required in an unexpected fashion. We have shown that ORP1L can alter its conformation as a response to binding cholesterol (Chapter 12). In a non-cholesterol-associated conformation, ORP1L can recruit VAP (an ER resident protein) to interact with ORP1L in trans. VAP subsequently removes p150Glued from the Rab7-RILP-ORP1L complex and the vesicle localizes at the cell periphery. If cholesterol levels are high on the cytosolic leaflet of these vesicles, ORP1L does not recruit VAP, preventing a dissociation of p150Glued which leads to a clustering of these vesicles at the perinuclear region. Thus, the cholesterol levels regulate ORP1L conformation which regulates vesicular positioning in the cell.

A clear message from this chapter is that low resolution techniques, such as light microscopy, should not be over interpreted. One would conclude from the CLSM data in this paper that the endoplasmatic reticulum can fuse with lysosomal compartments. Monitoring now the same structures using electron mi-
croscopy led to the conclusion that the endoplasmatic reticulum aligns to, rather then fuses with, vesicular compartments. What factors enable this alignment, and thereby the VAP/ORP1L interactions, remains to be discovered. The formation of this endoplasmatic reticulum-vesicle synapse may be governed solely by VAP/ORP1L interactions, but could also be mediated by fur further unknown proteins. Also more processes may be regulated at this endoplasmatic reticulum-vesicle contact site, next to the removal of the p150Glued subunit from the motor complex, which are currently under investigation.

Another point of discussion is whether bacteria make use of the cholesterol-dependence of vesicle localization and possibly lysosomal maturation. This could be the case for Salmonella typhimurium. This bacterium secretes the factor SseJ, that induces an esterification of cholesterol. Although no data has been published about the physiological role of this cholesterol esterification, it may be related to the ORP1L-mediated vesicle localization. Through this modification, the cholesterol moiety may not be recognisable for ORP1L, preventing the maturation of the phagosome. This way, the cholesterol-driven vesicle-localization in cells can be influenced manipulated by bacteria in order to survive in host cells.

Next to chaperoning MHC class II, Ii can also have an influence on other factors. We have shown that Ii directly and functionally interacts with CD70, a Tumor Necrosis Factor (TNF)-related costimulatory ligand that - via its receptor CD27 - directs CD4+ and CD8+ T cell responses (Chapter 13). In the absence of Ii, CD70 transports by default to the plasma membrane. When Ii is coexpressed, CD70 can interact with Ii in the ER, after which Ii targets CD70 to the MIIC. Ii controls the kinetics and extent of CD70 cell surface expression and thereby regulates delivery of CD27-mediated survival signals to CD8+ T cells via MHC Class I. Additionally, now that both antigen-loaded MHC class II and CD70 are on the same structure, fusion of the vesicle with the plasma membrane results in a spatially and temporally restricted delivery of both antigen-loaded complex and a costimulatory response. This way, CD4+ T cells can not only detect the antigen-loaded MHC class II, but also in the same complex allow the buildup of an immunological synapse between CD27 and CD70, which serves to activate CD4+ T cells to provide help for the CD8+ T cell response and to dictate the extent of CD8+ T cell clonal expansion.

The results presented here illustrated an antigen-loading microdomain within MIIC and showed how the Rab7/RILP/ORP1L complex cooperatively orchestrates motor complex recruitment and vesicle positioning. In addition, we described that Ii mediates CD70 targeting to the MIIC, where timed and controlled delivery of both antigen-loaded MHC class II and CD70 determines CD4+ and CD8+ T cell responses. Still, a number of questions remain unanswered. What factors define the antigen-loading microdomain? What renders the internal vesicles of the multivesicular structure functionally different from the limiting membrane? Candidate factors are CD63 and CD82, tetraspanin molecules that are described to localize mainly on the internal vesicles of the MIIC and are reported to directly interact with both HLA-DR and HLA-DM. Another question raised concerns the existence of other potential factors which might directly influence antigen loading and transport, possibly fine-tuning the sophisticated MHC class II pathway. This would also pave the way to developing novel chemical inhibitors which could be applied in the clinical setting to treat MHC class II presentation-related diseases, such as auto-immune diseases and certain leukemias. Another obvious question is covering all the chapters discussed here: to what degree do these seemingly different processes interconnect? Do vesicles destined to fuse with lysosomes expose high cholesterol levels on their cytosolic leaflet, and after antigen loading and CD70 acquisition decrease their cholesterol content, in order to move to and fuse with the plasma membrane?

Further research will certainly focus on these and other questions, shedding light on the complex and tightly regulated cellular machinery involved in MHC class II antigen presentation.