Novel insights in MHC class II antigen presentation

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Chapter 5
Summary and Discussion

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Current Knowledge

Major Histocompatibility Complex class II (MHC-II) antigen presentation is involved in the regulation of immune responses against infections and tumours. It is also strongly linked to autoimmune diseases. A better understanding of the regulation of this process is needed to develop new therapies to improve immune responses against infection and cancer or to reduce these in case of autoimmunity.
In the past 20 years, many groups contributed to our current understanding of the MHC-II pathway in antigen presenting cells, as reviewed in chapter 1 of this thesis. Successful antigen presentation by MHC-II in the result of a set of tightly regulated cellular processes. It starts with transcription under the control of the MHC-II master regulator CIITA, followed by synthesis and assembly of MHC-II complexes in the endoplasmic reticulum (ER), where it also acquires the Invariant Chain (Ii). MHC-II is transported to the MHC-II loading compartment (MIIC) with the help of Ii. The MIIC is a late endosomal compartment defined by the presence of MHC-II and its chaperone HLA-DM (DM). Here, Ii is degraded and DM facilitates exchange of the Ii fragment, CLIP, for antigenic peptide. Antigens are delivered via the endocytic route and processed by MIIC resident proteases giving rise to peptides that will be loaded on MHC-II. Then, MHC-II has to be transported to the plasma membrane to presents its cargo to the immune system. This requires motor protein driven transport pathways involving tubular structures, vesicle budding, fusion with the plasma membrane and release of exosomes (the intraluminal vesicles of MIIC released to the extracellular space). MHC-II presentation can be terminated by internalization from the plasma membrane by degradation or recycling.

The Unknown

The scaffold is set, but subtle details are still missing. However, these details often involve regulatory steps, which are important starting points in drug, antibody- or peptide-based therapy design. To deepen our understanding of the pathway's mechanism two strategies can be applied. One is to describe one niche in the MHC-II pathway in detail, using high resolution, state-of-the-art techniques. This will usually yield static and descriptive data only. The alternative is to apply a genome-wide unbiased approach combined with systems biology, which will define new players and reveal their complex relationship with known factors. Therefore, combining the information of different databases with in-depth investigation on novel factors controlling MHC-II antigen presentation will uncover 'new biology'. In this thesis we show examples for both strategies.

Strategy One – Zooming in to details

Peptide loading of MHC-II occurs in the MIIC, a late endosomal multivesicular

body, consisting of a limiting membrane and luminal vesicles (ILV) (Neefjes et al., 1990; Peters et al., 1991). The MIIC is enriched for MHC-II, its chaperone DM and proteases, which generate a pool of peptides from endocytosed material. The interaction between MHC-II and DM occurs most efficiently on the ILVs, as detected in FRET studies that suggests them as the location of peptide loading (Zwart et al., 2005). We hypothesise that tetraspanin proteins, known for their tendency to be organized in protein microdomains, function as a scaffold for protein interactions, hence facilitating the interaction between MHC-II and DM. The tetraspanins CD63 and CD82 are good candidates to build such microdomains, as they are enriched on ILVs (Escola et al., 1998; Wubbolts et al., 2003) and interact with both MHC-II and DM (Hammond et al., 1998). We visualized the efficiency of dynamic homo- and hetero-typic interactions between CD63 and CD82 using sensitized emission Fluorescence Resonance Energy Transfer, seFRET (van Rheenen et al., 2004) described in chapter 2. While the two tetraspanins interacted more efficiently with each other on the limiting membrane, both MHC-II and DM preferably interacted with CD63 on ILVs. At the limiting membrane of an MVB, MHC-II favoured interactions with CD82, which could be also observed at the plasma membrane. For the last few years, only few MHC-II related tetraspanins functions have been postulated. Lately, involvement of the tetraspanin CD9 in MHC-II sorting on ILVs has been proposed (Buschow et al., 2009). At the plasma membrane of mouse cells, CD9 is involved in clustering of two MHC-II variants building up the so-called immune synapse (Unternaehrer et al., 2007). We propose that CD63 on internal vesicles facilitates the interaction between MHC-II and DM. CD82 might play a role in sorting of peptide-MHC-II on the limiting membrane for transport towards the plasma membrane. Our studies are the first to show the dynamics of tetraspanin networks within MIIC and may explain the spatial distribution of MHC-II /DM complexes within these intracellular compartments.

Strategy Two – High Throughput Genome Wide Screen

To find new players involved in the route of MHC-II antigen presentation, we performed a genome-wide , unbiased RNAi screen for the effects on MHC-II surface expression and peptide loading by flow cytometry creating a MHC-II cell surface expression database as described in chapter 3. We defined 276 novel MHC-II regulators, which are expressed in primary immune cells as determined by microarray analysis yielding an mRNA expression database. Ten percent of these are known to associate with MHC-II and eight percent are related to autoimmune diseases. Searching existing protein interaction databases did not yield new pathways because many hits have an unknown function. Therefore, we aimed at assigning in an unbiased manner a function to proteins in the MHC-II pathway to all our novel MHC-II regulators.

Functional Screens

To place hits in pathways, we designed two additional screens; (1) to analyse MHC-II transcription and (2) to visualize MHC-II transport including sorting (Golgi) and antigen delivery (Early Endosomes). Nine hits were found to influence the

transcription of MHC-II or related genes. We designed novel assays to place these hits in complex feedback networks, wherein transcription of the various hits is connected. Microscopic analysis after silencing the hits revealed different phenotypes, which could be classified by supervised machine learning software (CP analyst 2). This yielded our MHC-II trafficking database.

Systems Biology: Integrating Databases

The databases generated so far were integrated to form a tree diagram. One branch contained hits with an MHC-II redistribution phenotype resembling maturing DC. To confirm that these genes influence antigen presentation in professional APC, we studied the effects of downregulation on MHC-II expression and intracellular localization in immature DCs.

Candidates were silenced in human primary monocytes that were subsequently differentiated into immature DCs. Indeed, MHC-II was redistributed to the plasma membrane, a phenotype typical for mature DCs. The virally transduced cells lacked cell surface expression of maturation markers, indicating that MHC-II redistribution to the plasma membrane is the result of silencing the targeted genes and not maturation. We thus generated imDC with an mDC phenotype with respect to MHC-II distribution.

Resolving the mechanism of new players

One of the MHC-II redistribution genes confirmed in DCs was the unknown GTPase ARF7/ARL14. It triggered our interest, because of the known involvement of ARF proteins in transport events. This GTPase localized to MHC-II containing compartments. GTPases are active in a GEF-dependent GTP-bound state and inactive in a GAP-dependent GDP-bound state. By searching in our databases for GEF motifs, we found an immune-specific ARF-GEF, PSD4, showing the MHC-II redistribution phenotype upon down regulation. PSD4 was able to load ARF7/ARL14 with GTP and to bind to PIP_2. This phospoinositide is the product of combined efforts of PI3K and PIP5K (two other MHC-II redistributing genes), defining a small network of PI3KR2, PIP5K1A, ARF7/ARL14 and the GEF: PSD4. GTPases exert their function via interaction with effector proteins. In a yeast two hybrid assay we identified an unknown ARF7/ARL14 effector, C11orf46 (named ARF7EP). We could link the GTPase pathway to the actin cytoskeleton, by showing an interaction between ARF7EP and the actin motor MYO1E in PBMCs after GST-pull down and mass-spectrometry. This interaction was also confirmed by immunoprecipitation. Combining data sets with wet lab experiments allows the swift identification of novel signalling pathways controlling essential processes in the immune system such as the secretion of MHC-II following activation of human DC.

From Systems Biology to New Biology

The question is whether we can analyze cell biology in a high throughput manner to decipher new pathways. At the present state of technology, we can use and combine datasets and databases to make predictions of proteins involved in particular pathways. In addition, we may assume that proteins involved in the same phenotypic pathway are operating in the same signalling pathway. Large datasets

become available in the public domain and are combined in prediction programs for networks such as STRING and Humannet. Making use of these prediction programs will accelerate the identification of molecular pathways controlling many processes such as antigen presentation. Though, laboratory experiments are still required to test or validate novel interactions, or to investigate the order of control of proteins acting in the same pathway. We illustrate this by deciphering a novel transcription pathway that controls MHC-II expression. By integration information of three databases, the MHC-II cell surface expression database, the mRNA expression database and the MHC-II trafficking database, we could select of a group of genes with the same phenotype; MHC-II redistribution to the plasma membrane. Some of them were experimentally confirmed in Dendritic cells. By combining database information and biochemical experiments we could identify the proteins interacting with ARF7/ARL14 and the function of this group of proteins in MHC-II transport.

Outlook

A better understanding of subtle regulation of MHC-II antigen presentation is essential as this process is one of the critical regulators of immune responses. MHC-II is a central player in defence against infectious diseases. An effective B-cell response is needed to fight infections. This requires optimal antigen presentation by MHC-II. Some of our hits will be perfect candidates to influence MHC-II antigen presentation. These hits could function as target molecules for drug design.

MHC-II is also strongly related to the development of autoimmune diseases. It is likely that some of the novel MHC-II regulators identified in this study are involved in the development and the severity of autoimmune diseases (some proteins related to autoimmune diseases are identified in our screen as well; see Figure 2C in Chapter 3). These targets should be further studied to identify complete pathways involved in or perhaps even causal for autoimmune diseases. This will open new possibilities for design of drug-, antibody- or peptide-based therapy strategies to treat patients suffering from these diseases.

As a first step, it will be important to determine the influence of downregulation or overexpression of the novel MHC-II regulators on the (auto) immune reaction by B- and T-cells. It will be challenging to experimentally verify the function of those novel regulators of MHC-II antigen presentation in the context of the total immune system. The result of a relevant change in defence against infectious disease should be measured by studying the targets in a mouse model. The novel regulators that effect MHC-II antigen presentation and influence defence against pathogens should be subjected to further studies. Drug design is an expensive and complicated process. It is important to determine the ideal target. Identification of the functional pathways of these novel regulators might uncover more possible drugable targets, working in the same pathway and yielding the same result. The best target from such a pathway should be selected, thereby positively contributing to the development of new drugs.

Design of drugs can also be approached from a different site: screening compound libraries and determining the effect on MHC-II antigen presentation. Hereby,

new leads can be identified, which have to be further developed into drugs that manipulate MHC-II antigen presentation. The list of novel regulators identified in this study could help to discover the correct target-lead combination. This can be the start of the development of new drugs for manipulating the immune response. The ultimate goal is to improve it in infections and cancer, and to dampen it in case of autoimmune diseases. These efforts may translate fundamental scientific information into application in the clinic.
Some of the latest attempts to manipulate MHC-II antigen presentation by compounds, antibodies and/or peptides have been summarized in the introduction of this thesis. With this study we hope to provide a basis for development of new methodologies that can influence MHC-II antigen presentation. This can be of interest for the immunological community, as it provides molecular understanding of a number of essential steps in immune responses. But it may also be translated to clinical applications when the targets identified will be used to define leads for further development.

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