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Chemical biology of antigen presentation by MHC molecules

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MHC class I and MHC class II molecules present peptides to the immune system to drive proper T cell responses. Pharmacological modulation of T-cell responses can offer treatment options for a range of immune-related diseases. Pharmacological downregulation of MHC molecules may find application in treatment of auto-immunity and transplantation rejection while pharmacological activation of antigen presentation would support immune responses to infection and cancer. Since the cell biology of MHC class I and MHC class II antigen presentation is understood in great detail, many potential targets for manipulation have been defined over the years. Here, we discuss how antigen presentation by MHC molecules can be modulated by pharmacological agents and how chemistry can further support the study of antigen presentation in general. The chemical biology of antigen presentation by MHC molecules shows surprising options for immune modulation and the development of future therapies.

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An update on MHC class I antigen presentation

The early events: proteins for peptides

MHC class I molecules present peptides from intracellular origin to the immune system (Figure 1) [1]. Successful presentation involves a number of sequential steps, many of which can in principle be targeted by chemical compounds. The process starts with the recognition of protein substrates for degradation by proteasomes. The protein substrates can either be stable proteins at the end of their natural lifetime but they can also be misfolded or mistranslated proteins (so-called DRiPs) [2]. Many properly folded old proteins will also contribute to the MHC class I-associated peptide pool [3]. Whether cells can increase the contribution of DRiPs is unclear. Yet, an increase in the error rate of translation and folding may likely induce the contribution of DRiPs to the peptide pool for MHC class I molecules. Such conditions may include oxidation (e.g. following IFNγ exposure [4] or radiation [5]), chemical interference with folding and higher temperatures (fever) and the mode of translation from endogenous or viral templates [6]. The proteins are then degraded by the ubiquitin-proteasome system and peptides are generated. A critical role for the proteasome in peptide generation for MHC class I molecules is undisputed.

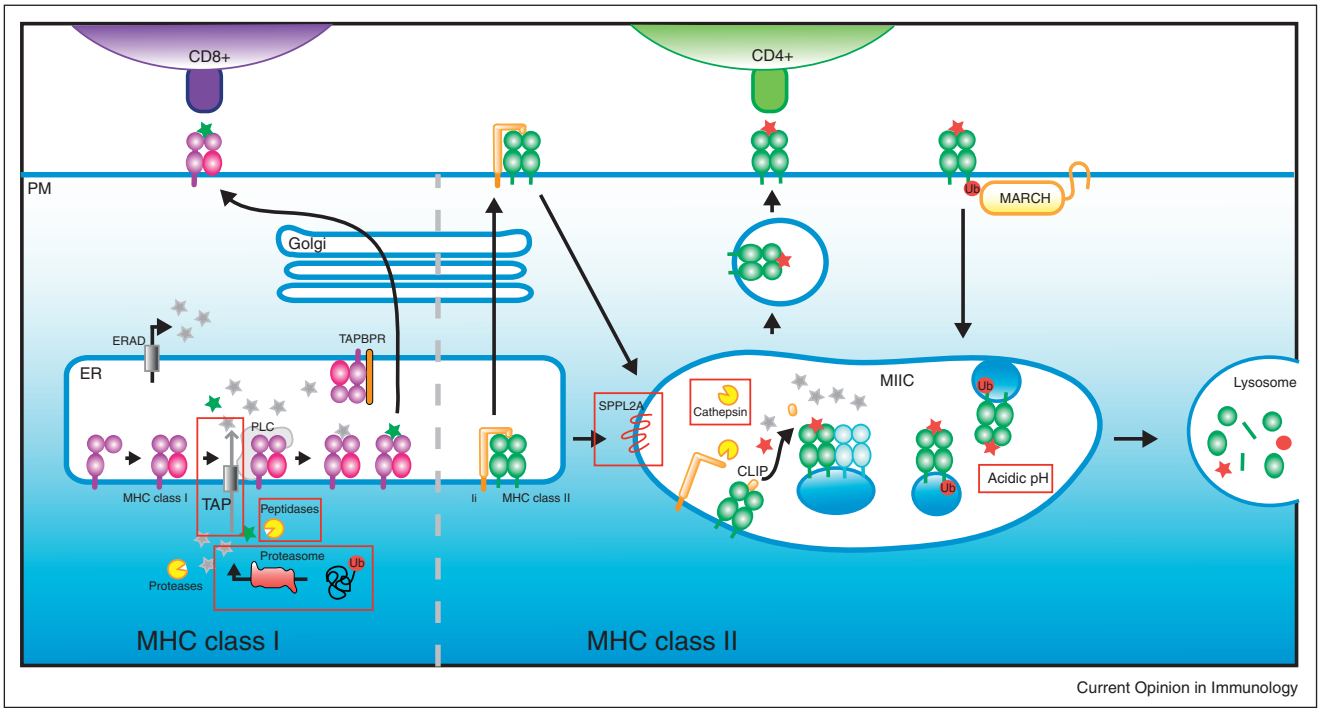
One or many proteasomes?

The substrate is unfolded by the 19S cap of one of the now three recognized forms of the proteasome; the constitutive proteasome as found in all cells, the immunoproteasome which can be induced by interferon-γ and highly expressed in immune cells and the thymoproteasome in thymic cortical epithelial cells where T cell selection occurs [7]. Three of the total 14 different 20S proteasome subunits differ between these forms and represent the proteolytically active subunits. Consequently, the fine specificity of the proteasomal digestion product differs [8]. Mice deficient in all immunoproteasome subunits lower MHC class I expression, antigen presentation and T cell responses to infection [9]. Mice deficient for the thymoproteasomal subunit β5t have a different peptide repertoire and decreased $CD8^+$ T cell repertoire against pathogens [10]. Yet, why an altered peptide repertoire by different proteasomes is essential for shaping better immune responses, is unclear. The different proteasomes may have additional functions. Immunoproteasomes, for example, seem more active and thus critical for the degradation of substrates under conditions of surplus waste following oxidative stress by IFN-γ exposure [4]. Whether this is indeed the function of immunoproteasomes is disputed [11].

The proteasome degrades proteins with a particular specificity to peptides of 3-18 amino acids [12]. During the proteolytic cleavage a reversal of proteolysis may occur resulting in the splicing and ligation of two fragments into a new peptide that is not encoded genetically [13–15]. Whether this process of chemical ligation is equally active in all cells and tissues, and whether all proteasomes (constitutive-, immuno- and thymo-) are equally efficient in this process, is unclear.

Degradation continues following the proteasome [16]. The majority of peptides generated by proteasomes will be destroyed in seconds by the set of aminopeptidases

Figure 1



Druggable nodes in the MHC class I and MHC class II antigen presentation pathways. MHC class I and MHC class II molecules are assembled in the ER. They bind peptides produced at different sites in the cell. Boxed are targets that can be druggable for manipulation of expression of MHC molecules and manipulation of the peptide repertoire. In general terms these include proteases involved in peptide generation, proteases involved in cleavage of the invariant chain and TAP.

present in the cytosol [17]. Some specialization may exist. For example, the huge protease TPPII destroys large peptide fragments [18]. Other cytosolic peptidases may be critical for generating or destroying peptides for presentation include nardilysin [19], TPPII [20], Leucine aminopeptidase (LAP) and Thimet OligoPeptidase (TOP) [21,22]. However, none of these peptidases is solely critical in antigen presentation suggesting that they collectively generate, trim or destroy peptides.

And finally…meeting MHC class I

TAP requires peptides longer than 7 amino acids (like MHC class I) but also allows transfer of peptides of over 40 amino acids in length (unlike MHC class I that usually binds peptides of around 9 amino acids and rarely longer peptides) [23,24]. Following translocation, peptides encounter TAP associated MHC class I molecules that are stabilized and kept in place by a complex consisting of two dedicated chaperones tapasin/TAP complex [25•] and one or two MHC class I molecules in complex with one protein disulfide reductase ER60 and one lectin, calreticulin. Tapasin is a classical chaperone that promotes peptide on-rates and off-rates from many (but not all) MHC class I alleles [26]. Peptides can bind directly to MHC class I in this so-called Peptide Loading Complex (PLC). Peptides that are too long for binding MHC class I in the PLC can be further trimmed by the ER resident amino-peptidases ERAP1 and ERAP2 (ERAAP in mice) to a size proper for MHC class I molecules [27]. Various MHC class I alleles ignore the PLC yet find peptides in the ER for presentation [28]. Recently, a tapasin-related protein TAPBPR was identified that binds MHC class I molecules in the ER without involvement of the PLC and transports these toward the cell surface [29•]. How TAPBR complements the PLC, is unclear.

Sadly, both MHC class I molecules that fail to acquire peptide cargo (which can be a substantial number), the free H chain and free peptides will be cleared from the ER lumen (β_2m is secreted) by the ERAD system [30]. This ERAD system involves recognition of the glycosylated MHC class I H chain by EDEM1 before dislocation in the cytosol to allow ubiquitination by HRD1 for degradation by proteasomes [31].

And then… the end

MHC class I-peptide combinations will usually stay at the cell surface for 4–18 h dependent on cell type. The exact molecular mechanism for this difference is unclear but may involve expression of MARCH ubiquitin ligases that modify the cytoplasmic tail of MHC class I by one or more Ub molecules for internalization and degradation in lyso-

somes [32]. Of note, a fraction of MHC class I can escape lysosomal destruction. They may lose peptides at the buoyant acidic pH (around pH 5.5) and then acquire a new peptide generated in endosomes for presentation at the plasma membrane [33]. This is a form of cross-presentation of peptides from exogenous antigens generated by endosomal proteases as for MHC class II.

Targets and drugs effective in MHC class I antigen presentation

With this detailed understanding of the MHC class I biology, selective targets for inhibition by compounds to modulate expression and function can be defined. In fact, this has already been exploited by nature: various viruses downregulate MHC class I to escape recognition by the immune system [34]. Many of these viral proteins inhibit TAP [35], or affect folding and degradation of MHC class. Why would inhibition of MHC class I be of interest for drug development? Downregulating MHC class I would obviously be useful to prevent transplant rejection, but also in particular auto-immune diseases such as Bechterew that is strongly linked to the MHC class I allele HLA-B27.05 [36].

Proteasome inhibitors are used in the treatment of Multiple Myeloma (MM) cancer patients. Velcade was the first inhibitor introduced in the clinic and Kyprolis, developed by Onyx pharmaceuticals was approved earlier this year. It is noteworthy that both inhibitors are so-called covalent binders (Figure 2). Covalent drugs tend to be avoided by Pharma, since side reactions are difficult to control, but these compounds may change this dogma. Both Velcade and Kyprolis target mainly β5 and β2 activities in proteasomes, their activities on immunoproteasomes have not been documented very well. Inhibition of all proteasome activities may not be required for proper anticancer effects (or simply lethal) [37]. More selective immunoproteasome inhibitors should have selectivity for immune cells such as dendritic cells while cells lacking immunoproteasomes would be left untouched. Thymoproteasome inhibitors may have effects of T cell selection and of interest in auto-immune diseases. Although the first immunoproteasome specific inhibitor has been reported [37], many improvements in design and selectivity are to be expected.

Cytosolic peptidases are difficult proteases to inhibit selectively, as they are mostly metalloproteases (generally inhibited by non-selective metal-ligating reagents, such as thioleucine and phenanthroline). In addition, such inhibitors may alter the peptide repertoire which may induce new auto-immune responses.

Inhibition of the **peptide transporter TAP** would selectively affect MHC class I antigen presentation. Peptides with long side chains are competitive TAP inhibitors, as they block the pore in TAP during translocation [38]. Yet better inhibitors may be generated based on inhibitors made against ABC transporter family members of TAP, notably multidrug transporter MDR1. One such inhibitor is cyclosporine that also prevents activation of T cells. Non-immunosuppressive cyclosporins have been developed [39], but not tested for TAP-inhibition.

Finally, it may be difficult to selectively interfere with peptide loading of MHC class I molecules. But inhibiting the ER associated peptidases **ERAAP/ERAP** may be useful to trim MHC class I expression at the cell surface as apparently many peptides require a final trimming step in the ER, as concluded from genetic silencing and the inhibition of this metallo-peptidase with thioleucine [40] (Figure 2). Targeting ERAAP/ERAP may be highly relevant in the case of rheumatoid arthritis that has been linked to genetic variants of this peptidase [41•]. The first inhibitors for ERAP1 have now been developed [42,43]. How these inhibitors act in living systems is as yet unclear.

The MHC class I pathway reveals many critical enzymes and transporters that could be targeted by small compounds for regulation of MHC class I expression.

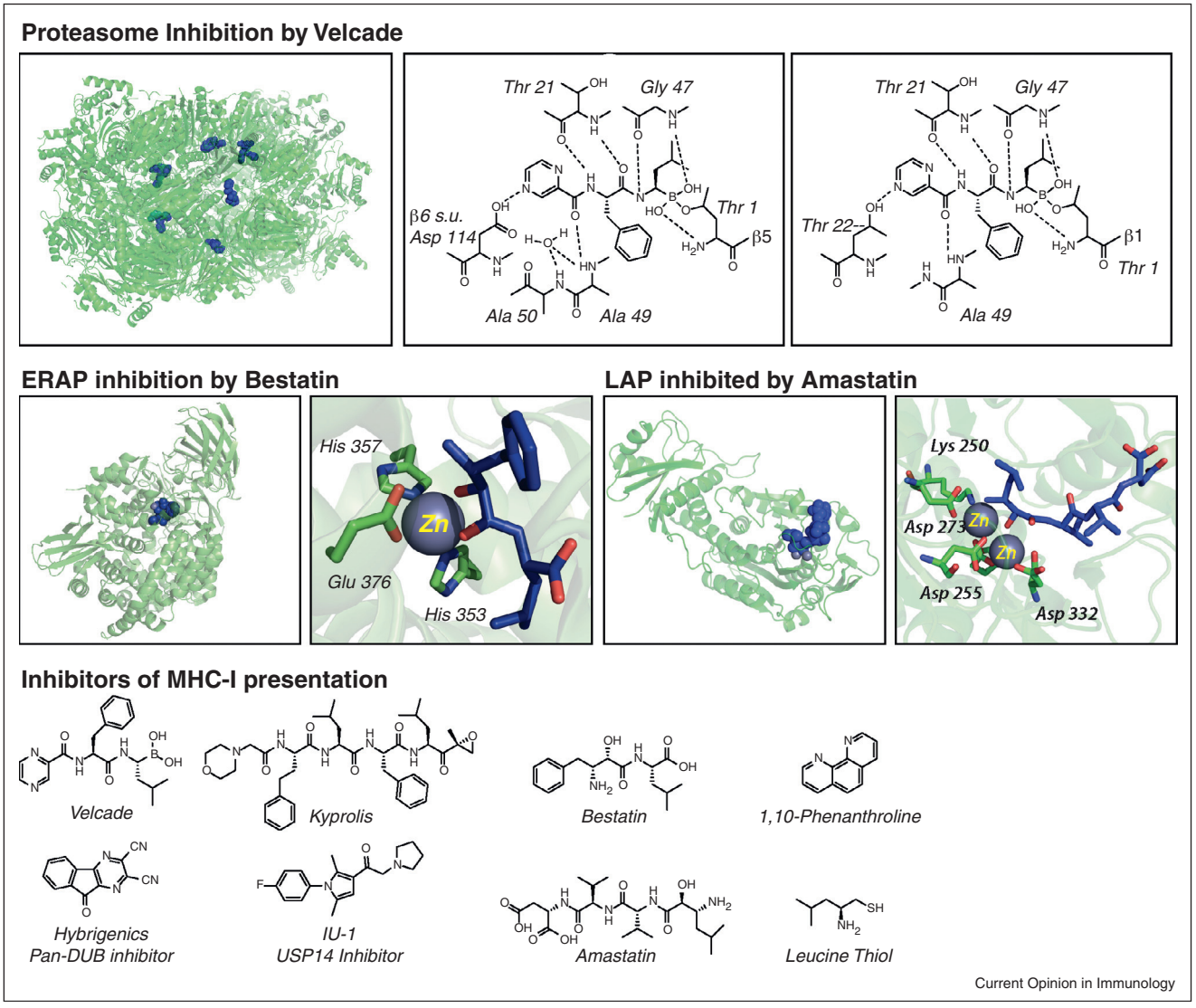
Updating MHC class II antigen presentation

The basics

MHC class II has a similar architecture as MHC class I yet they differ in tissue distribution and in the source of peptides presented that are mainly derived from proteins degraded in the endocytic pathway. Firstly, MHC class II is assembled in the ER and pairs with the invariant chain Ii. Ii then supports transport to late endosomes, generally named MIIC [44]. Here, the luminal domain of Ii is degraded by resident proteases (especially cathepsin S and L) [45] and the transmembrane part of Ii ultimately by the transmembrane protease SPPL2a [46•,47•]. Released MHC class II then associates with dedicated chaperone DM possibly on the internal vesicles within MIIC but also the limiting membrane [48,49] (Figure 1). A recent crystal structure of the MHC class II (HLA-DR1)-DM complex, shows that DM affects the beginning of the peptide-binding groove (the P1P2 pockets) and more specifically a Trp residue that is then pointing towards HLA-DM. This conformation allows efficient peptide exchange until high affinity peptides bind and occupy the P1P2 pockets in HLA-DR1. The Trp residue may then flip inwards, lock the peptide and prevent further DM interactions [50••]. This explains how a chaperone (DM) promotes the selection of 'high affinity' peptides by MHC class and it is likely that tapasin acts in a similar manner (tapasin and DM are both MHC look-alikes with a very similar overall structure [51]).

Some immune cells (such as immature B cells) express an addition chaperone called DO that tightly interacts with DM. In fact, DM is required for DO to leave the ER for

Figure 2



Drugs targeting the MHC-I pathway. **(a)** Crystal structure of proteasome inhibitor Velcade in the yeast 20S proteasome [98]. β1 and β5 catalytic subunits are targeted through multiple H-bonding interactions; **(b)** Inhibition of the metalloproteases ERAP [99] and LAP [100,101] by their inhibitors bestatin and amastatin works through ligation of cis-heteroatoms in the inhibitors with the catalytic Zn-atoms in the enzymes catalytic site; and **(c)** chemical structures of inhibitors of MHC class I antigen presentation, as discussed in part 2.

transport to MIIC. DO appears to act as an MHC class II molecule and the crystal structure shows it binding at the same sites to DM as MHC class II would [52••]. Consequently, DO alters the MHC class II associated peptidome and quenches MHC class II antigen presentation. Yet, it may promote presentation of exogenous antigens, as deduced from mouse studies in DO-deficient mice [53]. Most auto-immune diseases are strongly associated to MHC class II alleles. DO is probably important to reduce auto-immune responses [53,54,23] but may have other surprising functions as well.

Ultimately, MHC class II is transported to the plasma membranes either via tubules emanating from MIIC [55] or by direct fusion of MIIC to the plasma membrane [56]. Whether this involves some sort of quality control system allowing only proper MHC class II to leave MIIC, is unclear as such a system is unknown in the endosomal pathway.

Controlling MHC class II transport

MHC class II export from MIIC can be controlled as exemplified for macrophages exposed to IL-10 and maturing DC. IL-10 quenches MHC transport to the

plasma membrane of human monocytes and thus reduces immune responses [57].

Dendritic cells (DC) strongly upregulate MHC class II expression at the plasma membrane following maturation. This is the result of induced transport of MHC class II from MIIC to the plasma membrane and – at the same time – a strongly increased half-life at the cell surface [58]. A recent siRNA screen identified many factors controlling MHC class II biology [23]. This screen integrated expression arrays, microscopy and flow cytometry to predict proteins involved in controlling the MHC class II redistribution in maturing DC. Nine proteins were identified that – when silenced in immature DC – produced a MHC class II distribution as seen in mature DC. One pathway was particularly well resolved. The GTPase Arl14/ARF7 on MIIC interacts with effector ARF7EP that coupled to actin-binding protein Myo1e to control transport of MIIC in DC. Myo1e is a 'single-leg' actin binding motor that does not move but attach cargo to actin. The pathway then shows how MIIC is retained by a mechanism attaching these vesicles to actin thus preventing transport to the cell surface. Loss of this retention mechanism yields immature DC with a mature MHC class II phenotype, as MHC class II is now mostly at the cell surface [23].

The end

Once at the cell surface, MHC class II may display remarkably different half-lives, and is strongly increased following differentiation of mature DC. This coincides with high cell surface expression of MHC class II and prolonged presentation of antigen. The transmembrane E3 ubiquitin-ligase MARCH-1 controls the cell surface half-life of MHC class II in DC. MARCH-1 ubiquitinates MHC class II for internalization and degradation (Figure 1) [59]. Immature DC in MARCH-1 deficient mice present with stable and higher MHC class II levels [60,61]. However, MARCH-1 also locates to MIIC [62] and may have other effects on MHC class II biology since engineered mice with MHC class II mutations to prevent ubiquitination show normal antigen presentation responses [63].

Targets for pharmacological modulation of MHC class II antigen presentation

As MHC class II is strongly associated to almost all auto-immune diseases, pharmacological modulation to control expression may have direct application in these diseases. Surprisingly, peptides from denatured protein (fragments) may be presented differently than those derived from intact antigen and then contribute to autoimmunity [64]. Controlling this may also yield solutions for autoimmune diseases. The current understanding of the MHC class II pathway also reveals some druggable targets (Figure 1). These include the proteases involved in the destruction of Ii (cathepsin S, cathepsin L, AEP [65] and SPPL2a [46•,66•,67]). Endosomal proteases involved in specific generation of disease-associated peptides may be targeted [68,69]. Also, endosomal enzymes involved in the preparation of antigens for destruction are drug targets. This includes GILT, a gamma interferon-inducible lysosomal thiolreductase that controls responses to dust mite allergens [70] and auto-immune responses against Trp1[71]. This may be a reasonable target as GILT-deficient mice are fully viable (yet poor in cross-presentation) [72].

Are there more options for defining druggable targets that control MHC class II? The genome-wide siRNA screen for factors controlling MHC class II expression and peptide loading revealed 287 with about 140 proteins with unknown function [23]. These include many druggable proteins. The quest is to define inhibitors with sufficient specificity, selectivity and *in vivo* activity to manipulate MHC class II responses without serious side effects for application in auto-immune diseases like MS, T1D and Coeliac disease that all have strong links to MHC class II alleles.

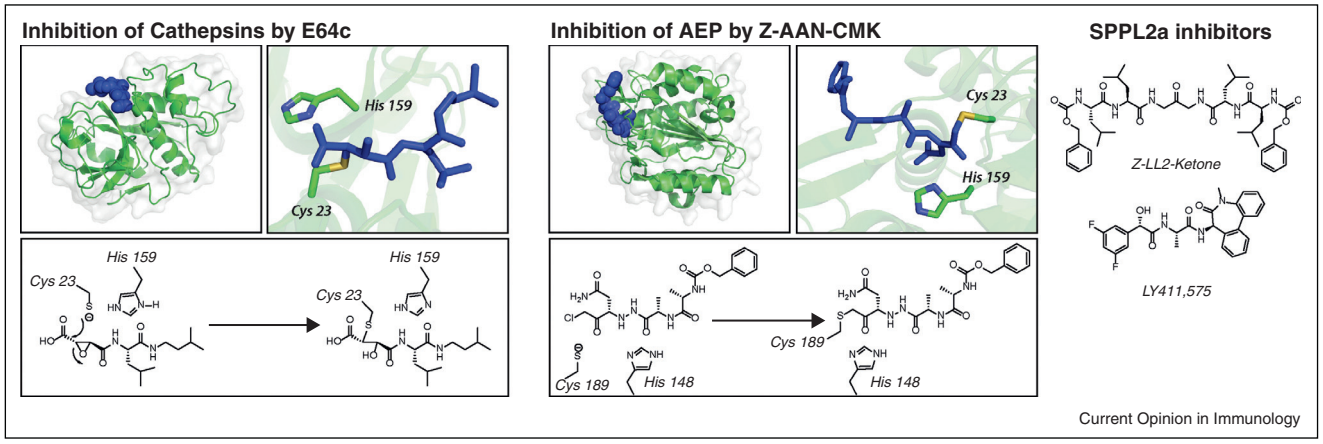
Small molecule modulation of MHC class II antigen presentation

Modulating endosomal homeostasis or specific endosomal proteases can alter MHC class II antigen presentation [73]. Chloroquine (inhibits lysosomal acidification [74]) and the general cysteine protease inhibitor leupeptin [75] both inhibit MHC class II antigen presentation albeit fairly unspecific (for structures and mode of inhibition; Figure 3). Selective inhibitors of cathepsin S interfere with processing of Ii [76] and reduced presentation of auto-antigens [77]. These inhibitors are in clinical trials for treatment of auto-immune disorders [78,79]. Other proteases may merely affect processing of antigens. Inhibitors of aspartyl proteases and AEP [65] can alter the presentation of defined epitopes [68,69]. These studies illustrate the complexity of protease inhibition to manipulate MHC class II: Inhibiting proteases may both prevent epitope generation and induce the generation of other epitopes that trigger (different) self-reactive T cells and then induce auto-immune responses.

In addition, endosomal proteases act in a network [80]. They are required for removal of propeptides to activate other proteases and inhibiting one protease then affects the activity levels of other endosomal proteins. For example, cathepsin S regulates the activity of cathepsin L and the turnover of GILT [81]. But GILT then regulates the degradation of cathepsin S. Highly specific single-protease inhibitors may thus have broad effects on endo-lysosomal protease activities [82]. Broad cathepsin-specific inhibitors have been developed that affect endosomal destruction of EGF-receptor. The effects on MHC class II are unknown [83].

Another protease-target may be SPPL2a that cleaves the last fragment of Ii before allowing peptide loading of MHC class II [66•,67,84]. Selective inhibitors of SPPL2a

Figure 3



Mechanisms of some endosomal protease inhibitors. **(a)** Crystal structure of the cathepsin inhibitor E64c in complex with papain, the archetypal cysteine protease and the mechanism of inhibition [102]; **(b)** crystal structure of AEP in complex with a Z-AAN-CMK inhibitor and the mechanism of inhibition [103]; and **(c)** reported SPP inhibitors [67] that may be a start-point for further development of more selective SPPL2a inhibitors.

and b have not been reported, but general inhibitors of multimembrane aspartyl proteases, such as LY411575 [85] and zLL2k (initially reported as a γ-secretase but also an SPPL2 inhibitor) [67,86] can inhibit the final stages of Ii processing. These compounds can be the basis for more selective SPPL2a inhibitors.

Further targets in the MHC class II pathway are endo-lysosomal enzymes that post-translationally edit peptide epitopes. For example, the major collagen epitope in rheumatoid arthritis is hydroxylated and glycosylated at lysine residues. In rheumatoid arthiritis, these appear to be deglycosylated [87]. The responsible glycosidase is an interesting target for rheumatoid arthritis treatment [88]. Other post-translational modifications on antigenic peptides (citrullination, tyrosine nitration) also yield neo-epitopes that can induce auto-immunity. Citrullinated peptides are detected by T cells in rheumatoid arthritis patients [89].

Chemistry for innovative vaccines

With the recent drive to the development of more effective vaccines, chemical synthesis and characterization is essential in their rational design. Results in the form of long peptide vaccines and synthetic TLR ligands are only illustrations of chemical options. Fully synthetic glycopeptide tumor vaccines are generated [90]. TLR-ligands are conjugated to peptides for combining innate signaling and antigen to the same endosome in the same DC. Such conjugates can induce robust immune responses [91–93].

The design of vaccines has relied so far on immunization with either inactivated live strains or components thereof. Vaccination has made an enormous contribution to mankind. Chemical modification of MHC presented antigenic peptides may provide a future entry to peptide-based formulations that provide safe and long-lasting immune responses [94] against cancer and infection diseases.

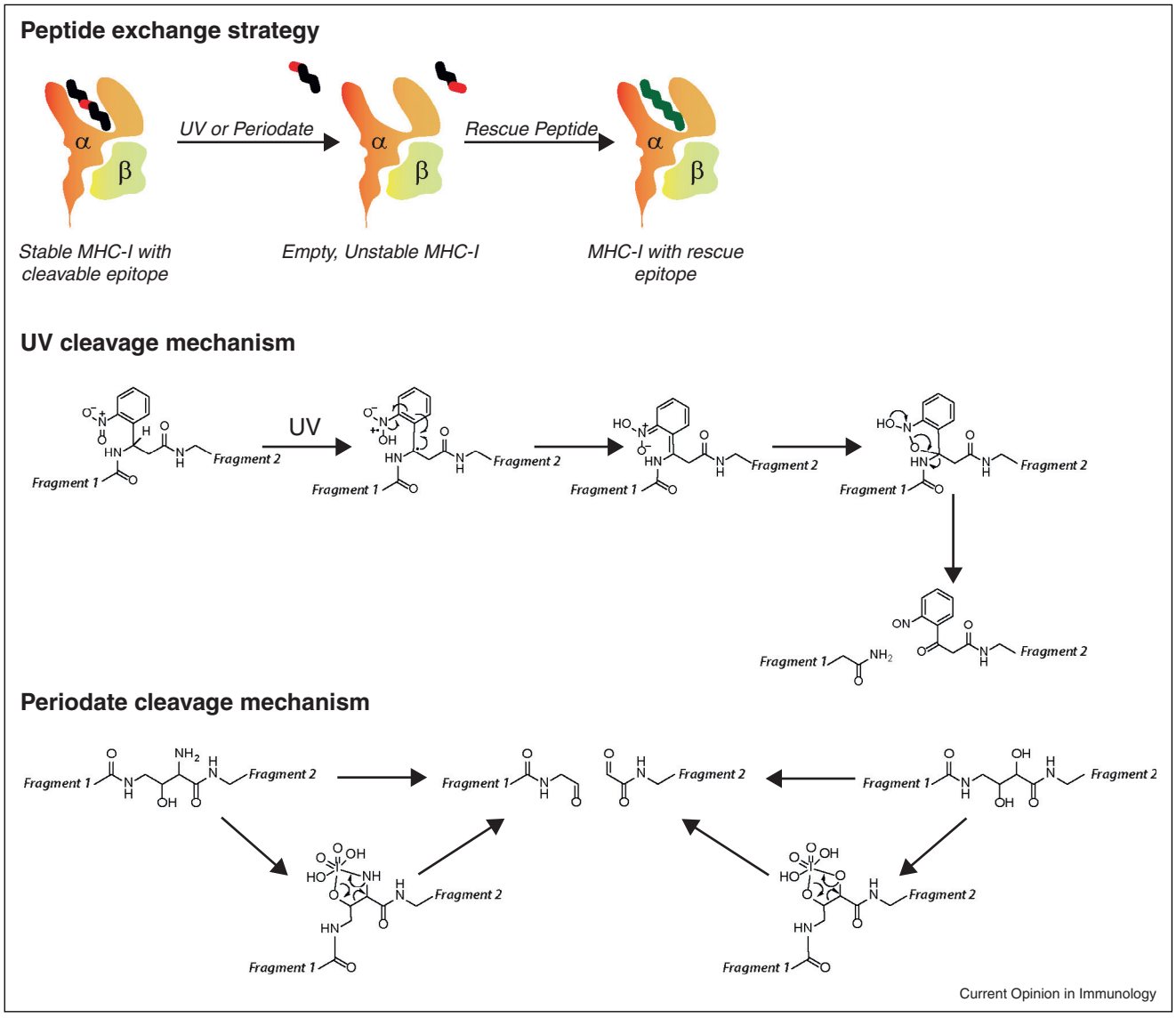
What chemistry can do for immunologists: new chemical tools

An enormous number of reagents used by immunologists during culturing and measuring immune responses are the result of a serious chemistry activity. F-moc chemistry to produce peptides is one such example. These peptides are used to induce immune responses but also to load *in vitro* folded MHC molecules to form tetramers for monitoring T cell responses. However, a tetramer for each and every different peptide has to be produced. Chemists generated peptides with UV and (later) chemical unstable linkages [95,96] (Figure 4). Tetramers with these peptides can be exposed to UV or periodate in the presence of peptides of choice. The tetramer-associated peptide will be split and dissociates to be replaced by the exogenous peptide. This allows the generation of one peptide-tetramer batch for loading with any peptide of choice. This allowed combinatorial coding of broad T cell responses by tetramers [97].

What immunologists can do for chemists and chemists for immunologists

There is probably no field beyond immunology where molecules of a different nature have a biological impact. Many unique molecules are produced by the various pathogens to which the host immune system responds. These can provide inspiration to bio-organic chemists to synthesize and combine bioactive structures evolved in the battle between host and pathogen. Such molecules may provide options for radically new drug design with applications in immuno-modulation and improved and

Figure 4



Peptide exchange in MHC-tetramers. Peptide exchange in MHC-tetramers. **(a)** MHC-I complexes can be folded with a single cleavable peptide. Treatment with periodate or UV-light will result in cleavage of this starter epitope into two low affinity fragments that can be displaced by a 'rescue'-peptide [104,105]. This approach allows for the rapid generation of libraries of MHC-peptide complexes; and **(b)** the chemistries available are based on ortho-nitrophenyl sidechains, which cleave upon UV-treatment or are based on cis-diols [106] or cis hydroxylamines [107], which can be cleaved upon treatment with periodate via the mechanisms shown.

innovative vaccines. Organic chemistry will be essential to arrive at such compounds, not only for therapy but also for diagnostics. Diagnostic imaging with novel fluorophores and reading immune response using MHC or TCR tetramers are just examples of these. Many of the examples outlined above, have been the results of interactions between the fields of immunology and bioorganic chemistry. Although they have different language, an accepted Esperanto seems nearby. To make the next steps towards new drugs, new immunotherapies, new imaging techniques, better arraying options and more, immunology and chemistry communities should embrace each other's worlds.

Concluding remarks: a bright future for chemical immunology

Chemical immunology is an upcoming field with no representation at significant Immunology or Chemistry meetings. Yet, the power of chemical tools and bioactive small molecules in the immunology field is undisputed as is the width of immunology as an inspiration for organic chemistry. The current scientific climate promotes

translational research and chemistry is essential to arrive at bioactive compounds to modulate immune responses. New molecules always yielded new biology and it may be anticipated that chemical immunology will generate both better understanding and manipulating of immunological processes such as antigen presentation by MHC molecules.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Neefjes J, Jongsma ML, Paul P, Bakke O: **Towards a systems understanding of MHC class I and MHC class II antigen presentation**. *Nature Reviews: Immunology* 2011, **11**:823-836.
2. Yewdell JW, Princiotta MF: **All roads lead to ome: defining the DRiPome**. *Blood* 2012, **119**:6179-6180.
3. Farfan-Arribas DJ, Stern LJ, Rock KL: **Using intein catalysis to probe the origin of major histocompatibility complex class I-presented peptides**. *Proceedings of the National Academy of Sciences USA* 2012, **109**:16998-17003.
4. Seifert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M *et al.*: **Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress**. *Cell* 2010, **142**:613-624.
5. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J *et al.*: **Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy**. *J Exp Med* 2006, **203**:1259-1271.
6. Lu X, Gibbs JS, Hickman HD, David A, Dolan BP, Jin Y, Kranz DM, Bennink JR, Yewdell JW, Varma R: **Endogenous viral antigen processing generates peptide-specific MHC class I cell-surface clusters**. *Proceedings of the National Academy of Sciences U.S.A.* 2012, **109**:15407-15412.
7. Forster F, Unverdorben P, Sledz P, Baumeister W: **Unveiling the Long-Held Secrets of the 26S proteasome**. *Structure* 2013, **21**:1551-1562.
8. Vigneron N, Van den Eynde BJ: **Proteasome subtypes and the processing of tumor antigens: increasing antigenic diversity**. *Curr Opin Immunol* 2012, **24**:84-91.
9. Kincaid EZ, Che JW, York I, Escobar H, Reyes-Vargas E, Delgado JC, Welsh RM, Karow ML, Murphy AJ, Valenzuela DM *et al.*: **Mice completely lacking immunoproteasomes show major changes in antigen presentation**. *Nat Immunol* 2011, **13**:129-135.
10. Nitta T, Murata S, Sasaki K, Fujii H, Ripen AM, Ishimaru N, Koyasu S, Tanaka K, Takahama Y: **Thymoproteasome shapes immunocompetent repertoire of CD8$^+$ T cells**. *Immunity* 2010, **32**:29-40.
11. Nathan JA, Spinnenhirn V, Schmidtke G, Basler M, Groettrup M, Goldberg AL: **Immuno- and constitutive proteasomes do not differ in their abilities to degrade ubiquitinated proteins**. *Cell* 2013, **152**:1184-1194.
12. Cascio P, Hilton C, Kisselev AF, Rock KL: **Goldberg AL: 26S proteasomes and immunoproteasomes produce mainly N-extended versions of an antigenic peptide**. *EMBO J* 2001, **20**:2357-2366.
13. Hanada K, Yewdell JW, Yang JC: **Immune recognition of a human renal cancer antigen through post-translational protein splicing**. *Nature* 2004, **427**:252-256.
14. Vigneron N, Stroobant V, Chapiro J, Ooms A, Degiovanni G, Morel S, van der Bruggen P, Boon T, Van den Eynde BJ: **An antigenic peptide produced by peptide splicing in the proteasome**. *Science* 2004, **304**:587-590.
15. Warren EH, Vigneron NJ, Gavin MA, Coulie PG, Stroobant V, Dalet A, Tykodi SS, Xuereb SM, Mito JK, Riddell SR *et al.*: **An antigen produced by splicing of noncontiguous peptides in the reverse order**. *Science* 2006, **313**:1444-1447.
16. York IA, Goldberg AL, Mo XY, Rock KL: **Proteolysis class I major histocompatibility complex antigen presentation**. *Immunol Rev* 1999, **172**:49-66.
17. Reits E, Griekspoor A, Neijssen J, Groothuis T, Jalink K, van Veelen P, Janssen H, Calafat J, Drijfhout JW, Neefjes J: **Peptide diffusion, protection, and degradation in nuclear and cytoplasmic compartments before antigen presentation by MHC class I**. *Immunity* 2003, **18**:97-108.
18. York IA, Bhutani N, Zendzian S, Goldberg AL, Rock KL: **Tripeptidyl peptidase II is the major peptidase needed to trim long antigenic precursors, but is not required for most MHC class I antigen presentation**. *J Immunol* 2006, **177**:1434-1443.
19. Kessler JH, Khan S, Seifert U, Le Gall S, Chow KM, Paschen A, Bres-Vloemans SA, de Ru A, van Montfoort N, Franken KL *et al.*: **Antigen processing by nardilysin and thimet oligopeptidase generates cytotoxic T cell epitopes**. *Nat Immunol* 2011, **12**:45-53.
20. Seifert U, Maranon C, Shmueli A, Desoutter JF, Wesoloski L, Janek K, Henklein P, Diescher S, Andrieu M, de la Salle H *et al.*: **An essential role for tripeptidyl peptidase in the generation of an MHC class I epitope**. *Nat Immunol* 2003, **4**:375-379.
21. Kawahara M, York IA, Hearn A, Farfan D, Rock KL: **Analysis of the role of tripeptidyl peptidase II in MHC class I antigen presentation in vivo**. *J Immunol* 2009, **183**:6069-6077.
22. York IA, Mo AX, Lemerise K, Zeng W, Shen Y, Abraham CR, Saric T, Goldberg AL, Rock KL: **The cytosolic endopeptidase, thimet oligopeptidase, destroys antigenic peptides and limits the extent of MHC class I antigen presentation**. *Immunity* 2003, **18**:429-440.
23. Paul P, van den Hoorn T, Jongsma ML, Bakker MJ, Hengeveld R, Janssen L, Cresswell P, Egan DA, van Ham M, Ten Brinke A *et al.*: **A genome-wide multidimensional RNAi screen reveals pathways controlling MHC class II antigen presentation**. *Cell* 2011, **145**:268-283.
24. Chang SC, Momburg F, Bhutani N, Goldberg AL: **The ER aminopeptidase, ERAP1, trims precursors to lengths of MHC class I peptides by a molecular ruler mechanism**. *Proc Natl Acad Sci USA* 2005, **102**:17107-17112.
25. • Panter MS, Jain A, Leonhardt RM, Ha T, Cresswell P: **Dynamics of major histocompatibility complex class I association with the human peptide-loading complex**. *J Biol Chem* 2012, **287**:31172-31184.

Panter *et al*. Biophysical study on the build up on the PLC that addressed the long standing issue of the number of tapasin molecules associated to one TAP.

26. Geironson L, Thuring C, Harndahl M, Rasmussen M, Buus S, Roder G, Paulsson KM: **Tapasin facilitation of natural HLA-A and -B allomorphs is strongly influenced by peptide length, depends on stability, and separates closely related allomorphs**. *J Immunol* 2013.
27. Hammer GE, Gonzalez F, Champsaur M, Cado D, Shastri N: **The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules**. *Nat Immunol* 2006, **7**:103-112.
28. Neisig A, Wubbolts R, Zang X, Melief C, Neefjes J: **Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing**. *J Immunol* 1996, **156**:3196-3206.
29. • Boyle LH, Hermann C, Boname JM, Porter KM, Patel PA, Burr ML, Duncan LM, Harbour ME, Rhodes DA, Skjodt K *et al.*: **Tapasin-related protein TAPBPR is an additional component of the MHC class I presentation pathway**. *Proc Natl Acad Sci USA* 2013, **110**:3465-3470.

Boyle *et al*. Identification of a tapasin look-alike that transports MHC class I from the ER. The exact function in relation to the classical PLC is unclear.

30. Koopmann JO, Albring J, Huter E, Bulbuc N, Spee P, Neefjes J, Hammerling GJ, Momburg F: **Export of antigenic peptides from**

the endoplasmic reticulum intersects with retrograde protein translocation through the Sec61p channel. *Immunity* 2000, **13**:117-127.

31. Burr ML, van den Boomen DJ, Bye H, Antrobus R, Wiertz EJ, Lehner PJ: **MHC class I molecules are preferentially ubiquitinated on endoplasmic reticulum luminal residues during HRD1 ubiquitin E3 ligase-mediated dislocation**. *Proc Natl Acad Sci USA* 2013, **110**:14290-14295.

32. Nathan JA, Lehner PJ: **The trafficking and regulation of membrane receptors by the RING-CH ubiquitin E3 ligases**. *Exp Cell Res* 2009, **315**:1593-1600.

33. Gromme M, Uytdehaag FG, Janssen H, Calafat J, van Binnendijk RS, Kenter MJ, Tulp A, Verwoerd D, Neefjes J: **Recycling MHC class I molecules and endosomal peptide loading**. *Proc Natl Acad Sci USA* 1999, **96**:10326-10331.

34. Ressing ME, Luteijn RD, Horst D, Wiertz EJ: **Viral interference with antigen presentation: trapping TAP**. *Mol Immunol* 2013, **55**:139-142.

35. Parcej D, Tampe R: **ABC proteins in antigen translocation and viral inhibition**. *Nat Chem Biol* 2010, **6**:572-580.

36. Sorrentino R, Bockmann RA, Fiorillo MT: **HLA-B27 and antigen presentation: at the crossroads between immune defense and autoimmunity**. *Mol Immunol* 2014, **57**:22-27.

37. Huber EM, Basler M, Schwab R, Heinemeyer W, Kirk CJ, Groettrup M, Groll M: **Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity**. *Cell* 2012, **148**:727-738.

38. Gromme M, van der Valk R, Sliedregt K, Vernie L, Liskamp R, Hammerling G, Koopmann JO, Momburg F, Neefjes J: **The rational design of TAP inhibitors using peptide substrate modifications and peptidomimetics**. *Eur J Immunol* 1997, **27**:898-904.

39. Gaveriaux C, Boesch D, Boelsterli JJ, Bollinger P, Eberle MK, Hiestand P, Payne T, Traber R, Wenger R, Loor F: **Overcoming multidrug resistance in Chinese-Hamster ovary cells-invitro by Cyclosporine-A (Sandimmune) and non-immunosuppressive derivatives**. *Br J Cancer* 1989, **60**:867-871.

40. James E, Bailey I, Sugiyarto G, Elliott T: **Induction of protective antitumor immunity through attenuation of ERAAP function**. *J Immunol* 2013, **190**:5839-5846.

41. • Evans DM, Spencer CC, Pointon JJ, Su Z, Harvey D, Kochan G, Oppermann U, Dilthey A, Pirinen M, Stone MA *et al.*: **Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility**. *Nat Genet* 2011, **43**:761-767.
Evans *et al*., Genetic data suggest a link between antigen processing for MHC class I molecules and auto-immune disease AS.

42. Papakyriakou A, Zervoudi E, Theodorakis EA, Saveanu L, Stratikos E, Vourloumis D: **Novel selective inhibitors of aminopeptidases that generate antigenic peptides**. *Bioorg Med Chem Lett* 2013, **23**:4832-4836.

43. Zervoudi E, Papakyriakou A, Georgiadou D, Evnouchidou I, Gajda A, Poreba M, Salvesen GS, Drag M, Hattori A, Swevers L *et al.*: **Probing the S1 specificity pocket of the aminopeptidases that generate antigenic peptides**. *Biochem J* 2011, **435**:411-420.

44. Neefjes J, Jongsma ML, Paul P, Bakke O: **Towards a systems understanding of MHC class I and MHC class II antigen presentation**. *Nat Rev: Immunol* 2011, **11**:823-836.

45. Chapman HA: **Endosomal proteases in antigen presentation**. *Curr Opin Immunol* 2006, **18**:78-84.

46. • Schneppenheim J, Dressel R, Huettl S, Luellmann-Rauch R, Engelke M, Dittmann K, Wienands J, Eskelinen E-L, Hermans-Borgmeyer I, Fluhrer R *et al.*: **The intramembrane protease SPPL2a promotes B cell development and controls endosomal traffic by cleavage of the invariant chain**. *J Exp Med* 2013, **210**:41-58.
Schneppenheim *et al.*; Beisner *et al*.; Bergmann *et al*., identification of the endosomal intramembrane protease SPPL2a for the final cut in Ii and description of its function in APC development and immune responses.

47. • Beisner DR, Langerak P, Parker AE, Dahlberg C, Otero FJ, Sutton SE, Poirot L, Barnes W, Young MA, Niessen S *et al.*: **The intramembrane protease Sppl2a is required for B cell and DC development and survival via cleavage of the invariant chain**. *J Exp Med* 2013, **210**:23-30.
Schneppenheim *et al.*; Beisner *et al*.; Bergmann *et al*., identification of the endosomal intramembrane protease SPPL2a for the final cut in Ii and description of its function in APC development and immune responses.

48. Zwart W, Griekspoor A, Kuijl C, Marsman M, van Rheenen J, Janssen H, Calafat J, van Ham M, Janssen L, van Lith M *et al.*: **Spatial separation of HLA-DM/HLA-DR interactions within MIIC and phagosome-induced immune escape**. *Immunity* 2005, **22**:221-233.

49. Bosch B, Heipertz EL, Drake JR, Roche PA: **Major histocompatibility complex (MHC) class II-peptide complexes arrive at the plasma membrane in cholesterol-rich microclusters**. *J Biol Chem* 2013, **288**:13236-13242.

50. •• Pos W, Sethi DK, Call MJ, Schulze MS, Anders AK, Pyrdol J, Wucherpfennig KW: **Crystal structure of the HLA-DM-HLA-DR1 complex defines mechanisms for rapid peptide selection**. *Cell* 2012, **151**:1557-1568.
Pos *et al*. Design of the HLA-DR1-DM complex for crystallization to define the mechanism of HLA-DM mediated peptide exchange for optimal peptide binding by HLA-DR1. Masterpiece!

51. Dong G, Wearsch PA, Peaper DR, Cresswell P, Reinisch KM: **Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer**. *Immunity* 2009, **30**:21-32.

52. •• Guce AI, Mortimer SE, Yoon T, Painter CA, Jiang W, Mellins ED, Stern LJ: **HLA-DO acts as a substrate mimic to inhibit HLA-DM by a competitive mechanism**. *Nat Struct Mol Biol* 2013, **20**:90-98.
Guce *et al*., The structure of the HLA-DO in complex to HLA-DM that showed why HLA-DO prevented HLA-DM supporting peptide loading onto MHC class II. HLA-DO occupies a site similar are MHC class II on HLA-DM.

53. Gu Y, Jensen PE, Chen X: **Immunodeficiency and autoimmunity in H2-O-deficient mice**. *J Immunol* 2013, **190**:126-137.

54. Yi W, Seth NP, Martillotti T, Wucherpfennig KW, Sant'Angelo DB, Denzin LK: **Targeted regulation of self-peptide presentation prevents type I diabetes in mice without disrupting general immunocompetence**. *J Clin Invest* 2010, **120**:1324-1336.

55. Kleijmeer M, Ramm G, Schuurhuis D, Griffith J, Rescigno M, Ricciardi-Castagnoli P, Rudensky AY, Ossendorp F, Melief CJ, Stoorvogel W *et al.*: **Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells**. *J Cell Biol* 2001, **155**:53-63.

56. Wubbolts R, Fernandez-Borja M, Oomen L, Verwoerd D, Janssen H, Calafat J, Tulp A, Dusseljee S, Neefjes J: **Direct vesicular transport of MHC class II molecules from lysosomal structures to the cell surface**. *J Cell Biol* 1996, **135**:611-622.

57. Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R: **Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling**. *Immunity* 1997, **7**:861-871.

58. Pierre P, Turley SJ, Gatti E, Hull M, Meltzer J, Mirza A, Inaba K, Steinman RM, Mellman I: **Developmental regulation of MHC class II transport in mouse dendritic cells**. *Nature* 1997, **388**:787-792.

59. Shin JS, Ebersold M, Pypaert M, Delamarre L, Hartley A, Mellman I: **Surface expression of MHC class II in dendritic cells is controlled by regulated ubiquitination**. *Nature* 2006, **444**:115-118.

60. Walseng E, Furuta K, Bosch B, Weih KA, Matsuki Y, Bakke O, Ishido S, Roche PA: **Ubiquitination regulates MHC class II-peptide complex retention and degradation in dendritic cells**. *Proc Natl Acad Sci USA* 2010, **107**:20465-20470.

61. Matsuki Y, Ohmura-Hoshino M, Goto E, Aoki M, Mito-Yoshida M, Uematsu M, Hasegawa T, Koseki H, Ohara O, Nakayama M *et al.*: **Novel regulation of MHC class II function in B cells**. *EMBO J* 2007, **26**:846-854.

62. van Niel G, Wubbolts R, Ten Broeke T, Buschow SI, Ossendorp FA, Melief CJ, Raposo G, van Balkom BW, Stoorvogel W: **Dendritic cells regulate exposure of MHC class II at their plasma membrane by oligoubiquitination**. *Immunity* 2006, **25**:885-894.

63. McGehee AM, Strijbis K, Guillen E, Eng T, Kirak O, Ploegh HL: **Ubiquitin-dependent control of class II MHC localization is dispensable for antigen presentation and antibody production**. *PLoS One* 2011, **6**:e18817.

64. Mohan JF, Unanue ER: **Unconventional recognition of peptides by T cells and the implications for autoimmunity**. *Nat Rev: Immunol* 2012, **12**:721-728.

65. Manoury B, Mazzeo D, Li DN, Billson J, Loak K, Benaroch P, Watts C: **Asparagine endopeptidase can initiate the removal of the MHC class II invariant chain chaperone**. *Immunity* 2003, **18**:489-498.

66. • Bergmann H, Yabas M, Short A, Miosge L, Barthel N, Teh CE, Roots CM, Bull KR, Jeelall Y, Horikawa K *et al.*: **B cell survival, surface BCR and BAFFR expression, CD74 metabolism, and CD8(-) dendritic cells require the intramembrane endopeptidase SPPL2A**. *J Exp Med* 2013, **210**:31-40.
Schneppenheim *et al.*; Beisner *et al.*; Bergmann *et al.*, identification of the endosomal intramembrane protease SPPL2a for the final cut in Ii and description of its function in APC development and immune responses.

67. Beisner DR, Langerak P, Parker AE, Dahlberg C, Otero FJ, Sutton SE, Poirot L, Barnes W, Young MA, Niessen S *et al.*: **The intramembrane protease Sppl2a is required for B cell and DC development and survival via cleavage of the invariant chain**. *J Exp Med* 2013, **210**:23-30.

68. Moss CX, Villadangos JA, Watts C: **Destructive potential of the aspartyl protease cathepsin D in MHC class II-restricted antigen processing**. *Eur J Immunol* 2005, **35**:3442-3451.

69. Manoury B, Mazzeo D, Fugger L, Viner N, Ponsford M, Streeter H, Mazza G, Wraith DC, Watts C: **Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP**. *Nat Immunol* 2002, **3**:169-174.

70. West LC, Grotzke JE, Cresswell P: **MHC class II-restricted presentation of the major house dust mite allergen Der p 1 Is GILT-dependent: implications for allergic asthma**. *PLoS One* 2013, **8**:e51343.

71. Rausch MP, Irvine KR, Antony PA, Restifo NP, Cresswell P, Hastings KT: **GILT accelerates autoimmunity to the melanoma antigen tyrosinase-related protein 1**. *J Immunol* 2010, **185**:2828-2835.

72. Singh R, Cresswell P: **Defective cross-presentation of viral antigens in GILT-free mice**. *Science* 2010, **328**:1394-1398.

73. Burster T: **Processing and regulation mechanisms within antigen presenting cells: a possibility for therapeutic modulation**. *Curr Pharmaceut Des* 2013, **19**:1029-1042.

74. Loss GE Jr, Sant AJ: **Invariant chain retains MHC class II molecules in the endocytic pathway**. *J Immunol* 1993, **150**:3187-3197.

75. Neefjes JJ, Ploegh HL: **Inhibition of endosomal proteolytic activity by leupeptin blocks surface expression of MHC class II molecules and their conversion to SDS resistance alpha beta heterodimers in endosomes**. *EMBO J* 1992, **11**:411-416.

76. Riese RJ, Wolf PR, Bromme D, Natkin LR, Villadangos JA, Ploegh HL, Chapman HA: **Essential role for cathepsin S in MHC class II—associated invariant chain processing and peptide loading**. *Immunity* 1996, **4**:357-366.

77. Saegusa K, Ishimaru N, Yanagi K, Arakaki R, Ogawa K, Saito I, Katunuma N, Hayashi Y: **Cathepsin S inhibitor prevents autoantigen presentation and autoimmunity**. *J Clin Invest* 2002, **110**:361-369.

78. Samokhin AO, Lythgo PA, Gauthier JY, Percival MD, Broemme D: **Pharmacological inhibition of cathepsin S decreases atherosclerotic lesions in apoe−/− mice**. *J Cardiovasc Pharmacol* 2010, **56**:98-105.

79. Holsinger LJ, Mocci S, Link JO, Elrod K, Green MJ, Booth R, Dalrymple SA: **Characterization of VBY-129, a cathepsin S inhibitor efficacious in a mouse model of psoriasis**. *J Invest Dermatol* 2009, **129** pp. S58–S58.

80. Nagler DK, Menard R: **Family C1 cysteine proteases: biological diversity or redundancy?** *Biol Chem* 2003, **8**:3847-4843.

81. Honey K, Duff M, Beers C, Brissette WH, Elliott EA, Peters C, Maric M, Cresswell P, Rudensky A:: **Cathepsin S regulates the expression of cathepsin L and the turnover of gamma-interferon-inducible lysosomal thiol reductase in B lymphocytes**. *J Biol Chem* 2001, **276**:22573-22578.

82. Phipps-Yonas H, Semik V, Hastings KT: **GILT expression in B cells diminishes cathepsin S steady-state protein expression and activity**. *Eur J Immunol* 2013, **43**:65-74.

83. van Kasteren SI, Berlin I, Colbert JD, Keane D, Ovaa H, Watts C: **A multifunctional protease inhibitor to regulate endolysosomal function**. *ACS Chem Biol* 2011, **6**:1198-1204.

84. Schneppenheim J, Dressel R, Huttl S, Lullmann-Rauch R, Engelke M, Dittmann K, Wienands J, Eskelinen EL, Hermans-Borgmeyer I, Fluhrer R *et al.*: **The intramembrane protease SPPL2a promotes B cell development and controls endosomal traffic by cleavage of the invariant chain**. *J Exp Med* 2013, **210**:41-58.

85. Fauq AH, Simpson K, Maharvi GM, Golde T, Das P: **A multigram chemical synthesis of the gamma-secretase inhibitor LY411575 and its diastereoisomers**. *Bioorg Med Chem Lett* 2007, **17**:6392-6395.

86. Nyborg AC, Jansen K, Ladd TB, Fauq A, Golde TE: **A signal peptide peptidase (SPP) reporter activity assay based on the cleavage of type II membrane protein substrates provides further evidence for an inverted orientation of the SPP active site relative to presenilin**. *J Biol Chem* 2004, **279**:43148-43156.

87. Dzhambazov B, Holmdahl M, Yamada H, Lu S, Vestberg M, Holm B, Johnell O, Kihlberg J, Holmdahl R: **The major T cell epitope on type II collagen is glycosylated in normal cartilage but modified by arthritis in both rats and humans**. *Eur J Immunol* 2005, **35**:357-366.

88. Hamazaki H, Hotta K: **Purification and characterization of an alpha-glucosidase specific for hydroxylysine-linked disaccharide of collagen**. *J Biol Chem* 1979, **254**:9682-9687.

89. Aggarwal A, Srivastava R, Agrawal S: **T cell responses to citrullinated self-peptides in patients with rheumatoid arthritis**. *Rheumatol Int* 2013, **33**:2359-2363.

90. Wilson RM, Danishefsky SJ: **A vision for vaccines built from fully synthetic tumor-associated antigens: from the laboratory to the clinic**. *J Am Chem Soc* 2013, **135**:14462-14472.

91. Zom GG, Khan S, Filippov DV, Ossendorp F: **TLR ligand-peptide conjugate vaccines: toward clinical application**. *Adv Immunol* 2012, **114**:177-201.

92. Khan S, Weterings JJ, Britten CM, de Jong AR, Graafland D, Melief CJ, van der Burg SH, van der Marel G, Overkleeft HS, Filippov DV *et al.*: **Chirality of TLR-2 ligand Pam3CysSK4 in fully synthetic peptide conjugates critically influences the induction of specific CD8$^+$ T-cells**. *Mol Immunol* 2009, **46**:1084-1091.

93. Khan S, Bijker MS, Weterings JJ, Tanke HJ, Adema GJ, van Hall T, Drijfhout JW, Melief CJ, Overkleeft HS, van der Marel GA *et al.*: **Distinct uptake mechanisms but similar intracellular processing of two different toll-like receptor ligand-peptide conjugates in dendritic cells**. *J Biol Chem* 2007, **282**:21145-21159.

94. Kenter GG, Welters MJP, Valentijn ARPM, Lowik MJG, Berends-van der Meer DMA, Vloon APG, Essahsah F, Fathers LM, Offringa R, Drijfhout JW *et al.*: **Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia**. *New Engl J Med* 2009, **361**:1838-1847.

95. Toebes M, Rodenko B, Ovaa H, Schumacher TN: **Generation of peptide MHC class I monomers and multimers through ligand exchange**. *Curr Protocols Immunol* 2009. Chapter 18: Unit 18 16.

96. Grotenbreg GM, Roan NR, Guillen E, Meijers R, Wang JH, Bell GW, Starnbach MN, Ploegh HL: **Discovery of CD8$^+$ T cell epitopes in**

***Chlamydia trachomatis* infection through use of caged class I MHC tetramers**. *Proc Natl Acad Sci USA* 2008, **105**:3831-3836.

97. Andersen RS, Kvistborg P, Frosig TM, Pedersen NW, Lyngaa R, Bakker AH, Shu CJ, Straten P, Schumacher TN, Hadrup SR: **Parallel detection of antigen-specific T cell responses by combinatorial encoding of MHC multimers**. *Nat Protocols* 2012, **7**:891-902.

98. Groll M, Berkers CR, Ploegh HL, Ovaa H: **Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome**. *Structure* 2006, **14**:451-456.

99. Kochan G, Krojer T, Harvey D, Fischer R, Chen L, Vollmar M, von Delft F, Kavanagh KL, Brown MA, Bowness P *et al.*: **Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis for N-terminal peptide trimming**. *Proc Natl Acad Sci USA* 2011, **108**:7745-7750.

100. Kim H, Lipscomb WN: **Differentiation and identification of the two catalytic metal binding sites in bovine lens leucine aminopeptidase by x-ray crystallography**. *Proc Natl Acad Sci USA* 1993, **90**:5006-5010.

101. Kim H, Lipscomb WN: **X-ray crystallographic determination of the structure of bovine lens leucine aminopeptidase complexed with amastatin: formulation of a catalytic mechanism featuring a gem-diolate transition state**. *Biochemistry* 1993, **32**:8465-8478.

102. Kim MJ, Yamamoto D, Matsumoto K, Inoue M, Ishida T, Mizuno H, Sumiya S, Kitamura K: **Crystal structure of papain-E64-c complex. Binding diversity of E64-c to papain S2 and S3 subsites**. *Biochem J* 1992, **287(Pt 3)**:797-803.

103. Dall E, Brandstetter H: **Mechanistic and structural studies on legumain explain its zymogenicity, distinct activation pathways, and regulation**. *Proc Natl Acad Sci USA* 2013, **110**:10940-10945.

104. Rodenko B, Toebes M, Hadrup SR, van Esch WJ, Molenaar AM, Schumacher TN, Ovaa H: **Generation of peptide-MHC class I complexes through UV-mediated ligand exchange**. *Nat Protocols* 2006, **1**:1120-1132.

105. Toebes M, Coccoris M, Bins A, Rodenko B, Gomez R, Nieuwkoop NJ, van de Kasteele W, Rimmelzwaan GF, Haanen JB, Ovaa H *et al.*: **Design and use of conditional MHC class I ligands**. *Nat Med* 2006, **12**:246-251.

106. Rodenko B, Toebes M, Celie PH, Perrakis A, Schumacher TN: **Ovaa H: Class I major histocompatibility complexes loaded by a periodate trigger**. *J Am Chem Soc* 2009, **131**:12305-12313.

107. Amore A, Wals K, Koekoek E, Hoppes R, Toebes M, Schumacher TN, Rodenko B, Ovaa H: **Development of a hypersensitive periodate-cleavable amino acid that is methionine- and disulfide-compatible and its application in MHC exchange reagents for T cell characterisation**. *Chembiochem* 2013, **14**:123-131.