HHS Public Access

Author manuscript

Curr Opin Immunol. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Curr Opin Immunol. 2016 June ; 40: 70–77. doi:10.1016/j.coi.2016.03.004.

The melting pot of the MHC II peptidome

Lawrence J. Stern[1] and **Laura Santambrogio**[2]

[1]Department of Pathology, Department of Biochemistry & Molecular Pharmacology, and Graduate Program in Immunology and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655

[2]Department of Pathology and Department of Microbiology & Immunology, Albert Einstein College of Medicine, NY 10461

Abstract

Recent advances in mass spectrometry technology have facilitated detailed examination of MHC-II immunopeptidomes, i.e. the repertoires of peptides bound to MHC-II molecules expressed in antigen presenting cells. These studies have deepened our view of MHC-II presentation. Other studies have broadened our view of pathways leading up to peptide loading. Here we review these recent studies in the context of earlier work on conventional and non-conventional MHC-II processing. The message that emerges is that sources of antigen beyond conventional endosomal processing of endocytosed proteins are important for generation of cellular immune responses to pathogens and maintenance of central and peripheral tolerance. The multiplicity of pathways results in a broad MHC II immunopeptidome that conveys the sampled environment to patrolling T cells.

Presentation of endogenous and exogenous antigens

From the very first characterizations of eluted peptides [1–3], MHC II molecules have been shown to sample both endogenous and exogenous antigens. The inclusion into routine mass spectrometry workflows of once-esoteric techniques such as ion trapping, electron-transfer dissociation, ion-mobility fractionation, and data-independent acquisition strategies has allowed several groups to characterize very large sets of naturally-processed peptides bound to MHC II molecules [4–7]. Such sets can comprise several thousands of individual peptides derived from hundreds of different proteins, and now rival (or even exceed) in terms of numbers of confidently sequenced eluted peptides the most detailed studies of MHC I molecules [8–10]. By combining several analysis strategies, Mommen et al were able to identify almost 14,000 peptides bound to three HLA-DR allotypes expressed by a myelo-monocytic leukemia cell line [4]. The peptides derived from almost 2000 different source proteins, and appear to represent the most complete MHC II peptidome to date. Bergseng et al characterized up to 7400 HLA-DQ-bound peptides from each of several EBV-transformed

B-lymphoblastoid cell lines, and used these to define peptide binding motifs for three celiac disease-associated DQ allotypes [5]. Similarly Sofron et al identified over 1000 peptides from the murine A20 lymphoma line, helping to refine the I-A^d motif [6]. In each of these studies, the majority of eluted peptides derived from endogenous proteins, with a substantial fraction (up to 20%) derived from exogenous proteins brought into the cell by endocytosis. Of the endogenous sources of peptides, membrane proteins from plasma membrane or endo/lysosomal compartments were well represented, but so were nuclear, mitochondrial, ER/golgi, and cytosolic compartments.

Some studies characterized immunopeptidomes of antigen presenting cells as isolated directly from tissues. These should more closely reflect the distribution of source proteins and in vivo peptide repertoire than do the cell lines studies described above. Sofron et al reported almost 3000 I-A^d binding peptides from BALB/c spleen, and a similar number of I-A^b-binding peptides from C57BL/6 spleen [6]. As in the cell line studies described above, and as described previously for I-A^b from murine splenocytes [11], peptides bound to MHC II in vitro derive from proteins present throughout the cell. Collado et al identified 247 different peptides in three samples of human thymus [12], extending a much earlier study of mouse thymus that showed generally similar sets of self-peptides presented in thymus and spleen [13]. A subsequent study from the same group identified, among the peptides presented in human thymus, two peptides derived from AIRE-dependent expression of prostate and axon specific antigens in medullary epithelial cells [14], directly confirming the role of AIRE in providing antigens for presentation by MHC II molecules as part of central tolerance mechanisms. Clement et al identified more than 3000 MHCII-bound peptides presented *in vivo* on dendritic cells from HLA-DR1-transgenic mice [7]. Among the ~10% of peptides identified in this study that derived from extracellular protein sources, several corresponded to peptides that were also identified in lymphatic fluid where they had been generated by extracellular tissue remodeling, cellular apoptosis, plasma membrane editing and complement/coagulation protease cascade.

Lymph as a source of antigen for MHCII presentation

Lymph derives from the extracellular fluid bathing all tissues in the body. It collects the products of metabolic and catabolic activities underway in parenchymal organs [15] and so it provides a snap shot of the cellular and extracellular proteome in physiological and pathological conditions. In addition to intact antigens, lymph contains many peptides, generated by extracellular matrix reprocessing, cell-surface receptor editing, and other processes including release of material generated by cell death pathways [16]. Clement et al compared peptides present in lymph and peptides eluted from conventional (splenic) dendritic cells, and in many cases found similar species, often with termini that matched known sites of extracellular processing [7]. For example, a peptide derived from complement 3 was found in identical form both in lymph and bound to HLA-DR1 presented on splenic dendritic cells. The source protein is present in many tissues including spleen but is not expressed in dendritic cells; moreover its processing is known to require complement 1 as part of the complement activation pathway. In vitro processing studies showed that the observed peptide termini were not generated by digestion of purified C3 with isolated endosomes or mixtures of cathepsins, although cleavage at many other sites was observed

[7]. These observations indicated that the dendritic cells were able to capture and present a pre-processed version of the epitope. Several other such peptides were identified, from gelsolin, thymosin β4, and collagen II [7]. Overall lymph-carried peptides were shown to be generated by a variety of processing pathways including cathepsins, caspases, granzymes, and matrix metalloproteases among others. In many cases the eluted peptides were highly sensitive to DM-mediated peptide exchange, supporting the idea that the pathway by which they were loaded and presented did not include DM-containing endosomes. One potential mechanism for loading lymph-derived peptides onto MHCII proteins could involve the cell surface [17] or shallow recycling compartments [18–20] (Figure 1). MHC II loading could be achieved through exchange with low affinity/low stability peptides or on empty MHC II molecules that are expressed on the surface of immature dendritic cells [21,22]. Peptide-free MHCII proteins convert between peptide-receptive and peptide-averse conformations [23–26], and receptive MHCII could readily bind peptide provided extracellularly [22]. Interestingly, certain class I MHC proteins are also relatively stable as peptide-free species [27], and it has been shown that APC expressing these proteins also are able to directly access soluble extracellular antigen [28].

MHC II loading of exogenous peptides delivered intrathymically [29], intravenously [30], or intraperitoneally [31] have been shown to be effective ways to achieve central and peripheral tolerance [30–39]. Such peptides likely are conveyed to dendritic cells via lymph. The role of migratory DC in transporting the injected peptides to the thymus to mediate negative selection [35] and to lymph nodes to mediate peripheral T cell anergy and Treg induction [36,40] also have been reported [35,41]. However, extracellularly processed peptides also can also be a target for autoimmune responses [42]. The balance between tolerance and autoimmunity likely is determined by differences in peptide MHC II binding affinity/stability, efficiency of thymic selection, Treg induction, peptide generation by *de novo* processing pathways, and nature of the presenting APC. It is likely that several different processing pathways are utilized to generate the full variety of self-peptides required to maintain effective central and peripheral tolerance.

Autophagy and the MHCII peptidome

Many peptides identified in MHCII peptidomes derive from proteins found outside the classic endosomal/lysosomal pathway. Indeed, peptides derived from nuclear and cytosolic proteins together comprised 25% to 55% of the total HLA-DR-bound peptide sequences identified in two recent high-resolution studies [4,7]. It is likely that these antigens intersected the MHCII antigen processing pathway via an autophagy pathway. Three forms of autophagy have been described in immune cells, macroautophagy, microautophagy, and chaperone-mediated autophagy [43]. Macroautophagy is a “bulk” form of transport characterized by the sequestration of cargo from the cytosol into double membrane vesicles called autophagosomes, which fuse with late endosomes (to generate amphisomes) or lysosomes (to generate autophagolysosomes) [43,44]. Through this pathway cytosolic organelles are disposed, mitochondria (mitophagy), ribosomes (ribophagy), protein aggregates (aggregophagy), as well as sequestered cytosolic proteins [45,46]. Whereas in most cell types macroautophagy is induced, notably by starvation, in DC there is a constitutive macrophautophagic flux to deliver cytosolic self-proteins for

immunosurveillance [47]. Pathogens also can be delivered to MHC II compartments through macroautophagy when the pathogens are present in the cytosol or when the autophagy machinery aids the fusion of the phagosome with endo-lysosomal compartments [48–50]. Macroautophagy-deficient mice have defective immunity to mycobacteria and decreased response to yellow fever virus and BCG vaccination [51–54]. Microautophagy derives from the inward invagination and pinching off of the late endosomal or lysosomal limiting membrane. In late endosomes, the process is ESCRT-mediated and the resulting vesicles contain soluble cytosolic proteins. The same vesicles can then be released as exosomes or degraded and release their cargo into the lumen of the late endosomal compartment [55]. Chaperone-mediated autophagy relies on hsc-70 recognition of selected cargo proteins (KFERQ-like motives) which are delivered to lysosomes through the LAMP-2A translocation complex [56]. Interestingly all forms of autophagy rely on the hsc-70 chaperone to transport cytosolic peptides, misfolded polypeptides, or ubiquitinated proteins into MHC II-containing organelles [43,57]. In DC a decrease in endosomal transport of cytosolic self-antigens has been observed upon knock down of macroautophagy and ESCRT-mediated microautophagy [55], in thymic epithelial cells macroautophagy has been shown to shape the T cell repertoire [58], and in a B cell line knock down of chaperone-mediated autophagy decreased MHC II presentation of a cytosolic protein [59]. Overall the three forms of autophagy are the major pathway by which cytosolic and organelle-derived self-proteins as well as certain pathogens are delivered to MHC II compartments [48].

Endosomal and non-endosomal processing pathways

Many studies over the last two decades have highlighted the role of non-canonical processing in the MHCII pathway. MHCII can acquire peptide in intracellular compartments other than the classical late endosomes and lysosomes rich in cathepsins and DM. Early work from several groups identified an early endosomal/plasma membrane compartment where peptide loading occurs on recycled MHC II molecules [20,28,60–62]. Processing in different compartments led to presentation of different epitopes [62–64]. In early endosomes, invariant chain could be removed from MHC II and exachanged for peptides or unfolded proteins in a cathepsin-independent, DM-independent manner [20]. Capture of peptides and proteins at the plasma membrane also has been reported [65–67]. The feasibility of a capture-first model for antigen processing was highlighted by recent work from Sadegh-Nasseri and Kim in their development of a mimimalist in vitro antigen processing system that coupled proteolysis and loading steps [68,69].

An important role for non-endosomal processing in generation of the MHC II immunopeptidome was emphasized by recent work by Bosch et al, in studies of dendritic cells with disrupted multivesicular bodies which nonetheless were able to process and load peptides for MHC II presentation via an early endosome or cell surface peptide loading pathway [70]. For at least some antigens, components of the MHC I processing pathway can be involved. Spencer et al showed recently that the profile of peptides eluted from I-A^b was substantially altered in splenocytes from TAP−/− or ERAAP−/− mice, with effects on CD4 T cell recognition of epitopes derived from Listeria or vaccinia virus infection [71]. Tewari et al earlier had identified E^d restricted influenza epitopes similarly dependent on proteasome and TAP-mediated processing [72]. How antigens processed by the MHC I

pathway intersect MHC II proteins is not clear, but processing machinery from both pathways can be found together in some cell types [73–75]. Altogether these studies delineate how diverse processing mechanisms in conventional late endosomes and elsewhere ensure that a broad peptidome representing many intracellular and extracellular proteins is presented by MHCII proteins.

DM-dependent and DM-independent loading

The ability of MHCII to load peptides in relatively shallow recycling compartments or at the cell surface suggests that loading can occur with little or no contribution from DM. Of course, many in vitro studies have shown that DM can stabilize peptide-free MHCII molecules and promote peptide exchange [76–83]. Recently, Yin et al reexamined this issue, and found that DM's role in stabilizing peptide-free HLA-DR was facilitative rather than obligatory [84]. Both the rate of conversion between receptive and averse conformations, and the rate of peptide loading to receptive molecules, were increased by DM, but these processes were observed to occur efficiently also in the absence of DM. In vitro peptide exchange clearly is facilitated by DM, but with different rate enhancements for different peptides [85–92]. Clement et al reported DM susceptibilities for some abundant peptides eluted from splenic DC, as part of their study on the contribution of lymph peptides to the MHC II peptidome [7]. A substantial fraction of the eluted peptides had rapid exchange rates even in the absence of DM. Thus some MHCII molecules at the surface or in shallow recycling compartments should be able to bind peptide even if DM were absent or inhibited by DO. In fact, mice lacking DM are capable of loading exogenously added peptides despite allele-dependent increases in CLIP levels and compromised ability to process intact antigen [93–95], The original phenotype described for DM-deficient cells was alteration in the ability to present native exogenous protein antigens but peptides derived from these same proteins were presented effectively [96]. Even in DM-sufficient mice, removal of CLIP and peptide loading in recycling or early endosomes can occur independently of DM [20,62]. In dendritic cells, processing in DM-dependent or DM-independent pathways can be regulated by TLR ligands and type I interferon [97,98]. Different epitopes are targeted in DM-deficient and DM-sufficient mice [64,99,100], although DM can be required for pathogen clearance [101]. Thus, MHC II peptide loading can occur in many scenarios in the absence of DM, and a lack of DM expression should not be considered to rule out MHC II presentation. An important role for DM-independent antigen processing in the antiviral response was demonstrated recently by Miller et al, who showed that a majority of I-Ab restricted epitopes in the C57BL/6 response to influenza were processed by non-canonical pathways including those not dependent on DM or invariant chain, with considerable diversity in the pathways accessed by particular epitopes [102]. Overall, in several scenarios, DM-independent loading can broaden the MHC II peptidomes presented at the cell surface.

Concluding Remarks

Peptides destined for loading onto MHC II proteins are generated by both endosomal and non-endosomal processing, and derive from proteins present in a wide variety of intracellular and extracellular compartments. Peptides produced by non-endosomal processing have been shown to have key roles in maintenance of peripheral tolerance and

immunity to pathogens. Continuing work is needed to elucidate how the MHCII peptidomes changes during inflammatory conditions. Many peptides identified in the MHCII immunopeptidome studies carry post-translational modifications such as acetylation, amidation/deamidation, carboxylation, citrullation, hydroxylation/dehydration, methylation/demethylation, oxidation, and phosphorylation [7,9,103,104]. How these modifications impact tolerance and immune recognition in many cases remains to be determined. Because of the increased role of DM in MHC II processing under inflammatory conditions, the abundance of many self-peptides displayed at the cell surface is likely to change, with implications for the regulation of self-tolerance. Methodology for the reliable quantitative measurement of peptide abundances in complex samples is available, and very recently has begun to be applied to immunopeptidomes [105,106]. Thus we hope to learn in even greater detail about the distribution of peptides in the MHC II melting pot, and how they regulate T cell activity.

Acknowledgments

Supported by grants AI038996 (LJS) and AG045223 (LS).

References and Recommended Reading

References of • special or •• outstanding interest are noted.

1. Chicz RM, Urban RG, Lane WS, Gorga JC, Stern LJ, Vignali DA, Strominger JL. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. Nature. 1992; 358:764–768. [PubMed: 1380674]

2. Hunt DF, Michel H, Dickinson TA, Shabanowitz J, Cox AL, Sakaguchi K, Appella E, Grey HM, Sette A. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-Ad. Science. 1992; 256:1817–1820. [PubMed: 1319610]

3. Rudensky A, Preston-Hurlburt P, Hong SC, Barlow A, Janeway CA Jr. Sequence analysis of peptides bound to MHC class II molecules. Nature. 1991; 353:622–627. [PubMed: 1656276]

4••. Mommen GP, Marino F, Meiring HD, Poelen MC, van Gaans-van den Brink JA, Mohammed S, Heck AJ, van Els CA. Sampling from the proteome to the HLA-DR ligandome proceeds via high specificity. Mol Cell Proteomics. 2016 High-density combined peptidome for HLA-DRB1*10/DRB1*11/DRB3*01 from MUTZ3 cells, a myelo-monocytic leukemia line serving as a monocyte/dendritic cell model, with analysis of protease activity responsible for peptide processing.

5••. Bergseng E, Dorum S, Arntzen MO, Nielsen M, Nygard S, Buus S, de Souza GA, Sollid LM. Different binding motifs of the celiac disease-associated HLA molecules DQ2.5, DQ2.2, and DQ7. 5 revealed by relative quantitative proteomics of endogenous peptide repertoires. Immunogenetics. 2015; 67:73–84. High-density peptidomes for HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*02:01/DQB1*02:02, and HLA-DQA1*05/DQB1*03:01, derived from analysis of nine B-lymphoblastoid cell lines, with relative quantitative analysis of differences between peptidomes. [PubMed: 25502872]

6••. Sofron A, Ritz D, Neri D, Fugmann T. High-resolution analysis of the murine MHC class II immunopeptidome. Eur J Immunol. 2016; 46:319–328. High-density peptidomes for murine I-Ad from A20 lymphoma cell line, I-Ad from BALB/c spleen, and I-Ab from C57BL/6 spleen. I-Ad peptide binding motif improved. [PubMed: 26495903]

7••. Clement CC, Becerra A, Yin L, Zolla V, Huang L, Merlin S, Follenzi A, Shaffer SA, Stern LJ, Santambrogio L. The Dendritic Cell MHC II Peptidome Derives from a Variety of Processing Pathways and Includes Peptides with a Broad Spectrum of HLA-DM Sensitivity. J Biol Chem. 2016 High-density peptidome for HLA-DRB1*01:01 as expressed *in vivo* by dendritic cells of a

HLA-transgenic mouse. First report showing that lymph peptides contribute to the MHC II peptidome, with analysis of proteases responsible for generation of particular eptiopes.

8. Schellens IM, Hoof I, Meiring HD, Spijkers SN, Poelen MC, van Gaans-van den Brink JA, van der Poel K, Costa AI, van Els CA, van Baarle D, et al. Comprehensive Analysis of the Naturally Processed Peptide Repertoire: Differences between HLA-A and B in the Immunopeptidome. PLoS One. 2015; 10:e0136417. [PubMed: 26375851]
9. Mommen GP, Frese CK, Meiring HD, van Gaans-van den Brink J, de Jong AP, van Els CA, Heck AJ. Expanding the detectable HLA peptide repertoire using electron-transfer/higher-energy collision dissociation (EThcD). Proc Natl Acad Sci U S A. 2014; 111:4507–4512. [PubMed: 24616531]
10. Bassani-Sternberg M, Pletscher-Frankild S, Jensen LJ, Mann M. Mass spectrometry of human leukocyte antigen class I peptidomes reveals strong effects of protein abundance and turnover on antigen presentation. Mol Cell Proteomics. 2015; 14:658–673. [PubMed: 25576301]
11. Bozzacco L, Yu H, Zebroski HA, Dengjel J, Deng H, Mojsov S, Steinman RM. Mass spectrometry analysis and quantitation of peptides presented on the MHC II molecules of mouse spleen dendritic cells. J Proteome Res. 2011; 10:5016–5030. [PubMed: 21913724]
12. Collado JA, Alvarez I, Ciudad MT, Espinosa G, Canals F, Pujol-Borrell R, Carrascal M, Abian J, Jaraquemada D. Composition of the HLA-DR-associated human thymus peptidome. Eur J Immunol. 2013; 43:2273–2282. [PubMed: 23719902]
13. Marrack P, Ignatowicz L, Kappler JW, Boymel J, Freed JH. Comparison of peptides bound to spleen and thymus class II. J Exp Med. 1993; 178:2173–2183. [PubMed: 8245790]
14••. Alvarez I, Collado JA, Colobran R, Carrascal M, Ciudad MT, Canals F, James EA, Kwok WW, Gartner M, Kyewski B, et al. Central T cell tolerance: Identification of tissue-restricted autoantigens in the thymus HLA-DR peptidome. J Autoimmun. 2015; 60:12–19. Detection of two tissue-specific antigens in the human thymus HLA-DR peptidome. Expression of both epitopes was restricted to medullary thymic epithelium, and one was dependent on AIRE. [PubMed: 25911201]
15. Hansen KC, D'Alessandro A, Clement CC, Santambrogio L. Lymph formation, composition and circulation: a proteomics perspective. Int Immunol. 2015; 27:219–227. [PubMed: 25788586]
16. Clement CC, Cannizzo ES, Nastke MD, Sahu R, Olszewski W, Miller NE, Stern LJ, Santambrogio L. An expanded self-antigen peptidome is carried by the human lymph as compared to the plasma. PLoS One. 2010; 5:e9863. [PubMed: 20360855]
17. Santambrogio L, Sato AK, Carven GJ, Belyanskaya SL, Strominger JL, Stern LJ. Extracellular antigen processing and presentation by immature dendritic cells. Proc Natl Acad Sci U S A. 1999; 96:15056–15061. [PubMed: 10611337]
18. Amria S, Hajiaghamohseni LM, Harbeson C, Zhao D, Goldstein O, Blum JS, Haque A. HLA-DM negatively regulates HLA-DR4-restricted collagen pathogenic peptide presentation and T cell recognition. Eur J Immunol. 2008; 38:1961–1970. [PubMed: 18506881]
19. Pathak SS, Lich JD, Blum JS. Cutting edge: editing of recycling class II:peptide complexes by HLA-DM. J Immunol. 2001; 167:632–635. [PubMed: 11441064]
20. Villadangos JA, Driessen C, Shi GP, Chapman HA, Ploegh HL. Early endosomal maturation of MHC class II molecules independently of cysteine proteases and H-2DM. EMBO J. 2000; 19:882–891. [PubMed: 10698930]
21. Santambrogio L, Sato AK, Fischer FR, Dorf ME, Stern LJ. Abundant empty class II MHC molecules on the surface of immature dendritic cells. Proc Natl Acad Sci U S A. 1999; 96:15050–15055. [PubMed: 10611336]
22. Potolicchio I, Chitta S, Xu X, Fonseca D, Crisi G, Horejsi V, Strominger JL, Stern LJ, Raposo G, Santambrogio L. Conformational variation of surface class II MHC proteins during myeloid dendritic cell differentiation accompanies structural changes in lysosomal MIIC. J Immunol. 2005; 175:4935–4947. [PubMed: 16210595]
23. Natarajan SK, Assadi M, Sadegh-Nasseri S. Stable peptide binding to MHC class II molecule is rapid and is determined by a receptive conformation shaped by prior association with low affinity peptides. J Immunol. 1999; 162:4030–4036. [PubMed: 10201925]

24. Rabinowitz JD, Vrljic M, Kasson PM, Liang MN, Busch R, Boniface JJ, Davis MM, McConnell HM. Formation of a highly peptide-receptive state of class II MHC. Immunity. 1998; 9:699–709. [PubMed: 9846491]
25. Rupp B, Gunther S, Makhmoor T, Schlundt A, Dickhaut K, Gupta S, Choudhary I, Wiesmuller KH, Jung G, Freund C, et al. Characterization of structural features controlling the receptiveness of empty class II MHC molecules. PLoS One. 2011; 6:e18662. [PubMed: 21533180]
26. Joshi RV, Zarutskie JA, Stern LJ. A three-step kinetic mechanism for peptide binding to MHC class II proteins. Biochemistry. 2000; 39:3751–3762. [PubMed: 10736175]
27. Schumacher TN, Heemels MT, Neefjes JJ, Kast WM, Melief CJ, Ploegh HL. Direct binding of peptide to empty MHC class I molecules on intact cells and in vitro. Cell. 1990; 62:563–567. [PubMed: 2199065]
28. Eisen HN, Hou XH, Shen C, Wang K, Tanguturi VK, Smith C, Kozyrytska K, Nambiar L, McKinley CA, Chen J, et al. Promiscuous binding of extracellular peptides to cell surface class I MHC protein. Proc Natl Acad Sci U S A. 2012; 109:4580–4585. [PubMed: 22403068]
29. Oluwole SF, Jin MX, Chowdhury NC, Engelstad K, Ohajekwe OA, Hardy MA. Evidence for the role of host antigen-presenting cells in the induction of specific unresponsiveness to allografts by intrathymic inoculation of allopeptides. Transplant Proc. 1995; 27:132–133. [PubMed: 7878898]
30. Liblau RS, Tisch R, Shokat K, Yang X, Dumont N, Goodnow CC, McDevitt HO. Intravenous injection of soluble antigen induces thymic and peripheral T-cells apoptosis. Proc Natl Acad Sci U S A. 1996; 93:3031–3036. [PubMed: 8610163]
31. Bonasio R, Scimone ML, Schaerli P, Grabie N, Lichtman AH, von Andrian UH. Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus. Nat Immunol. 2006; 7:1092–1100. [PubMed: 16951687]
32. Campbell JD, Buckland KF, McMillan SJ, Kearley J, Oldfield WL, Stern LJ, Gronlund H, van Hage M, Reynolds CJ, Boyton RJ, et al. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. J Exp Med. 2009; 206:1535–1547. [PubMed: 19528258]
33. Chowdhury NC, Jin MX, Hardy MA, Oluwole SF. Donor-specific unresponsiveness to murine cardiac allografts induced by intrathymic-soluble alloantigens is dependent on alternate pathway of antigen presentation. J Surg Res. 1995; 59:91–96. [PubMed: 7630143]
34. Gallegos AM, Bevan MJ. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. J Exp Med. 2004; 200:1039–1049. [PubMed: 15492126]
35. Goldschneider I, Cone RE. A central role for peripheral dendritic cells in the induction of acquired thymic tolerance. Trends Immunol. 2003; 24:77–81. [PubMed: 12547504]
36. Oluwole SF, Jin MX, Chowdhury NC, Engelstad K, Ohajekwe OA, James T. Induction of peripheral tolerance by intrathymic inoculation of soluble alloantigens: evidence for the role of host antigen-presenting cells and suppressor cell mechanism. Cell Immunol. 1995; 162:33–41. [PubMed: 7704908]
37. Shimomura K, Hardy MA, Oluwole SF. Tolerance induction to cardiac allografts by simultaneous or sequential intrathymic inoculation of disparate alloantigens. Transplantation. 1995; 60:806–811. [PubMed: 7482739]
38. Zal T, Volkmann A, Stockinger B. Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. J Exp Med. 1994; 180:2089–2099. [PubMed: 7964486]
39. Martin S, Bevan MJ. Antigen-specific and nonspecific deletion of immature cortical thymocytes caused by antigen injection. Eur J Immunol. 1997; 27:2726–2736. [PubMed: 9368633]
40. Idoyaga J, Fiorese C, Zbytnuik L, Lubkin A, Miller J, Malissen B, Mucida D, Merad M, Steinman RM. Specialized role of migratory dendritic cells in peripheral tolerance induction. J Clin Invest. 2013; 123:844–854. [PubMed: 23298832]
41. Donskoy E, Goldschneider I. Two developmentally distinct populations of dendritic cells inhabit the adult mouse thymus: demonstration by differential importation of hematogenous precursors under steady state conditions. J Immunol. 2003; 170:3514–3521. [PubMed: 12646612]
42. Lovitch SB, Walters JJ, Gross ML, Unanue ER. APCs present A beta(k)-derived peptides that are autoantigenic to type B T cells. J Immunol. 2003; 170:4155–4160. [PubMed: 12682247]

43. Cuervo AM, Macian F. Autophagy and the immune function in aging. Curr Opin Immunol. 2014; 29:97–104. [PubMed: 24929664]
44. Hamasaki M, Shibutani ST, Yoshimori T. Up-to-date membrane biogenesis in the autophagosome formation. Curr Opin Cell Biol. 2013; 25:455–460. [PubMed: 23578367]
45. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. Curr Opin Cell Biol. 2010; 22:124–131. [PubMed: 20034776]
46. Bernard A, Jin M, Xu Z, Klionsky DJ. A large-scale analysis of autophagy-related gene expression identifies new regulators of autophagy. Autophagy. 2015; 11:2114–2122. [PubMed: 26649943]
47. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. Immunity. 2007; 26:79–92. [PubMed: 17182262]
48. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol. 2013; 13:722–737. [PubMed: 24064518]
49. Bradfute SB, Castillo EF, Arko-Mensah J, Chauhan S, Jiang S, Mandell M, Deretic V. Autophagy as an immune effector against tuberculosis. Curr Opin Microbiol. 2013; 16:355–365. [PubMed: 23790398]
50. Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell. 2005; 120:159–162. [PubMed: 15680321]
51. Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, Jiang S, Delgado-Vargas M, Timmins GS, Bhattacharya D, Yang H, et al. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. Proc Natl Acad Sci U S A. 2012; 109:E3168–3176. [PubMed: 23093667]
52. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell. 2012; 150:803–815. [PubMed: 22901810]
53. Ravindran R, Khan N, Nakaya HI, Li S, Loebbermann J, Maddur MS, Park Y, Jones DP, Chappert P, Davoust J, et al. Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation. Science. 2014; 343:313–317. [PubMed: 24310610]
54. Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL Jr, Eissa NT. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. Nat Med. 2009; 15:267–276. [PubMed: 19252503]
55. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, Potolicchio I, Nieves E, Cuervo AM, Santambrogio L. Microautophagy of cytosolic proteins by late endosomes. Dev Cell. 2011; 20:131–139. [PubMed: 21238931]
56. Orenstein SJ, Cuervo AM. Chaperone-mediated autophagy: molecular mechanisms and physiological relevance. Semin Cell Dev Biol. 2010; 21:719–726. [PubMed: 20176123]
57. Deffit SN, Blum JS. A central role for HSC70 in regulating antigen trafficking and MHC class II presentation. Mol Immunol. 2015; 68:85–88. [PubMed: 25953005]
58. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. Nature. 2008; 455:396–400. [PubMed: 18701890]
59. Zhou D, Li P, Lin Y, Lott JM, Hislop AD, Canaday DH, Brutkiewicz RR, Blum JS. Lamp-2a facilitates MHC class II presentation of cytoplasmic antigens. Immunity. 2005; 22:571–581. [PubMed: 15894275]
60. Haque MA, Hawes JW, Blum JS. Cysteinylation of MHC class II ligands: peptide endocytosis and reduction within APC influences T cell recognition. J Immunol. 2001; 166:4543–4551. [PubMed: 11254711]
61. Lovitch SB, Pu Z, Unanue ER. Amino-terminal flanking residues determine the conformation of a peptide-class II MHC complex. J Immunol. 2006; 176:2958–2968. [PubMed: 16493054]
62. Pu Z, Lovitch SB, Bikoff EK, Unanue ER. T cells distinguish MHC-peptide complexes formed in separate vesicles and edited by H2-DM. Immunity. 2004; 20:467–476. [PubMed: 15084275]
63. Griffin JP, Chu R, Harding CV. Early endosomes and a late endocytic compartment generate different peptide-class II MHC complexes via distinct processing mechanisms. J Immunol. 1997; 158:1523–1532. [PubMed: 9029086]

64. Nanda NK, Bikoff EK. DM peptide-editing function leads to immunodominance in CD4 T cell responses in vivo. J Immunol. 2005; 175:6473–6480. [PubMed: 16272301]

65. Vergelli M, Pinet V, Vogt AB, Kalbus M, Malnati M, Riccio P, Long EO, Martin R. HLA-DR-restricted presentation of purified myelin basic protein is independent of intracellular processing. Eur J Immunol. 1997; 27:941–951. [PubMed: 9130648]

66. Pinet V, Vergelli M, Martin R, Bakke O, Long EO. Antigen presentation mediated by recycling of surface HLA-DR molecules. Nature. 1995; 375:603–606. [PubMed: 7540726]

67. Nygard NR, Giacoletto KS, Bono C, Gorka J, Kompelli S, Schwartz BD. Peptide binding to surface class II molecules is the major pathway of formation of immunogenic class II-peptide complexes for viable antigen presenting cells. J Immunol. 1994; 152:1082–1093. [PubMed: 8301118]

68•. Kim A, Hartman IZ, Poore B, Boronina T, Cole RN, Song N, Ciudad MT, Caspi RR, Jaraquemada D, Sadegh-Nasseri S. Divergent paths for the selection of immunodominant epitopes from distinct antigenic sources. Nat Commun. 2014; 5:5369. A reductionist cell-free system of MHC II, DM, and cathepsin was used to study processing of pathogen-derived proteins and autoantigens. Immunodominance patterns were related to differential susceptibility to cathepsins or resistance to DM-mediated dissociation. [PubMed: 25413013]

69. Sadegh-Nasseri S, Kim A. Exogenous antigens bind MHC class II first, and are processed by cathepsins later. Mol Immunol. 2015; 68:81–84. [PubMed: 26254987]

70. Bosch B, Berger AC, Khandelwal S, Heipertz EL, Scharf B, Santambrogio L, Roche PA. Disruption of multivesicular body vesicles does not affect major histocompatibility complex (MHC) class II-peptide complex formation and antigen presentation by dendritic cells. J Biol Chem. 2013; 288:24286–24292. [PubMed: 23846690]

71. Spencer CT, Dragovic SM, Conant SB, Gray JJ, Zheng M, Samir P, Niu X, Moutaftsi M, Van Kaer L, Sette A, et al. Sculpting MHC class II-restricted self and non-self peptidome by the class I Ag-processing machinery and its impact on Th-cell responses. Eur J Immunol. 2013; 43:1162–1172. [PubMed: 23386199]

72. Tewari MK, Sinnathamby G, Rajagopal D, Eisenlohr LC. A cytosolic pathway for MHC class II-restricted antigen processing that is proteasome and TAP dependent. Nat Immunol. 2005; 6:287–294. [PubMed: 15711549]

73. Adiko AC, Babdor J, Gutierrez-Martinez E, Guermonprez P, Saveanu L. Intracellular Transport Routes for MHC I and Their Relevance for Antigen Cross-Presentation. Front Immunol. 2015; 6:335. [PubMed: 26191062]

74. Segura E, Amigorena S. Cross-Presentation in Mouse and Human Dendritic Cells. Adv Immunol. 2015; 127:1–31. [PubMed: 26073982]

75. Schuette V, Burgdorf S. The ins-and-outs of endosomal antigens for cross-presentation. Curr Opin Immunol. 2014; 26:63–68. [PubMed: 24556402]

76. Denzin LK, Hammond C, Cresswell P. HLA-DM interactions with intermediates in HLA-DR maturation and a role for HLA-DM in stabilizing empty HLA-DR molecules. J Exp Med. 1996; 184:2153–2165. [PubMed: 8976171]

77. Denzin LK, Cresswell P. HLA-DM induces CLIP dissociation from MHC class II alpha beta dimers and facilitates peptide loading. Cell. 1995; 82:155–165. [PubMed: 7606781]

78. Kropshofer H, Arndt SO, Moldenhauer G, Hammerling GJ, Vogt AB. HLA-DM acts as a molecular chaperone and rescues empty HLA-DR molecules at lysosomal pH. Immunity. 1997; 6:293–302. [PubMed: 9075930]

79. Vogt AB, Kropshofer H, Moldenhauer G, Hammerling GJ. Kinetic analysis of peptide loading onto HLA-DR molecules mediated by HLA-DM. Proc Natl Acad Sci U S A. 1996; 93:9724–9729. [PubMed: 8790398]

80. Sloan VS, Cameron P, Porter G, Gammon M, Amaya M, Mellins E, Zaller DM. Mediation by HLA-DM of dissociation of peptides from HLA-DR. Nature. 1995; 375:802–806. [PubMed: 7596415]

81. Zarutskie JA, Busch R, Zavala-Ruiz Z, Rushe M, Mellins ED, Stern LJ. The kinetic basis of peptide exchange catalysis by HLA-DM. Proc Natl Acad Sci U S A. 2001; 98:12450–12455. [PubMed: 11606721]

82. 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. [PubMed: 23260142]

83. Anders AK, Call MJ, Schulze MS, Fowler KD, Schubert DA, Seth NP, Sundberg EJ, Wucherpfennig KW. HLA-DM captures partially empty HLA-DR molecules for catalyzed removal of peptide. Nat Immunol. 2011; 12:54–61. [PubMed: 21131964]

84•. Yin L, Maben ZJ, Becerra A, Stern LJ. Evaluating the Role of HLA-DM in MHC Class II-Peptide Association Reactions. J Immunol. 2015; 195:706–716. Comprehensive kinetic study of the role of DM in promoting peptide binding to MHC II and converting MHC II between peptide-receptive and peptide-averse forms. [PubMed: 26062997]

85. Fallang LE, Roh S, Holm A, Bergseng E, Yoon T, Fleckenstein B, Bandyopadhyay A, Mellins ED, Sollid LM. Complexes of two cohorts of CLIP peptides and HLA-DQ2 of the autoimmune DR3-DQ2 haplotype are poor substrates for HLA-DM. J Immunol. 2008; 181:5451–5461. [PubMed: 18832702]

86. Belmares MP, Busch R, Wucherpfennig KW, McConnell HM, Mellins ED. Structural factors contributing to DM susceptibility of MHC class II/peptide complexes. J Immunol. 2002; 169:5109–5117. [PubMed: 12391227]

87•. Zhou Z, Reyes-Vargas E, Escobar H, Rudd B, Rockwood AL, Delgado JC, He X, Jensen PE. Type 1 diabetes associated HLA-DQ2 and DQ8 molecules are relatively resistant to HLA-DM mediated release of invariant chain-derived CLIP peptides. Eur J Immunol. 2015 Diabetes-associated HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*03:01/DQB1*03:02, and corresponding trans-heterodimers are relatively resistant to DM-mediated exchange, as shown by mass spectrometry of peptides eluted from transfected 293T cells and peptide exchange studies with purified soluble proteins.

88. Weber DA, Evavold BD, Jensen PE. Enhanced dissociation of HLA-DR-bound peptides in the presence of HLA-DM. Science. 1996; 274:618–620. [PubMed: 8849454]

89. Lazarski CA, Chaves FA, Sant AJ. The impact of DM on MHC class II-restricted antigen presentation can be altered by manipulation of MHC-peptide kinetic stability. J Exp Med. 2006; 203:1319–1328. [PubMed: 16682499]

90. Nanda NK, Sant AJ. DM determines the cryptic and immunodominant fate of T cell epitopes. J Exp Med. 2000; 192:781–788. [PubMed: 10993909]

91••. Yin L, Trenh P, Guce A, Wieczorek M, Lange S, Sticht J, Jiang W, Bylsma M, Mellins ED, Freund C, et al. Susceptibility to HLA-DM protein is determined by a dynamic conformation of major histocompatibility complex class II molecule bound with peptide. J Biol Chem. 2014; 289:23449–23464. Biochemcial and biophysical study of why some MHC II-peptide complexes are resistant to DM whereas others are susceptible. Conformational flexibility rather than sequence or pocket occupancy was identified as the key factor determining susceptibility to DM-mediated peptide exchange. [PubMed: 25002586]

92. Yin L, Calvo-Calle JM, Dominguez-Amorocho O, Stern LJ. HLA-DM constrains epitope selection in the human CD4 T cell response to vaccinia virus by favoring the presentation of peptides with longer HLA-DM-mediated half-lives. J Immunol. 2012; 189:3983–3994. [PubMed: 22966084]

93. Tourne S, Miyazaki T, Wolf P, Ploegh H, Benoist C, Mathis D. Functionality of major histocompatibility complex class II molecules in mice doubly deficient for invariant chain and H-2M complexes. Proc Natl Acad Sci U S A. 1997; 94:9255–9260. [PubMed: 9256469]

94. Martin WD, Hicks GG, Mendiratta SK, Leva HI, Ruley HE, Van Kaer L. H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. Cell. 1996; 84:543–550. [PubMed: 8598041]

95. Koonce CH, Wutz G, Robertson EJ, Vogt AB, Kropshofer H, Bikoff EK. DM loss in k haplotype mice reveals isotype-specific chaperone requirements. J Immunol. 2003; 170:3751–3761. [PubMed: 12646641]

96. Mellins E, Smith L, Arp B, Cotner T, Celis E, Pious D. Defective processing and presentation of exogenous antigens in mutants with normal HLA class II genes. Nature. 1990; 343:71–74. [PubMed: 1967485]

97. Lovitch SB, Esparza TJ, Schweitzer G, Herzog J, Unanue ER. Activation of type B T cells after protein immunization reveals novel pathways of in vivo presentation of peptides. J Immunol. 2007; 178:122–133. [PubMed: 17182547]

98. Strong BS, Unanue ER. Presentation of type B peptide-MHC complexes from hen egg white lysozyme by TLR ligands and type I IFNs independent of H2-DM regulation. J Immunol. 2011; 187:2193–2201. [PubMed: 21788443]

99. Kamala T, Nanda NK. Protective response to Leishmania major in BALB/c mice requires antigen processing in the absence of DM. J Immunol. 2009; 182:4882–4890. [PubMed: 19342667]

100. Menges PR, Jenks SA, Bikoff EK, Friedmann DR, Knowlden ZA, Sant AJ. An MHC class II restriction bias in CD4 T cell responses toward I-A is altered to I-E in DM-deficient mice. J Immunol. 2008; 180:1619–1633. [PubMed: 18209058]

101. Nepal RM, Vesosky B, Turner J, Bryant P. DM, but not cathepsin L, is required to control an aerosol infection with Mycobacterium tuberculosis. J Leukoc Biol. 2008; 84:1011–1018. [PubMed: 18591414]

102••. Miller MA, Ganesan AP, Luckashenak N, Mendonca M, Eisenlohr LC. Endogenous antigen processing drives the primary CD4+ T cell response to influenza. Nat Med. 2015; 21:1216–1222. The CD4+ T cell response that protects C57BL/6 mice from influenza infection is driven principally by unconventional MHC II processing pathways. [PubMed: 26413780]

103. Depontieu FR, Qian J, Zarling AL, McMiller TL, Salay TM, Norris A, English AM, Shabanowitz J, Engelhard VH, Hunt DF, et al. Identification of tumor-associated, MHC class II-restricted phosphopeptides as targets for immunotherapy. Proc Natl Acad Sci U S A. 2009; 106:12073–12078. [PubMed: 19581576]

104. Engelhard VH, Altrich-Vanlith M, Ostankovitch M, Zarling AL. Post-translational modifications of naturally processed MHC-binding epitopes. Curr Opin Immunol. 2006; 18:92–97. [PubMed: 16343885]

105. Caron E, Espona L, Kowalewski DJ, Schuster H, Ternette N, Alpizar A, Schittenhelm RB, Ramarathinam SH, Lindestam Arlehamn CS, Chiek Koh C, et al. An open-source computational and data resource to analyze digital maps of immunopeptidomes. Elife. 2015; 4

106. Croft NP, Purcell AW, Tscharke DC. Quantifying epitope presentation using mass spectrometry. Mol Immunol. 2015; 68:77–80. [PubMed: 26118903]

107••. Jiang W, Strohman MJ, Somasundaram S, Ayyangar S, Hou T, Wang N, Mellins ED. pH-susceptibility of HLA-DO tunes DO/DM ratios to regulate HLA-DM catalytic activity. Sci Rep. 2015; 5:17333. Catalytically active free DM is released from inactive DO-DM complexes at late endosomal/lysosomal pH due to irreversible acid-promoted DO release and denaturation. [PubMed: 26610428]

Highlights

- MHCII present antigens from a variety of intracellular and extracellular sources
- Endosomal and non-endosomal processing pathways contribute to the MHCII peptidome
- Lymphatic fluid is a rich source of pre-processed antigens for MHCII presentation
- Multiple processing pathways promote presentation of a broad repertoire of peptides
- Presentation of a broad peptide repertoire might be important for tolerance

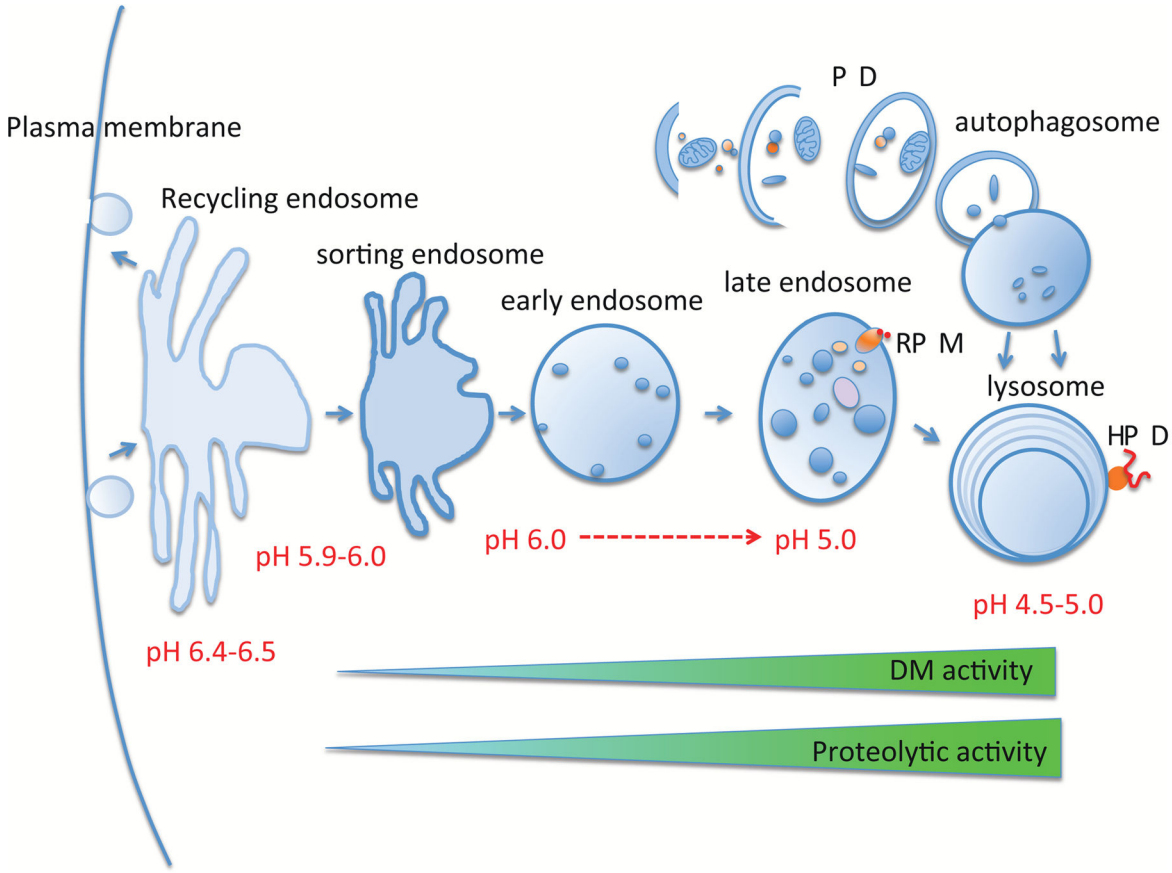

Fig 1.

Intracellular sites for conventional and non-conventional loading onto MHC II proteins. Extracellular antigens or cell-surface proteins can enter the MHC II pathway through a conventional route of endocytosis followed by proteolysis and MHC II loading in late endosomes or lysosomes. Alternate pathways for antigen entry include macroautophagy (MA), endosomal microautophagy (eMI), or chaperone-mediated autophagy (CMI), which bring proteins into late endosomes or lysosomes for processing and loading. Antigens can be loaded onto MHC II proteins also at the cell surface or in recycling, sorting, or early endosomes. Because of lower proteolytic activity in these compartments this pathway is likely to be more important for preprocessed peptides, for example from lymph, or for unfolded proteins. DM activity increases with endosomal maturation, in part because of DO dissociation from DM at low pH [107], and so DM-sensitive antigens are more likely to be loaded in early compartments, and loading in late compartments more likely to be restricted to DM-resistant antigens.