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Class II MHC Antigen Processing in Immune Tolerance and Inflammation

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Abstract

Presentation of peptide antigens by MHC-II proteins is prerequisite to effective CD4 T cell tolerance to self and to recognition of foreign antigens. Antigen uptake and processing pathways as well as expression of the peptide exchange factors HLA-DM and HLA-DO differ among the various professional and non-professional antigen-presenting cells and are modulated by cell developmental state and activation. Recent studies have highlighted the importance of these cell-specific factors in controlling the source and breadth of peptides presented by MHC-II under different conditions. During inflammation, increased presentation of selected self-peptides has implications for maintenance of peripheral tolerance and autoimmunity.

Keywords

Major Histocompatibility Complex; MHC protein; Antigen presentation; Immuno-peptidome

Introduction

Presentation of peptide antigens by class II Major Histocompatibility Complex (MHC-II) molecules is critical for maintenance of self-tolerance and for initiation of effective immune responses. Immunological tolerance to antigens presented on MHC-II molecules begins at the selection stage of T cell development in the thymus and continues in the periphery. CD4 T cells necessarily interact with self-ligands presented by MHC-II molecules for positive selection in the thymus as well as require tonic stimulation by MHC-II-peptide complexes in the periphery for survival. During the process of negative selection in the thymus, development of T cells with T cell receptor specificities overtly reactive to self is prevented, and cells with borderline reactivity are directed into the CD4 T regulatory lineage. A peripheral immune response is initiated when antigen-presenting cells (APCs) present peptides that alert the adaptive immune system to mount a targeted response against invading pathogens. Entry of self and foreign antigens into the endo/lysosomal pathway, proteolytic processing, and selection of peptides for presentation are influenced by APC

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Conflict of Interest

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Compliance with Ethical Standards

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type, maturation state, and external environment. Thus, the set of peptides presented by MHC-II molecules, known as the MHC-II immunopeptidome, is determined by these factors.

In this review, we discuss the details of MHC-II presentation and processing, with particular focus on the nonclassical MHC-II molecules HLA-DM and HLA-DO and their role in shaping the MHC-II immunopeptidome. We also discuss the influence of APC type and maturation state on the immunopeptidome, and how the peptides presented may change under inflammatory conditions. Finally, we explore implications of these changes in generation of immune responses to pathogens and maintenance of tolerance to self-antigens.

Overview of MHC-II synthesis and peptide loading pathways

MHC-II molecules are constitutively expressed on professional APCs such as dendritic cells, macrophages, and B cells, as well as thymic epithelia, and are upregulated on many other cell types by IFN- γ . The study of MHC-II synthesis and peptide loading pathways has been performed predominantly in B cells and dendritic cells. Expression of MHC-II genes is primarily regulated by the class II transactivator (CIITA), which interacts with multiple DNA-binding proteins to initiate transcription of MHC-II genes and MHC-II antigen processing pathway components (Masternak et al. 2000; Reith et al. 2005). The MHC-II antigen processing pathway is depicted schematically in Figure 1, and expression of antigen processing components in various APCs is summarized in Table 1. Following synthesis of MHC-II α and β subunits in the ER, nascent MHC-II molecules bind the invariant chain (Ii) protein, allowing for stabilization of the MHC heterodimer, spatial restriction of peptide loading, and transport to the endocytic compartment for peptide loading (Elliott et al. 1994; Roche and Cresswell 1990). The MHC-II-Ii complex then translocates through the Golgi complex into the endocytic pathway by way of a dileucine targeting motif present in the cytoplasmic domain of Ii (Cresswell 1996; Lotteau et al. 1990; Pieters et al. 1993). As endocytic vesicles acidify, endolysosomal proteases termed cathepsins acquire increased proteolytic activity and cleave the MHC-II-bound invariant chain until only a residual peptide (CLIP, or class II-associated invariant chain peptide) remains bound in the MHC-II peptide-binding site (Blum et al. 2013; Riese et al. 1998; Riese et al. 1996). Proteins transported into the lumen of the endosome unfold and are digested by endosomal proteases to allow for MHCII binding (Hsieh et al. 2002; van Kasteren and Overkleeft 2014; Villadangos et al. 1999; Villadangos and Ploegh 2000). The nonclassical MHC-II peptide exchange factor HLA-DM (H2-M in mice, hereafter referred to as DM) also resides in endosomal/lysosomal compartments and catalyzes the removal of CLIP from the peptide-binding groove of MHC-II molecules with resultant exchange of CLIP for endosomal peptides (Pos et al. 2012; Sloan et al. 1995; Vogt et al. 1996). DM can act on other peptides in addition to CLIP, such that multiple cycles of peptide exchange can occur (Kropshofer et al. 1996; Weber et al. 1996). The DM-MHC-II interaction has been shown to be transient (Anders et al. 2011; Narayan et al. 2009; Painter et al. 2011), increasing the rates of peptide binding to and peptide release from MHC-II without altering the equilibrium affinity, consistent with an enzyme-like catalytic activity (Vogt et al. 1996; Zarutskie et al. 2001). Because the efficiency of DM-mediated catalysis varies for different MHC-II-peptide complexes, the net effect of DM activity is to exchange peptides that interact more weakly

with MHC-II for those that interact with greater affinity. The precise features of the MHC-II-peptide interaction that are targeted by DM are controversial, with recent work focusing on conformational flexibility as an important determinant (Alvaro-Benito et al. 2018; Busch et al. 2012; Pos et al. 2012; Yin et al. 2014; Zhou et al. 2017). Overall, DM thus serves an editing function, selecting for peptides with optimal interaction with MHC-II. DM activity is competitively inhibited by another nonclassical MHC-II molecule, HLA-DO (H2-O in mice, hereafter referred to as DO), which tightly binds to DM in the endoplasmic reticulum and traffics together with DM into the endocytic pathway, preventing a fraction of the DM from interacting with MHC-II (Denzin et al. 1997; Deshaies et al. 2005; Guce et al. 2013; Kropshofer et al. 1998; Painter et al. 2011; Xiu et al. 2011; Yoon et al. 2012; Zarutskie et al. 2001). The implications of this inhibition are discussed below. Following removal of CLIP and loading of antigenic peptide, the MHC-II-peptide complex then traffics to the plasma membrane for presentation at the cell surface to CD4 T cells. While the basic mechanism of peptide loading appears to be shared by all cells that constitutively express MHC-II, the acquisition and processing of MHC-II antigens can occur via diverse pathways (Figure 1) to promote presentation of epitopes from a broad range of antigens.

The nonclassical MHC-II molecules HLA-DM and HLA-DO in immunopeptidome selection

The expression of DM has been shown to be critical for effective processing and presentation of MHC-II antigens. In mice deficient in DM, CLIP removal and peptide exchange are impaired, resulting in both a restricted MHC-II peptidome as well as defective negative selection of CD4 T cells (Fung-Leung et al. 1996; Martin et al. 1996; Miyazaki et al. 1996; Surh et al. 1997). In human transfected cell systems, reduced expression of DM leads to presentation of a set of peptides with lower MHC-II-peptide affinity (Alvaro-Benito et al. 2018; Zhou et al. 2017). Certain HLA-DQ alleles genetically linked to autoimmune disease exhibit reduced susceptibility to DM editing, suggesting that efficient DM editing is required for generation of a self-tolerant T cell pool (Busch et al. 2012; Fallang et al. 2008; Hou et al. 2011; Nguyen et al. 2017; Zhou et al. 2016). DO deficiency, while shown to have a significantly less dramatic effect on the peptide and T cell repertoires, lifts inhibition of DM and has been demonstrated to alter presentation of select epitopes, to affect CD4 T cell responses, and to result in mild autoimmunity and enhanced neutralizing antibody generation (Alfonso et al. 2003; Denzin et al. 2017; Denzin et al. 1997; Kremer et al. 2012; Liljedahl et al. 1998; Perraudau et al. 2000; Yi et al. 2010). The net DM activity resulting from the ratio of DM to DO protein levels is posited to influence the content of the immunopeptidome presented on MHC-II-expressing cells, such that presentation of particular epitopes is reduced by the presence and/or levels of DM (Ferrante et al. 2015; Kropshofer et al. 1996; Lovitch et al. 2003; Yin et al. 2012). These observations have led to development of a model of epitope immunodominance governed by DM, whereby epitopes resistant to DM-mediated exchange become more prevalent in the MHC-II peptidome as DM activity increases (Kim et al. 2014; Sant et al. 2005; Sant et al. 2013; Yin et al. 2012). Due to differing spatiotemporal levels of DM and DO in different APC subsets and during different stages of maturation (Table 1), the immunopeptidome presented on MHC-II may thus qualitatively vary with respect to its breadth and diversity (Denzin 2013). Differences in the particular peptides selected for presentation as well as the relative amounts of peptides that are selected are both expected to be important in APC interactions with T cells. In

particular, DO may play a role in negative selection in the thymus, based on its expression in medullary thymic epithelial cells (mTECs)(Douek and Altmann 1997; Douek and Altmann 2000; Karlsson et al. 1991; Kasai et al. 1998; Surh et al. 1992), where a diverse set of self-peptides would be most advantageous for efficient clonal deletion of autoreactive T cells. Expression of DM and DO can also modulate the density of particular epitopes, potentially further influencing clonal deletion or affecting diversion of CD4 T cells into the T regulatory (Treg) population. An alternative model for DO action suggests that rather than broadening the immunopeptidome through inhibition of DM editing, the primary function of DO is to focus antigen presentation on late endosomal compartments, based on the fact that the interaction of DM and DO is pH-sensitive (Jiang et al. 2015; Kropshofer et al. 1998; Liljedahl et al. 1996; Liljedahl et al. 1998; van Ham et al. 2000; Yoon et al. 2012). By tightly binding DM until the endosome becomes very acidic, DO has been suggested to prevent efficient peptide exchange from occurring in early endosomes, resulting in preferential loading of foreign antigens trafficking to late endosomes or lysosomes (Gondre-Lewis et al. 2001; Jiang et al. 2015; van Ham et al. 2000; Yi et al. 2010). A focus on late endosomes or lysosomes has been postulated to allow B cells to focus attention on antigens internalized through the B cell receptor (BCR), which presumably would not release bound cargo until relatively low pH (Alfonso et al. 2003; Denzin et al. 2005). These proposed functions of DO are not necessarily mutually exclusive, and further experimentation may prove that the mechanism of DO action in fact integrates the two models, such that inhibition of epitope selection by DO and pH susceptibility of the DM/DO interaction together shape the immunopeptidome.

Acquisition of antigens for processing in MHC-II pathways

MHC-II-expressing cells acquire antigen by distinct cellular processes that allow professional APCs to sample their external environment. Classically, extracellular proteins were thought to predominate as antigenic sources in MHC-II presentation, but many studies have demonstrated that the MHC-II peptidome largely consists of peptides derived from endogenous – rather than exogenous – source proteins (Bergseng et al. 2015; Mommen et al. 2016; Sofron et al. 2016). These endogenous peptides can derive from proteins expressed at the plasma membrane and in endo/lysosomal compartments, as expected from location of the MHC-II loading machinery in the endocytic pathway, but peptides from other intracellular compartments such as the nucleus, mitochondria, ER/Golgi, and cytosol also are found in abundance. The predominance of endogenous peptides has been shown to persist in inflammatory conditions (Draheim et al. 2017; Fugmann et al. 2017; Strug et al. 2008) and thus may have implications for maintenance of tolerance during an immune response. The fact that very low fractional occupancy of MHC-II by pathogen-derived peptides in virally-infected cells (Strug et al. 2008), which is also observed for MHC-I peptides (Schellens et al. 2015), is sufficient to elicit effective immunity demonstrates that minimal shifts in the immunopeptidome can have important immunological consequences.

Canonical acquisition and processing of MHC-II antigens

Capture of extracellular antigen occurs via multiple processes with varying degrees of efficiency in different types of antigen-presenting cells. Macropinocytosis, a nonspecific and

actin-dependent endocytic process that allows for fluid-phase uptake of extracellular material via invagination of the cell membrane, serves as a primary means of exogenous antigen internalization in immature DCs and macrophages (Sallusto et al. 1995; Steinman et al. 1976; West et al. 2000). Macropinocytotic activity has been shown to be predominantly regulated in response to growth factor stimulation and results in incorporation of extracellular solute into large vesicles termed macropinosomes, which mature before converging with the endocytic pathway (Lim and Gleeson 2011). Phagocytosis, an additional fundamental uptake mechanism that occurs principally in DCs and macrophages, is characterized by internalization of relatively large ($>0.3 \mu\text{m}$) particulate antigens (Savina and Amigorena 2007). In contrast to the nonspecific process of macropinocytosis, phagocytosis primarily occurs following recognition of surface receptors such as the C-type lectin pattern-recognition receptor DEC-205 or the opsonic Fc γ receptors (Allavena et al. 2004; Guillems et al. 2014; Jiang et al. 1995; Linehan et al. 1999). Signal transduction following ligation of different surface receptors varies but has been shown in all cases to culminate in actin polymerization and phagosome formation (Underhill and Goodridge 2012). The phagosome, derived primarily from the plasma membrane, then fuses with lysosomes to form phagolysosomes (Flannagan et al. 2012). Proteolysis of internalized proteins within the phagolysosome is accomplished in DCs by cathepsins and other endosomal proteases, while NOX2 is recruited to the phagosomal membrane to regulate antigen proteolysis (Savina et al. 2009). In macrophages, increased acidification of this compartment favors pathogen eradication through effects of antimicrobial factors (e.g. ROS, RNI, and lysozyme) (Myers et al. 2003; Wolf et al. 2011). In comparison, B cells principally depend upon receptor-mediated endocytosis to capture extracellular antigen (Adler et al. 2017; Lanzavecchia 1985; Rock et al. 1984). Antigen is internalized during this process after binding to the BCR, or to surface Fc or complement receptors, after which endocytosis – most often mediated by entry via clathrin-coated vesicles – occurs (Malhotra et al. 2009; Roche and Furuta 2015). Each APC subset thus preferentially adopts a particular mode or modes of internalization, all of which converge in the endocytic pathway for generation of peptides and presentation on MHC-II.

Nonconventional pathways of MHC-II processing

Evidence for acquisition of MHC-II antigenic source proteins via alternate pathways has accumulated over the past two decades. Perhaps chief among these is the process of autophagy, whereby cytoplasmic components are digested by the cell for degradation and processing (Klionsky 2007). Incorporation of the cytosol into autophagosomes was initially described in yeast as the cellular response to starvation, and as such, an essential energy source for cell survival (Takeshige et al. 1992). Since its discovery, however, autophagy has been determined to be critical for myriad cellular and organismal processes, including aging and development, as well as for antigen presentation (Brazil et al. 1997; Dengjel et al. 2005; Levine and Klionsky 2004). All forms of autophagy (macro-, micro-, or chaperone-mediated) intersect with the endocytic pathway and thus deliver nuclear, microsomal, and cytoplasmic proteins for presentation on MHC-II (Crotzer and Blum 2009; Kaushik and Cuervo 2018; Veerappan Ganesan and Eisenlohr 2017). Processing of antigens via macroautophagy has been demonstrated to be important in DCs, in which constitutive formation of autophagosomes has been shown, as well as in B cells, for presentation of both

self- and foreign antigens (Crotzer and Blum 2009; Ireland and Unanue 2011; Schmid et al. 2007; Zhou et al. 2005). Although antigen processing pathways in thymic epithelial cells (TECs) are considerably less well-examined than in professional APCs, TECs are thought to rely primarily upon constitutive macroautophagy to present MHC-II antigens (Liang et al. 2018; Nedjic et al. 2008; Wu et al. 2013). In mTECs, incorporation of antigens into autophagosomes has been shown to allow for presentation of the many tissue-specific antigens under the transcriptional control of AIRE (Aichinger et al. 2013). Colocalization of the autophagosomal marker LC3 with DM was demonstrated nearly a decade ago in cryosections of thymus samples (Kasai et al. 2009), and it was recently shown that clonal deletion of conventional T cells specific for the model antigens pigeon cytochrome c and (a GFP-LC3 fusion of) human C-reactive protein is dependent upon processing through the macroautophagy pathway (Aichinger et al. 2013). Another group reported that specific deletion of Atg7 in mTECs had little effect on selection of thymocytes (Sukseree et al. 2012), but this discrepancy may be due to differences in the autophagic machinery that intersects with the antigen presentation pathway, as Klein and colleagues examined the effect of Atg5 rather than Atg7 ablation (Aichinger et al. 2013). Antigen transfer via trogocytosis, in which an immune synapse forms between cells to mediate intercellular transfer of proteins, has also been shown to allow for capture and presentation of MHC-peptide complexes by both APCs and non-APCs (Cone et al. 1972; Joly and Hudrisier 2003; Nakayama 2014). In the thymus, peptides presented as a result of trogocytosis between DCs and mTECs is posited to aid in increasing presentation of rare thymic antigens by MHC-II (Klein et al. 2009; Kroger et al. 2017; Millet et al. 2008). Intercellular MHC-II transfer has been shown to occur in a number of other peripheral immune cell subsets as well, including between DCs and NK cells (Nakayama 2014; Nakayama et al. 2011). While the mechanism of transfer from DCs to many cell types remains unclear, MHC-II-peptide uptake by T cells has been demonstrated to be mediated via the TCR and the costimulatory molecule CD28 (Hwang et al. 2000). Recycling of MHC-II molecules also serves as an alternative means of presentation of MHC-II epitopes; peptide loading on recycled MHC-II following endocytosis has been shown to occur in early endosome compartments (Li et al. 2005; Pathak and Blum 2000; Reid and Watts 1990; Villadangos et al. 2000). Of note, the antigen acquisition processes of trogocytosis and recycling in early endosomes in most cases seem to be independent of DM and cathepsin activity, such that peptide species loaded or exchanged on recycled or trogocytosed MHC-II may be comprised of epitopes absent from the MHC-II peptidome generated by canonical pathways (Griffin et al. 1997; Lindner and Unanue 1996; Villadangos et al. 2000), although in some cases DM-mediated editing has been observed (Pathak et al. 2001). Still another mechanism capable of generating antigens presented on MHCII molecules relies on components of MHC-I machinery, including the proteasome, TAP, and ERAP (Spencer et al. 2013; Tewari et al. 2005); however, the mode of intersection of the MHC-I and MHC-II processing pathways has not been determined. It has thus become clear that the MHC-II immunopeptidome can be influenced by multiple processing pathways that serve to present antigens from endogenous and exogenous sources, and that canonical MHC-II acquisition and processing is not the sole determinant of the MHC-II-bound peptide repertoire.

Source proteins in the MHC-II peptidome

Recent advances in mass spectrometry technology have significantly improved both accuracy and sensitivity of detection of peptides eluted from purified MHC proteins, allowing for qualitative and quantitative characterization of MHC peptidomes. Mass spectrometry can thus be leveraged as a powerful tool to identify source proteins from which MHC-II peptides derive in order to assess effects of perturbations in MHC processing such as cellular maturation or infection, and the relative contributions of different processing pathways. Increases in the sensitivity of mass spectrometry has allowed studies of APCs characterized ex vivo (Clement et al. 2016; Fugmann et al. 2017; Olsson et al. 2018), likely reflective of in vivo peptidomes, as compared to classical immunopeptidome studies of cultured cells. Recent work has demonstrated that the MHC-II peptidome is substantially comprised of peptides derived from nuclear or cytosolic proteins (25–55% of total peptides eluted from HLA-DR) (Clement et al. 2016; Mommen et al. 2016), presumably largely due to the self-degradative autophagic process. Several studies have quantified peptides derived from extracellular source proteins as constituting only ~10–20% of the MHC-II peptidome, with the remaining peptides derived from endogenous source proteins located in various cellular compartments, including the plasma membrane, mitochondria, endosomes or lysosomes, and ER/Golgi (Bergseng et al. 2015; Clement et al. 2016; Mommen et al. 2016; Sofron et al. 2016). A caveat of these analyses is that classification of peptides as sourced from endogenous proteins does not preclude acquisition of (at least a portion of) these proteins via uptake of necrotic or apoptotic cells. Recent work examining source proteins in the peptidome of mouse lymphatic fluid compared to the HLADR1-bound peptidome of splenic dendritic cells posited that lymph-derived epitopes were loaded at the surface or in early endosomal compartments based on their sensitivity to DM-mediated exchange and endosomal digestion, suggesting a DM-independent mechanism for presentation of low affinity autoantigens derived from extracellular sources (Clement et al. 2016). In the context of infection or inflammation, the MHC-II peptidome has been shown to include only a very small fraction of foreign peptide sequences present within a much larger set of host-derived sequences, as characterized in the settings of experimentally-induced colitis in mice (~0.2% of 2188 total sequences), a mouse model of malaria infection (42 malaria-derived out of 372 sequences), or vaccinia infection of cultured B cells (1% of the peptidome, as estimated by comparing intensities of several hundred peaks) (Draheim et al. 2017; Fugmann et al. 2017; Strug et al. 2008). Further quantitative analysis is required to determine the proportion of foreign peptides presented on MHC-II molecules with respect to total peptide component (as compared to the fraction of different sequences), but these studies suggest that even during inflammation or infection, the MHC-II peptidome largely consists of peptides derived from self.

Implications of MHC-II processing for immune tolerance

Positive and negative selection of T cells in the thymus depends on interactions of developing T cells with local antigen-presenting cells, and so antigen presentation events in the thymus play a key role in guiding selection of CD4 T cells as well as in development of regulatory CD4 T cells. Differential processing and presentation pathways in various cell populations can result in distinct peptidomes in different tissues. Restraint of autoimmunity

to tissue-restricted antigens produced in this manner presents a challenge for central and peripheral tolerance mechanisms.

MHC-II presentation in central tolerance

Somatic gene arrangement of the T cell receptor (TCR) occurs in the thymus and generates T cells with diverse specificities for different MHC-II-peptide complexes. Presentation of self-ligands on MHC-II in the thymus positively selects only those T cells that express TCRs capable of recognizing the peptide-MHC complex (Huseby et al. 2005). Positive selection is mediated by cortical thymic epithelial cells (cTECs), which have been posited to present a unique MHC-II ligandome due to proteolytic activities of cathepsin L and thymus-specific serine protease (TSSP) (Gommeaux et al. 2009; Klein et al. 2009; Nakagawa et al. 1998) (Table 1). Evidence for a requirement for distinct peptide species in the positively-selecting MHC-II peptidome was demonstrated by rescue of the defect in CD4 T cell selection observed in cathepsin L-deficient mice (Nakagawa et al. 1998) via reconstitution with MHC-II-deficient bone marrow (Honey et al. 2002). These results suggested that in mice deficient in cathepsin L, the efficiency of negative selection of T cells is increased due to overlapping immunopeptidomes of MHC-II-expressing hematopoietic APCs and cTECs. A substantially reduced MHC-II peptide repertoire in H2-M-deficient mice demonstrated a similar effect on the CD4 T cell compartment (Martin et al. 1996; Miyazaki et al. 1996), highlighting the importance of (at least somewhat) distinct peptides presented by positively- and negatively-selecting APCs in effective thymic selection of CD4 T cells.

If positive selection is required to ensure that developing CD4 T cells can recognize MHC-II-peptides, the process of negative selection is necessary to remove CD4 T cells that are overtly reactive with self-ligands (Klein et al. 2014). By deleting T cells with exceedingly high affinity for MHC-II bound to peptide autoantigens in the thymus, peripheral immune responses to self-antigens can be subverted. MHC-II presentation by mTECs of peptides derived from self-peptides is mediated by promiscuous expression of tissue-restricted antigens by AIRE, which allows for deletion of CD4 T cells with high affinity for autoantigens expressed in peripheral tissues (Mathis and Benoist 2009). MHC-II processing and presentation of tissue-restricted antigens has been shown to involve nonconventional pathways such as macroautophagy and trogocytosis (discussed above). Recent analysis of thymus MHC-II peptidomes has confirmed the diverse spectrum of source proteins presented by thymic APCs (Adamopoulou et al. 2013; Collado et al. 2013), but whether the thymus-associated MHC-II immunopeptidome is comparatively unique relative to other tissues remains a subject for future work.

Presentation of ligands by MHC-II in the thymic medulla also controls development of thymocytes into an alternative CD4 T cell fate. Selection of the suppressive Foxp3-expressing T regulatory (Treg) population occurs in the thymic medulla through recognition of MHC-II-peptide complexes, at a threshold of affinity/avidity thought to lie above the threshold for conventional T cell selection but below the threshold for clonal deletion (Aschenbrenner et al. 2007; Benoist and Mathis 2012; Hsieh et al. 2012; Klein and Jovanovic 2011). Examination of T cell activation by the Hogquist group using reporter mice expressing Nur77, which is rapidly upregulated following TCR signaling,

demonstrated the importance of TCR specificity in selection of Tregs (Moran et al. 2011). The consequence of Treg selection within a high affinity or avidity window is suggested to result in activation of Tregs in the periphery at low agonist doses, so that they may outcompete conventional T cells for MHC-II ligand binding (Gubser et al. 2016; Onishi et al. 2008). Seminal work from the Hsieh group demonstrated through TCR retrogenic technology that Treg self-reactivity is observed over a broad range of affinity, indicating Tregs are likely to participate in suppression of immunity to both self and non-self (Lee et al. 2012). MHC-II peptide ligands responsible for Treg selection have not been determined, and identification of these peptides may provide greater understanding of the determinants of Treg selection, particularly with regard to ligand avidity and affinity.

Peripheral tolerance and the MHC-II immunopeptidome

Following thymic egress, mature CD4 T cells migrate to the periphery and populate the secondary lymphoid organs. A portion of clonotypes that exit the thymus are reactive to self, owing to the fact that negative selection is an imperfect process (Xing and Hogquist 2012). Peripheral tolerance mechanisms serve to dampen or eliminate autoreactive T cells by inducing anergy or mediating clonal deletion. T cells that bind their cognate ligands in the absence of positive costimulatory molecules become hyporesponsive (or anergic), thereby circumventing autoimmune attack of healthy tissue (Mueller 2010). Other costimulatory molecules such as PD-1 provide negative signals that inhibit T cell activation, effectively suppressing self-reactivity (Fife et al. 2009); in mouse models deficient in PD-1, autoimmune disease develops with features of lupus, including arthritis and glomerulonephritis (Nishimura et al. 1999). CD4 T cells also require tonic stimulation by MHC-II ligands to survive, and the absence of tonic signals results in clonal deletion by apoptotic cell death (Fletcher et al. 2011; Steinman et al. 2003). A third mechanism of peripheral tolerance occurs via Treg-mediated suppression of effector T cells. Thymic Tregs enter the periphery endowed with suppressive function, and peripheral induction of Tregs, particularly in sites such as the gut where naïve CD4 T cells encounter commensal and food-derived antigens, can help to restrain overactive or self-reactive T cell responses (Kanamori et al. 2016; Yadav et al. 2013). The precise mechanism(s) by which Treg-mediated suppression occurs is a matter of some debate (Shevach 2006; Vignali 2012), but models of Treg deficiency conclusively demonstrate the vital function of these cells in regulating the development of autoimmunity and in preventing excessive immunopathology during infection (Belkaid 2007; Gambineri et al. 2003; Ramsdell and Ziegler 2014; Sakaguchi et al. 1995).

Alterations in the MHC-II peptidome that can occur in specific tissues or in certain disease states have the potential to compromise the efficacy of peripheral tolerance mechanisms. Tissue- or cell-specific differences in antigenic sources, processing, or peptide loading may generate distinct peptide repertoires presented by particular APCs in particular locations. Local sources of captured exogenous antigen are likely to vary considerably in different organs, and disparate metabolic or gene expression profiles of tissue-specific APCs may further diversify the immunopeptidome in various locations throughout the body (Calderon et al. 2014; Fortier et al. 2008; Fugmann et al. 2017; Muixi et al. 2012). Such differences may have implications in maintenance of tolerance based on the weak expression of tissue-

restricted antigens mediated by AIRE in mTECs (Derbinski and Kyewski 2010; Villasenor et al. 2008), in cases where TCR agonist density increases sufficiently enough in the periphery to activate a T cell selected in the thymus with only weak affinity for its cognate antigen. In addition, post-translational modifications of self-peptides in the periphery can lead to generation of novel epitopes capable of binding and activating T cells that then initiate an autoimmune response. In such cases, if epitopes fail to be generated in the thymus, developing T cells with specificity for these antigens may escape negative selection. For example, citrullination of peptides or proteins has been observed in rheumatoid arthritis, multiple sclerosis, and type 1 diabetes (T1D) and have been implicated in the etiology of disease (Nguyen and James 2016; Sipila et al. 2017). Trans-splicing of peptides derived from proteins present in the insulin secretory granule has been suggested to generate new epitopes that can be loaded onto MHC-II and presented by pancreatic beta cells and recognized by diabetogenic CD4 T cells (Babon et al. 2016; Delong et al. 2016; Jin et al. 2015). Differential proteolytic activity in different cells or different cellular locations could alter proteolysis of antigens and result in a qualitatively different immunopeptidome, as suggested by altered processing of myelin basic protein (MBP) and insulin proteins in thymic APCs due to cathepsin S activity (Stoeckle et al. 2012). Differential expression of DM, or differential trafficking of antigens through DM-containing compartments, in the periphery as compared to thymus could further influence the content of the MHC-II peptidome and impact autoimmunity (Mohan et al. 2011; Mohan and Unanue 2012). Lastly, regulation of the MHC-II peptidome may also be conferred by differential resistance of MHC alleles to editing by DM, and this has been suggested to contribute to the genetic association of certain HLA-DR and HLA-DQ allotypes with autoimmunity in diabetes and celiac disease (Busch et al. 2012; Nguyen et al. 2017; Zhou et al. 2017).

MHC-II processing in inflammation

Professional APCs undergo a maturation process in the context of inflammation that results in alterations in MHC-II processing and presentation pathways. Changes in multiple components of the MHC-II presentation pathway in addition to upregulation of MHC-II expression may serve to focus the peptide repertoire by amplifying presentation of stable MHC-II-peptide complexes, which has been shown to be a feature of immunodominant epitopes derived from foreign antigens (Ferrante et al. 2015; Lazarski et al. 2005; Yin et al. 2012). Induction of CIITA by IFN- γ in non-professional APCs such as endothelial cells – which do not constitutively express MHCII molecules – results in substantial surface expression of MHC-II (McDouall et al. 1996; Pober et al. 1983). Such changes allow for efficient and widespread presentation of inflammation-associated epitopes derived from invading pathogens or autoantigens. Due to the preponderance of self-ligands in the MHC-II peptidome, however, these changes may also have implications for breakdown of tolerance under inflammatory conditions.

Effects of inflammation on antigen acquisition pathways

During an immune response, targeted and precise activation of T cells is necessary to eliminate pathogenic insult. Some processes of antigen acquisition are downmodulated when APCs are activated, representing a shift in priority from antigen sampling to more efficient

antigen presentation (Figure 1). Macropinocytotic activity in activated DCs has been shown to be reduced in some contexts and unaffected in others (Drutman and Trombetta 2010; Garrett et al. 2000), while activation of macrophages has been demonstrated to reprogram antigen uptake to favor macropinocytosis over receptor-mediated phagocytosis (Bosadasgupta and Pieters 2014). In contrast, DC activation appears not to affect the efficiency of antigen capture via receptor-mediated phagocytosis (Platt et al. 2010), although MHC-II trafficking pathways are altered to prevent MHC-II ubiquitination and targeted degradation in lysosomes, preserving captured MHC-II-peptide complexes for recycling (Cho et al. 2015). With regard to nonconventional antigen acquisition by autophagy in the context of inflammation, presentation of many bacterial and viral antigens has been shown to be dependent on macroautophagy (Jagannath et al. 2009; Lee et al. 2010; Paludan et al. 2005), supporting a continued role for this process during an immune response. The dominant EBV antigen EBNA-1 was shown to colocalize with LAMP-1 in EBV-transformed lymphoblastoid cells, and blockade of autophagy resulted in decreased EBNA-1-specific CD4 T cell activation (Paludan et al. 2005). Lysosomal degradation of *Mycobacterium tuberculosis* is enhanced by induction of autophagy and aids in eradication of the mycobacteria (Alonso et al. 2007). Some pathogens have been shown to be eliminated through noncanonical functions of the autophagy machinery, such as LC3-associated phagocytosis, which has been demonstrated to be necessary for clearance of the fungal pathogen *Aspergillus fumigatus* (Martinez et al. 2015). Altogether, the autophagic pathway has been shown to be necessary for efficient presentation of a multitude of foreign antigens to induce an effective CD4 T cell response.

Inflammation-associated alterations in MHC-II processing and presentation

Changes in the antigen processing and presentation machinery during inflammation serve to poise APCs to most efficiently orchestrate an effective immune response (Figure 1). Concurrent with alterations in antigen sampling, synthesis of MHC molecules is also regulated in APCs to maximize presentation of MHC-II epitopes during inflammation. Surface MHC-II levels are 10-fold higher in mature DCs compared to immature DCs, owing to a transient burst in MHC-II biosynthesis upon DC activation (Cella et al. 1997; Pierre et al. 1997). IFN-inducible CIITA-mediated upregulation of MHC-II and associated processing and loading machinery also confers antigen-presenting ability to endothelium and epithelium (Abrahimi et al. 2016; Cella et al. 1997; Chang et al. 1994; Thelemann et al. 2014). Redistribution of MHC-II molecules from endosomes and lysosomes to the plasma membrane in DCs and macrophages further maximizes antigen-presenting efficiency, as does the enhanced stability of surface peptide-MHC complexes upon DC maturation (Cella et al. 1997; Chow et al. 2002; Pierre et al. 1997). In addition to overall enhanced MHC-II presentation and upregulation of costimulatory molecules, inflammatory signals result in changes in antigen processing and peptide loading machinery that likely affect the spectrum of peptides presented on MHC-II. Cathepsins are redistributed to MHC-containing compartments, and antigen processing efficiency is enhanced in DC exposed to TLR ligands (Blander and Medzhitov 2006; Lautwein et al. 2002). Acquisition of cathepsin E activity has been shown in primary B cells activated with PMA or *Staphylococcus aureus* in vitro (Burster et al. 2008; Sealy et al. 1996). DO expression is downmodulated following DC maturation and B cell entry into germinal centers (Chen et al. 2002; Chen et al. 2006; Fallas

et al. 2004; Glazier et al. 2002; Hornell et al. 2006), effectively increasing DM activity. Removing inhibition of DM has the potential to allow for editing of a subset of the MHC-II peptidome, although the determinants that dictate sensitivity vs. resistance of peptides to DM-mediated editing remains a topic of active investigation (Alvaro-Benito et al. 2018; Chou and Sadegh-Nasseri 2000; Ferrante and Gorski 2010; Ferrante and Gorski 2012; Pos et al. 2013; Radrizzani et al. 1999; Yin et al. 2014; Zhou et al. 2009). These alterations, together with overall reduction in antigen acquisition during inflammation, result in augmentation of antigen-presenting capacity and presentation of stable MHC-peptide complexes to T cells.

Potential effects of inflammation on peripheral tolerance

MHC-II peptide elution studies suggest that epitopes derived from pathogens comprise only a small fraction of the overall peptide repertoire. Due to the predominance of self-ligands in the MHC-II peptidome, increased surface expression of MHC-II on APCs during inflammation will likely result in increased peptide density of certain self-peptides. In addition, proteins upregulated by the inflammatory process, for example by induction of IFN-stimulated genes, can enter the endo/lysosomal pathway and represent an additional source of antigens potentially upregulated in inflammatory immunopeptidomes. Thus, during an inflammatory response the increased expression of MHC-II molecules carrying particular self-peptides together with increased expression of costimulatory molecules, as well as enhanced secretion of cytokines and chemokines, may result in unwanted activation of T cells that under homeostatic conditions are only mildly reactive to self. Consistent with this idea, the etiology of autoimmune disorders has been linked to exposure to pathogens; initial onset of autoimmunity is shown in many instances to coincide with recent viral infection (Christen and von Herrath 2005; Fujinami 2001). Associations have been demonstrated between type 1 diabetes and Coxsackie virus, herpesvirus, and retrovirus, as well as between multiple sclerosis and EBV, HHV-6, and rubeola virus (Cusick et al. 2012). The mechanism whereby inappropriate activation of autoreactive T cells occurs in these contexts is often attributed to molecular mimicry (Cusick et al. 2012; Fujinami et al. 2006). Degenerate TCR specificity has indeed been shown in studies of MBP-reactive T cell clones, which were found to bind pathogen-derived peptides (Fujinami and Oldstone 1985; Hemmer et al. 1997; Wucherpfennig and Strominger 1995), and TCRs crossreactive for self- and foreign antigens have been observed in other contexts as well (Colf et al. 2007; Harkiolaki et al. 2009). Yet, given the considerable levels of self-pMHC on the surface of APCs during inflammation (Fugmann et al. 2017; Strug et al. 2008), activation of autoreactive T cell clones may also occur in an antigen-specific manner, such that increased ligand density in combination with costimulatory molecule expression initiates an inappropriate response to self. This idea shares features with the concept of bystander activation (Fujinami et al. 2006) associated with breakdown of tolerance, but is distinct in that additional uptake of self-antigen appears not to be required, given the predominance of self-pMHC in the immunopeptidome in both homeostatic and inflammatory conditions. Moreover, Tregs selected with higher affinity or avidity for self-antigen may be similarly activated by upregulated presentation of self-peptides, particularly if selection of Tregs is mediated by ubiquitous proteins or by antigens commonly overexpressed during inflammation. Thus, in addition to suppressing autoreactive T cell proliferation under homeostatic conditions,

thymically-derived Tregs may in an inflammatory context have an important role in suppressing unwanted self-reactivity. Identification of thymic Treg peptide ligands could lend support for this proposed function, as well as reinforce the basis for selection of Tregs within an avidity/affinity window above that of conventional T cells.

Conclusions and perspective

Antigen uptake, processing, and presentation pathways differ among the various types of antigen-presenting cells, their developmental states, and their tissue location. Self-peptides comprise the bulk of the MHC-II peptide in both resting and activated antigen-presenting cells. Substantial alterations in antigen processing pathways occur during inflammation in addition to upregulation of immune response genes and costimulatory functions, including changes in antigen acquisition and in the relative expression of the nonclassical MHC-II proteins DO and DM that mediate peptide editing/selection. Thus, inflammation is expected to result in considerable changes in the MHC-II self-peptidome, although detailed qualitative and quantitative studies will be required to elucidate these changes. Small changes in the peptide repertoire can have substantial effects on activation of pathogen-reactive T cells, suggesting possible immunological consequences of altered MHC-II peptidomes under inflammation, but whether the extreme sensitivity to peptide dose observed for pathogen-specific T cells also applies to self-reactive conventional T cells and to Tregs is unclear. The mechanisms whereby tolerance to upregulated or peripherally modified self-antigens is maintained or lost during inflammation are incompletely understood, and further knowledge of this process may provide insights into the etiology of autoimmune disease.

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References

- Abrahami P, Qin L, Chang WG, Bothwell AL, Tellides G, Saltzman WM, Pober JS (2016) Blocking MHC class II on human endothelium mitigates acute rejection. *JCI Insight* 1
- Adamopoulou E, Tenzer S, Hillen N, Klug P, Rota IA, Tietz S, Gebhardt M, Stevanovic S, Schild H, Tolosa E, Melms A, Stoeckle C (2013) Exploring the MHC-peptide matrix of central tolerance in the human thymus. *Nat Commun* 4:2039 [PubMed: 23783831]
- Adler LN, Jiang W, Bhamidipati K, Millican M, Macaubas C, Hung SC, Mellins ED (2017) The Other Function: Class II-Restricted Antigen Presentation by B Cells. *Front Immunol* 8:319 [PubMed: 28386257]
- Aichinger M, Wu C, Nedjic J, Klein L (2013) Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J Exp Med* 210:287–300 [PubMed: 23382543]
- Alfonso C, Williams GS, Han JO, Westberg JA, Winqvist O, Karlsson L (2003) Analysis of H2-O influence on antigen presentation by B cells. *J Immunol* 171:2331–7 [PubMed: 12928379]
- Allavena P, Chieppa M, Monti P, Piemonti L (2004) From pattern recognition receptor to regulator of homeostasis: the double-faced macrophage mannose receptor. *Crit Rev Immunol* 24:179–92 [PubMed: 15482253]

- Alonso S, Pethe K, Russell DG, Purdy GE (2007) Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc Natl Acad Sci U S A* 104:6031–6 [PubMed: 17389386]
- Alvaro-Benito M, Morrison E, Abualrous ET, Kuropka B, Freund C (2018) Quantification of HLA-DM-Dependent Major Histocompatibility Complex of Class II Immunoepitomes by the Peptide Landscape Antigenic Epitope Alignment Utility. *Front Immunol* 9:872 [PubMed: 29774024]
- Anders AK, Call MJ, Schulze MS, Fowler KD, Schubert DA, Seth NP, Sundberg EJ, Wucherpfennig KW (2011) HLA-DM captures partially empty HLA-DR molecules for catalyzed removal of peptide. *Nat Immunol* 12:54–61 [PubMed: 21131964]
- Arunachalam B, Phan UT, Geuze HJ, Cresswell P (2000) Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma-interferon-inducible lysosomal thiol reductase (GILT). *Proc Natl Acad Sci U S A* 97:745–50 [PubMed: 10639150]
- Aschenbrenner K, D'Cruz LM, Vollmann EH, Hinterberger M, Emmerich J, Swee LK, Rolink A, Klein L (2007) Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. *Nat Immunol* 8:351–8 [PubMed: 17322887]
- Babon JA, DeNicola ME, Blodgett DM, Crevecoeur I, Buttrick TS, Maehr R, Bottino R, Naji A, Kaddis J, Elyaman W, James EA, Haliyur R, Brissova M, Overbergh L, Mathieu C, Delong T, Haskins K, Pugliese A, Campbell-Thompson M, Mathews C, Atkinson MA, Powers AC, Harlan DM, Kent SC (2016) Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nat Med* 22:1482–1487 [PubMed: 27798614]
- Bania J, Gatti E, Lelouard H, David A, Cappello F, Weber E, Camosseto V, Pierre P (2003) Human cathepsin S, but not cathepsin L, degrades efficiently MHC class II-associated invariant chain in nonprofessional APCs. *Proc Natl Acad Sci U S A* 100:6664–9 [PubMed: 12748383]
- Barascuk N, Skjot-Arkil H, Register TC, Larsen L, Byrjalsen I, Christiansen C, Karsdal MA (2010) Human macrophage foam cells degrade atherosclerotic plaques through cathepsin K mediated processes. *BMC Cardiovasc Disord* 10:19 [PubMed: 20409295]
- Barrera C, Ye G, Espejo R, Gunasena S, Almanza R, Leary J, Crowe S, Ernst P, Reyes VE (2001) Expression of cathepsins B, L, S, and D by gastric epithelial cells implicates them as antigen presenting cells in local immune responses. *Hum Immunol* 62:1081–91 [PubMed: 11600214]
- Baumgart M, Moos V, Schuhbauer D, Muller B (1998) Differential expression of major histocompatibility complex class II genes on murine macrophages associated with T cell cytokine profile and protective/suppressive effects. *Proc Natl Acad Sci U S A* 95:6936–40 [PubMed: 9618517]
- Beers C, Burich A, Kleijmeer MJ, Griffith JM, Wong P, Rudensky AY (2005) Cathepsin S controls MHC class II-mediated antigen presentation by epithelial cells in vivo. *J Immunol* 174:1205–12 [PubMed: 15661874]
- Beers C, Honey K, Fink S, Forbush K, Rudensky A (2003) Differential regulation of cathepsin S and cathepsin L in interferon gamma-treated macrophages. *J Exp Med* 197:169–79 [PubMed: 12538657]
- Belkaid Y (2007) Regulatory T cells and infection: a dangerous necessity. *Nat Rev Immunol* 7:875–88 [PubMed: 17948021]
- Benoist C, Mathis D (2012) Treg cells, life history, and diversity. *Cold Spring Harb Perspect Biol* 4:a007021 [PubMed: 22952391]
- Bergmann H, Yabas M, Short A, Miosge L, Barthel N, Teh CE, Roots CM, Bull KR, Jeelall Y, Horikawa K, Whittle B, Balakishnan B, Sjollem G, Bertram EM, Mackay F, Rimmer AJ, Cornall RJ, Field MA, Andrews TD, Goodnow CC, Enders A (2013) B cell survival, surface BCR and BAFFR expression, CD74 metabolism, and CD8- dendritic cells require the intramembrane endopeptidase SPPL2A. *J Exp Med* 210:31–40 [PubMed: 23267016]
- Bergseng E, Dorum S, Arntzen MO, Nielsen M, Nygard S, Buus S, de Souza GA, Sollid LM (2015) 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* 67:73–84 [PubMed: 25502872]
- Blander JM, Medzhitov R (2006) Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* 440:808–12 [PubMed: 16489357]

- Blum JS, Wearsch PA, Cresswell P (2013) Pathways of antigen processing. *Annu Rev Immunol* 31:443–73 [PubMed: 23298205]
- Bosedasgupta S, Pieters J (2014) Inflammatory stimuli reprogram macrophage phagocytosis to macropinocytosis for the rapid elimination of pathogens. *PLoS Pathog* 10:e1003879 [PubMed: 24497827]
- Brazil MI, Weiss S, Stockinger B (1997) Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. *Eur J Immunol* 27:1506–14 [PubMed: 9209504]
- Burster T, Beck A, Tolosa E, Marin-Esteban V, Rotzschke O, Falk K, Lautwein A, Reich M, Brandenburg J, Schwarz G, Wiendl H, Melms A, Lehmann R, Stevanovic S, Kalbacher H, Driessen C (2004) Cathepsin G, and not the asparagine-specific endoprotease, controls the processing of myelin basic protein in lysosomes from human B lymphocytes. *J Immunol* 172:5495–503 [PubMed: 15100291]
- Burster T, Beck A, Tolosa E, Schnorrer P, Weissert R, Reich M, Kraus M, Kalbacher H, Haring HU, Weber E, Overkleef H, Driessen C (2005) Differential processing of autoantigens in lysosomes from human monocyte-derived and peripheral blood dendritic cells. *J Immunol* 175:5940–9 [PubMed: 16237087]
- Burster T, Reich M, Zaidi N, Voelter W, Boehm BO, Kalbacher H (2008) Cathepsin E regulates the presentation of tetanus toxin C-fragment in PMA activated primary human B cells. *Biochem Biophys Res Commun* 377:1299–303 [PubMed: 18996084]
- Busch R, De Riva A, Hadjinicolaou AV, Jiang W, Hou T, Mellins ED (2012) On the perils of poor editing: regulation of peptide loading by HLA-DQ and H2-A molecules associated with celiac disease and type 1 diabetes. *Expert Rev Mol Med* 14:e15 [PubMed: 22805744]
- Calderon B, Carrero JA, Unanue ER (2014) The central role of antigen presentation in islets of Langerhans in autoimmune diabetes. *Curr Opin Immunol* 26:32–40 [PubMed: 24556398]
- Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A (1997) Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 388:782–7 [PubMed: 9285591]
- Chain BM, Free P, Medd P, Swetman C, Tabor AB, Terrazzini N (2005) The expression and function of cathepsin E in dendritic cells. *J Immunol* 174:1791–800 [PubMed: 15699105]
- Chalouni C, Banchereau J, Vogt AB, Pascual V, Davoust J (2003) Human germinal center B cells differ from naive and memory B cells by their aggregated MHC class II-rich compartments lacking HLA-DO. *Int Immunol* 15:457–66 [PubMed: 12663675]
- Chang CH, Fontes JD, Peterlin M, Flavell RA (1994) Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. *J Exp Med* 180:1367–74 [PubMed: 7931070]
- Chen X, Laur O, Kambayashi T, Li S, Bray RA, Weber DA, Karlsson L, Jensen PE (2002) Regulated expression of human histocompatibility leukocyte antigen (HLA)-DO during antigen-dependent and antigen-independent phases of B cell development. *J Exp Med* 195:1053–62 [PubMed: 11956296]
- Chen X, Reed-Loisel LM, Karlsson L, Jensen PE (2006) H2-O expression in primary dendritic cells. *J Immunol* 176:3548–56 [PubMed: 16517723]
- Cho KJ, Walseng E, Ishido S, Roche PA (2015) Ubiquitination by March-I prevents MHC class II recycling and promotes MHC class II turnover in antigen-presenting cells. *Proc Natl Acad Sci U S A* 112:10449–54 [PubMed: 26240324]
- Chou CL, Sadegh-Nasseri S (2000) HLA-DM recognizes the flexible conformation of major histocompatibility complex class II. *J Exp Med* 192:1697–706 [PubMed: 11120767]
- Chow A, Toomre D, Garrett W, Mellman I (2002) Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. *Nature* 418:988–94 [PubMed: 12198549]
- Christen U, von Herrath MG (2005) Infections and autoimmunity--good or bad? *J Immunol* 174:7481–6 [PubMed: 15944245]
- Clement CC, Becerra A, Yin L, Zolla V, Huang L, Merlin S, Follenzi A, Shaffer SA, Stern LJ, Santambrogio L (2016) The Dendritic Cell Major Histocompatibility Complex II (MHC II)

- Peptidome Derives from a Variety of Processing Pathways and Includes Peptides with a Broad Spectrum of HLA-DM Sensitivity. *J Biol Chem* 291:5576–95 [PubMed: 26740625]
- Colf LA, Bankovich AJ, Hanick NA, Bowerman NA, Jones LL, Kranz DM, Garcia KC (2007) How a single T cell receptor recognizes both self and foreign MHC. *Cell* 129:135–46 [PubMed: 17418792]
- Collado JA, Alvarez I, Ciudad MT, Espinosa G, Canals F, Pujol-Borrell R, Carrascal M, Abian J, Jaraquemada D (2013) Composition of the HLA-DR-associated human thymus peptidome. *Eur J Immunol* 43:2273–82 [PubMed: 23719902]
- Cone RE, Sprent J, Marchalonis JJ (1972) Antigen-binding specificity of isolated cell-surface immunoglobulin from thymus cells activated to histocompatibility antigens. *Proc Natl Acad Sci U S A* 69:2556–60 [PubMed: 4506774]
- Cresswell P (1996) Invariant chain structure and MHC class II function. *Cell* 84:505–7 [PubMed: 8598037]
- Crotzer VL, Blum JS (2009) Autophagy and its role in MHC-mediated antigen presentation. *J Immunol* 182:3335–41 [PubMed: 19265109]
- Cusick MF, Libbey JE, Fujinami RS (2012) Molecular mimicry as a mechanism of autoimmune disease. *Clin Rev Allergy Immunol* 42:102–11 [PubMed: 22095454]
- Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ (1984) The detailed distribution of MHC Class II antigens in normal human organs. *Transplantation* 38:293–8 [PubMed: 6591602]
- Delong T, Wiles TA, Baker RL, Bradley B, Barbour G, Reisdorph R, Armstrong M, Powell RL, Reisdorph N, Kumar N, Elso CM, DeNicola M, Bottino R, Powers AC, Harlan DM, Kent SC, Mannering SI, Haskins K (2016) Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science* 351:711–4 [PubMed: 26912858]
- Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Muller M, Kreymborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee HG, Stevanovic S (2005) Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc Natl Acad Sci U S A* 102:7922–7 [PubMed: 15894616]
- Denzin LK (2013) Inhibition of HLA-DM Mediated MHC Class II Peptide Loading by HLA-DO Promotes Self Tolerance. *Front Immunol* 4:465 [PubMed: 24381574]
- Denzin LK, Fallas JL, Prendes M, Yi W (2005) Right place, right time, right peptide: DO keeps DM focused. *Immunol Rev* 207:279–92 [PubMed: 16181343]
- Denzin LK, Khan AA, Viridis F, Wilks J, Kane M, Beilinson HA, Dikiy S, Case LK, Roopenian D, Witkowski M, Chervonsky AV, Golovkina TV (2017) Neutralizing Antibody Responses to Viral Infections Are Linked to the Non-classical MHC Class II Gene H2-Ob. *Immunity* 47:310–322 e7 [PubMed: 28813660]
- Denzin LK, Sant'Angelo DB, Hammond C, Surman MJ, Cresswell P (1997) Negative regulation by HLA-DO of MHC class II-restricted antigen processing. *Science* 278:106–9 [PubMed: 9311912]
- Derbinski J, Kyewski B (2010) How thymic antigen presenting cells sample the body's self-antigens. *Curr Opin Immunol* 22:592–600 [PubMed: 20832274]
- Deshaias F, Brunet A, Diallo DA, Denzin LK, Samaan A, Thibodeau J (2005) A point mutation in the groove of HLA-DO allows egress from the endoplasmic reticulum independent of HLA-DM. *Proc Natl Acad Sci U S A* 102:6443–8 [PubMed: 15849268]
- Douek DC, Altmann DM (1997) HLA-DO is an intracellular class II molecule with distinctive thymic expression. *Int Immunol* 9:355–64 [PubMed: 9088974]
- Douek DC, Altmann DM (2000) T-cell apoptosis and differential human leucocyte antigen class II expression in human thymus. *Immunology* 99:249–56 [PubMed: 10692044]
- Draheim M, Wlodarczyk MF, Crozat K, Saliou JM, Alayi TD, Tomavo S, Hassan A, Salvioni A, Demarta-Gatsi C, Sidney J, Sette A, Dalod M, Berry A, Silvie O, Blanchard N (2017) Profiling MHC II immunopeptidome of blood-stage malaria reveals that cDC1 control the functionality of parasite-specific CD4 T cells. *EMBO Mol Med* 9:1605–1621 [PubMed: 28935714]
- Driessen C, Bryant RA, Lennon-Dumenil AM, Villadangos JA, Bryant PW, Shi GP, Chapman HA, Ploegh HL (1999) Cathepsin S controls the trafficking and maturation of MHC class II molecules in dendritic cells. *J Cell Biol* 147:775–90 [PubMed: 10562280]

- Driessen C, Lennon-Dumenil AM, Ploegh HL (2001) Individual cathepsins degrade immune complexes internalized by antigen-presenting cells via Fcγ receptors. *Eur J Immunol* 31:1592–601 [PubMed: 11465117]
- Drutman SB, Trombetta ES (2010) Dendritic cells continue to capture and present antigens after maturation in vivo. *J Immunol* 185:2140–6 [PubMed: 20644175]
- Elliott EA, Drake JR, Amigorena S, Elsemore J, Webster P, Mellman I, Flavell RA (1994) The invariant chain is required for intracellular transport and function of major histocompatibility complex class II molecules. *J Exp Med* 179:681–94 [PubMed: 8294875]
- Fallang LE, Roh S, Holm A, Bergseng E, Yoon T, Fleckenstein B, Bandyopadhyay A, Mellins ED, Sollid LM (2008) Complexes of two cohorts of CLIP peptides and HLA-DQ2 of the autoimmune DR3-DQ2 haplotype are poor substrates for HLA-DM. *J Immunol* 181:5451–5461 [PubMed: 18832702]
- Fallas JL, Tobin HM, Lou O, Guo D, Sant'Angelo DB, Denzin LK (2004) Ectopic expression of HLA-DO in mouse dendritic cells diminishes MHC class II antigen presentation. *J Immunol* 173:1549–60 [PubMed: 15265882]
- Fallas JL, Yi W, Draghi NA, O'Rourke HM, Denzin LK (2007) Expression patterns of H2-O in mouse B cells and dendritic cells correlate with cell function. *J Immunol* 178:1488–97 [PubMed: 17237397]
- Ferrante A, Gorski J (2010) Cutting edge: HLA-DM-mediated peptide exchange functions normally on MHC class II-peptide complexes that have been weakened by elimination of a conserved hydrogen bond. *J Immunol* 184:1153–8 [PubMed: 20038641]
- Ferrante A, Gorski J (2012) A Peptide/MHCII conformer generated in the presence of exchange peptide is substrate for HLA-DM editing. *Sci Rep* 2:386 [PubMed: 22545194]
- Ferrante A, Templeton M, Hoffman M, Castellini MJ (2015) The Thermodynamic Mechanism of Peptide-MHC Class II Complex Formation Is a Determinant of Susceptibility to HLA-DM. *J Immunol* 195:1251–61 [PubMed: 26116504]
- Fiebigler E, Meraner P, Weber E, Fang IF, Stingl G, Ploegh H, Maurer D (2001) Cytokines regulate proteolysis in major histocompatibility complex class II-dependent antigen presentation by dendritic cells. *J Exp Med* 193:881–92 [PubMed: 11304549]
- Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q, Azuma M, Krummel MF, Bluestone JA (2009) Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. *Nat Immunol* 10:1185–92 [PubMed: 19783989]
- Flannagan RS, Jaumouille V, Grinstein S (2012) The cell biology of phagocytosis. *Annu Rev Pathol* 7:61–98 [PubMed: 21910624]
- Fletcher AL, Malhotra D, Turley SJ (2011) Lymph node stroma broaden the peripheral tolerance paradigm. *Trends Immunol* 32:12–8 [PubMed: 21147035]
- Fortier MH, Caron E, Hardy MP, Voisin G, Lemieux S, Perreault C, Thibault P (2008) The MHC class I peptide repertoire is molded by the transcriptome. *J Exp Med* 205:595–610 [PubMed: 18299400]
- Fugmann T, Sofron A, Ritz D, Bootz F, Neri D (2017) The MHC Class II Immunoepitome of Lymph Nodes in Health and in Chemically Induced Colitis. *J Immunol* 198:1357–1364 [PubMed: 28011936]
- Fujinami RS (2001) Viruses and autoimmune disease--two sides of the same coin? *Trends Microbiol* 9:377–81 [PubMed: 11514220]
- Fujinami RS, Oldstone MB (1985) Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 230:1043–5 [PubMed: 2414848]
- Fujinami RS, von Herrath MG, Christen U, Whitton JL (2006) Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev* 19:80–94 [PubMed: 16418524]
- Fung-Leung WP, Surh CD, Liljedahl M, Pang J, Leturcq D, Peterson PA, Webb SR, Karlsson L (1996) Antigen presentation and T cell development in H2-M-deficient mice. *Science* 271:1278–81 [PubMed: 8638109]
- Gambineri E, Torgerson TR, Ochs HD (2003) Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by

mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 15:430–5 [PubMed: 12819471]

- Garrett WS, Chen LM, Kroschewski R, Ebersold M, Turley S, Trombetta S, Galan JE, Mellman I (2000) Developmental control of endocytosis in dendritic cells by Cdc42. *Cell* 102:325–34 [PubMed: 10975523]
- Glazier KS, Hake SB, Tobin HM, Chadburn A, Schattner EJ, Denzin LK (2002) Germinal center B cells regulate their capability to present antigen by modulation of HLA-DO. *J Exp Med* 195:1063–9 [PubMed: 11956297]
- Gommeaux J, Gregoire C, Nguessan P, Richelme M, Malissen M, Guerder S, Malissen B, Carrier A (2009) Thymus-specific serine protease regulates positive selection of a subset of CD4+ thymocytes. *Eur J Immunol* 39:956–64 [PubMed: 19283781]
- Gondre-Lewis TA, Moquin AE, Drake JR (2001) Prolonged antigen persistence within nonterminal late endocytic compartments of antigen-specific B lymphocytes. *J Immunol* 166:6657–64 [PubMed: 11359820]
- Gren ST, Rasmussen TB, Janciauskiene S, Hakansson K, Gerwien JG, Grip O (2015) A Single-Cell Gene-Expression Profile Reveals Inter-Cellular Heterogeneity within Human Monocyte Subsets. *PLoS One* 10:e0144351 [PubMed: 26650546]
- Griffin JP, Chu R, Harding CV (1997) Early endosomes and a late endocytic compartment generate different peptide-class II MHC complexes via distinct processing mechanisms. *J Immunol* 158:1523–32 [PubMed: 9029086]
- Gubser C, Schmalzer M, Rossi SW, Palmer E (2016) Monoclonal regulatory T cells provide insights into T cell suppression. *Sci Rep* 6:25758 [PubMed: 27210828]
- Guce AI, Mortimer SE, Yoon T, Painter CA, Jiang W, Mellins ED, Stern LJ (2013) HLA-DO acts as a substrate mimic to inhibit HLA-DM by a competitive mechanism. *Nat Struct Mol Biol* 20:90–8 [PubMed: 23222639]
- Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN (2014) The function of Fcγ receptors in dendritic cells and macrophages. *Nat Rev Immunol* 14:94–108 [PubMed: 24445665]
- Harkiolaki M, Holmes SL, Svendsen P, Gregersen JW, Jensen LT, McMahon R, Friese MA, van Boxel G, Etzensperger R, Tzartos JS, Kranc K, Sainsbury S, Harlos K, Mellins ED, Palace J, Esiri MM, van der Merwe PA, Jones EY, Fugger L (2009) T cell-mediated autoimmune disease due to low-affinity crossreactivity to common microbial peptides. *Immunity* 30:348–57 [PubMed: 19303388]
- Hemmer B, Fleckenstein BT, Vergelli M, Jung G, McFarland H, Martin R, Wiesmuller KH (1997) Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. *J Exp Med* 185:1651–9 [PubMed: 9151902]
- Hershberg RM, Framson PE, Cho DH, Lee LY, Kovats S, Beitz J, Blum JS, Nepom GT (1997) Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. *J Clin Invest* 100:204–15 [PubMed: 9202073]
- Honey K, Duff M, Beers C, Brissette WH, Elliott EA, Peters C, Maric M, Cresswell P, Rudensky A (2001) Cathepsin S regulates the expression of cathepsin L and the turnover of gamma-interferon-inducible lysosomal thiol reductase in B lymphocytes. *J Biol Chem* 276:22573–8 [PubMed: 11306582]
- Honey K, Nakagawa T, Peters C, Rudensky A (2002) Cathepsin L regulates CD4+ T cell selection independently of its effect on invariant chain: a role in the generation of positively selecting peptide ligands. *J Exp Med* 195:1349–58 [PubMed: 12021314]
- Hornell TM, Burster T, Jahnsen FL, Pashine A, Ochoa MT, Harding JJ, Macaubas C, Lee AW, Modlin RL, Mellins ED (2006) Human dendritic cell expression of HLA-DO is subset specific and regulated by maturation. *J Immunol* 176:3536–47 [PubMed: 16517722]
- Hou T, Macmillan H, Chen Z, Keech CL, Jin X, Sidney J, Strohma M, Yoon T, Mellins ED (2011) An insertion mutant in DQA1*0501 restores susceptibility to HLA-DM: implications for disease associations. *J Immunol* 187:2442–52 [PubMed: 21775680]
- Hsieh CS, deRoos P, Honey K, Beers C, Rudensky AY (2002) A role for cathepsin L and cathepsin S in peptide generation for MHC class II presentation. *J Immunol* 168:2618–25 [PubMed: 11884425]

- Hsieh CS, Lee HM, Lio CW (2012) Selection of regulatory T cells in the thymus. *Nat Rev Immunol* 12:157–67 [PubMed: 22322317]
- Huseby ES, White J, Crawford F, Vass T, Becker D, Pinilla C, Marrack P, Kappler JW (2005) How the T cell repertoire becomes peptide and MHC specific. *Cell* 122:247–60 [PubMed: 16051149]
- Hwang I, Huang JF, Kishimoto H, Brunmark A, Peterson PA, Jackson MR, Surh CD, Cai Z, Sprent J (2000) T cells can use either T cell receptor or CD28 receptors to absorb and internalize cell surface molecules derived from antigen-presenting cells. *J Exp Med* 191:1137–48 [PubMed: 10748232]
- Ireland JM, Unanue ER (2011) Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J Exp Med* 208:2625–32 [PubMed: 22162830]
- Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL, Jr., Eissa NT (2009) Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med* 15:267–76 [PubMed: 19252503]
- Jiang W, Strohmman MJ, Somasundaram S, Ayyangar S, Hou T, Wang N, Mellins ED (2015) pH-susceptibility of HLA-DO tunes DO/DM ratios to regulate HLA-DM catalytic activity. *Sci Rep* 5:17333 [PubMed: 26610428]
- Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM, Nussenzweig MC (1995) The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 375:151–5 [PubMed: 7753172]
- Jin N, Wang Y, Crawford F, White J, Marrack P, Dai S, Kappler JW (2015) N-terminal additions to the WE14 peptide of chromogranin A create strong autoantigen agonists in type 1 diabetes. *Proc Natl Acad Sci U S A* 112:13318–23 [PubMed: 26453556]
- Joly E, Hudrisier D (2003) What is trogocytosis and what is its purpose? *Nat Immunol* 4:815 [PubMed: 12942076]
- Kakehashi H, Nishioku T, Tsukuba T, Kadowaki T, Nakamura S, Yamamoto K (2007) Differential regulation of the nature and functions of dendritic cells and macrophages by cathepsin E. *J Immunol* 179:5728–37 [PubMed: 17947645]
- Kanamori M, Nakatsukasa H, Okada M, Lu Q, Yoshimura A (2016) Induced Regulatory T Cells: Their Development, Stability, and Applications. *Trends Immunol* 37:803–811 [PubMed: 27623114]
- Karlsson L, Surh CD, Sprent J, Peterson PA (1991) A novel class II MHC molecule with unusual tissue distribution. *Nature* 351:485–8 [PubMed: 1675431]
- Kasai M, Kominami E, Mizuochi T (1998) The antigen presentation pathway in medullary thymic epithelial cells, but not that in cortical thymic epithelial cells, conforms to the endocytic pathway. *Eur J Immunol* 28:1867–76 [PubMed: 9645368]
- Kasai M, Tanida I, Ueno T, Kominami E, Seki S, Ikeda T, Mizuochi T (2009) Autophagic compartments gain access to the MHC class II compartments in thymic epithelium. *J Immunol* 183:7278–85 [PubMed: 19915056]
- Kaushik S, Cuervo AM (2018) The coming of age of chaperone-mediated autophagy. *Nat Rev Mol Cell Biol* 19:365–381 [PubMed: 29626215]
- Kim A, Hartman IZ, Poore B, Boronina T, Cole RN, Song N, Ciudad MT, Caspi RR, Jaraquemada D, Sadegh-Nasseri S (2014) Divergent paths for the selection of immunodominant epitopes from distinct antigenic sources. *Nat Commun* 5:5369 [PubMed: 25413013]
- Klein L, Hinterberger M, Wirsberger G, Kyewski B (2009) Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat Rev Immunol* 9:833–44 [PubMed: 19935803]
- Klein L, Jovanovic K (2011) Regulatory T cell lineage commitment in the thymus. *Semin Immunol* 23:401–9 [PubMed: 21733719]
- Klein L, Kyewski B, Allen PM, Hogquist KA (2014) Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol* 14:377–91 [PubMed: 24830344]
- Klionsky DJ (2007) Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 8:931–7 [PubMed: 17712358]
- Kreisel D, Richardson SB, Li W, Lin X, Kornfeld CG, Sugimoto S, Hsieh CS, Gelman AE, Krupnick AS (2010) Cutting edge: MHC class II expression by pulmonary nonhematopoietic cells plays a

critical role in controlling local inflammatory responses. *J Immunol* 185:3809–13 [PubMed: 20810992]

- Kremer AN, van der Meijden ED, Honders MW, Goeman JJ, Wiertz EJ, Falkenburg JH, Griffioen M (2012) Endogenous HLA class II epitopes that are immunogenic in vivo show distinct behavior toward HLA-DM and its natural inhibitor HLA-DO. *Blood* 120:3246–55 [PubMed: 22889757]
- Kroger CJ, Spidale NA, Wang B, Tisch R (2017) Thymic Dendritic Cell Subsets Display Distinct Efficiencies and Mechanisms of Intercellular MHC Transfer. *J Immunol* 198:249–256 [PubMed: 27895179]
- Kropshofer H, Vogt AB, Moldenhauer G, Hammer J, Blum JS, Hammerling GJ (1996) Editing of the HLA-DR-peptide repertoire by HLA-DM. *EMBO J* 15:6144–54 [PubMed: 8947036]
- Kropshofer H, Vogt AB, Thery C, Armandola EA, Li BC, Moldenhauer G, Amigorena S, Hammerling GJ (1998) A role for HLA-DO as a co-chaperone of HLA-DM in peptide loading of MHC class II molecules. *EMBO J* 17:2971–81 [PubMed: 9606180]
- Lanzavecchia A (1985) Antigen-specific interaction between T and B cells. *Nature* 314:537–9 [PubMed: 3157869]
- Lautwein A, Burster T, Lennon-Dumenil AM, Overkleeft HS, Weber E, Kalbacher H, Driessen C (2002) Inflammatory stimuli recruit cathepsin activity to late endosomal compartments in human dendritic cells. *Eur J Immunol* 32:3348–57 [PubMed: 12432565]
- Lazarski CA, Chaves FA, Jenks SA, Wu S, Richards KA, Weaver JM, Sant AJ (2005) The kinetic stability of MHC class II:peptide complexes is a key parameter that dictates immunodominance. *Immunity* 23:29–40 [PubMed: 16039577]
- Lee HK, Mattei LM, Steinberg BE, Alberts P, Lee YH, Chervonsky A, Mizushima N, Grinstein S, Iwasaki A (2010) In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 32:227–39 [PubMed: 20171125]
- Lee HM, Bautista JL, Scott-Browne J, Mohan JF, Hsieh CS (2012) A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity* 37:475–86 [PubMed: 22921379]
- Lee J, Tam H, Adler L, Iltad-Minnihan A, Macaubas C, Mellins ED (2017) The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. *PLoS One* 12:e0183594 [PubMed: 28832681]
- Levine B, Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* 6:463–77 [PubMed: 15068787]
- Li P, Wang N, Zhou D, Yee CS, Chang CH, Brutkiewicz RR, Blum JS (2005) Disruption of MHC class II-restricted antigen presentation by vaccinia virus. *J Immunol* 175:6481–8 [PubMed: 16272302]
- Liang Z, Zhang L, Su H, Luan R, Na N, Sun L, Zhao Y, Zhang X, Zhang Q, Li J, Zhang L, Zhao Y (2018) MTOR signaling is essential for the development of thymic epithelial cells and the induction of central immune tolerance. *Autophagy* 14:505–517 [PubMed: 29099279]
- Liljedahl M, Kuwana T, Fung-Leung WP, Jackson MR, Peterson PA, Karlsson L (1996) HLA-DO is a lysosomal resident which requires association with HLA-DM for efficient intracellular transport. *EMBO J* 15:4817–24 [PubMed: 8890155]
- Liljedahl M, Winqvist O, Surh CD, Wong P, Ngo K, Teyton L, Peterson PA, Brunmark A, Rudensky AY, Fung-Leung WP, Karlsson L (1998) Altered antigen presentation in mice lacking H2-O. *Immunity* 8:233–43 [PubMed: 9492004]
- Lim JP, Gleeson PA (2011) Macropinocytosis: an endocytic pathway for internalising large gulps. *Immunol Cell Biol* 89:836–43 [PubMed: 21423264]
- Lin XP, Almquist N, Telemo E (2005) Human small intestinal epithelial cells constitutively express the key elements for antigen processing and the production of exosomes. *Blood Cells Mol Dis* 35:122–8 [PubMed: 16027013]
- Lindner R, Unanue ER (1996) Distinct antigen MHC class II complexes generated by separate processing pathways. *EMBO J* 15:6910–20 [PubMed: 9003767]
- Linehan SA, Martinez-Pomares L, Stahl PD, Gordon S (1999) Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: In situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. *J Exp Med* 189:1961–72 [PubMed: 10377192]

- Lotteau V, Teyton L, Peleraux A, Nilsson T, Karlsson L, Schmid SL, Quaranta V, Peterson PA (1990) Intracellular transport of class II MHC molecules directed by invariant chain. *Nature* 348:600–5 [PubMed: 2250716]
- Lovitch SB, Petzold SJ, Unanue ER (2003) Cutting edge: H-2DM is responsible for the large differences in presentation among peptides selected by I-Ak during antigen processing. *J Immunol* 171:2183–6 [PubMed: 12928360]
- Malhotra S, Kovats S, Zhang W, Coggeshall KM (2009) B cell antigen receptor endocytosis and antigen presentation to T cells require Vav and dynamin. *J Biol Chem* 284:24088–97 [PubMed: 19586920]
- Manoury B, Hewitt EW, Morrice N, Dando PM, Barrett AJ, Watts C (1998) An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. *Nature* 396:695–9 [PubMed: 9872320]
- Manoury B, Mazzeo D, Fugger L, Viner N, Ponsford M, Streeter H, Mazza G, Wraith DC, Watts C (2002) Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP. *Nat Immunol* 3:169–74 [PubMed: 11812994]
- Martin WD, Hicks GG, Mendiratta SK, Leva HI, Ruley HE, Van Kaer L (1996) H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell* 84:543–50 [PubMed: 8598041]
- Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, Orchard R, Guan JL, Tan H, Peng J, Kanneganti TD, Virgin HW, Green DR (2015) Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat Cell Biol* 17:893–906 [PubMed: 26098576]
- Masternak K, Muhlethaler-Mottet A, Villard J, Zufferey M, Steimle V, Reith W (2000) CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. *Genes Dev* 14:1156–66 [PubMed: 10809673]
- Mathis D, Benoist C (2009) Aire. *Annu Rev Immunol* 27:287–312 [PubMed: 19302042]
- McDouall RM, Yacoub M, Rose ML (1996) Isolation, culture, and characterisation of MHC class II-positive microvascular endothelial cells from the human heart. *Microvasc Res* 51:137–52 [PubMed: 8778570]
- Millet V, Naquet P, Guinamard RR (2008) Intercellular MHC transfer between thymic epithelial and dendritic cells. *Eur J Immunol* 38:1257–63 [PubMed: 18412162]
- Miyazaki T, Wolf P, Tourne S, Waltzinger C, Dierich A, Barois N, Ploegh H, Benoist C, Mathis D (1996) Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell* 84:531–41 [PubMed: 8598040]
- Mohan JF, Petzold SJ, Unanue ER (2011) Register shifting of an insulin peptide-MHC complex allows diabetogenic T cells to escape thymic deletion. *J Exp Med* 208:2375–83 [PubMed: 22065673]
- Mohan JF, Unanue ER (2012) Unconventional recognition of peptides by T cells and the implications for autoimmunity. *Nat Rev Immunol* 12:721–8 [PubMed: 22955843]
- Mommen GP, Marino F, Meiring HD, Poelen MC, van Gaans-van den Brink JA, Mohammed S, Heck AJ, van Els CA (2016) Sampling From the Proteome to the Human Leukocyte Antigen-DR (HLA-DR) Ligandome Proceeds Via High Specificity. *Mol Cell Proteomics* 15:1412–23 [PubMed: 26764012]
- Moran AE, Holzapfel KL, Xing Y, Cunningham NR, Maltzman JS, Punt J, Hogquist KA (2011) T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J Exp Med* 208:1279–89 [PubMed: 21606508]
- Moss CX, Villadangos JA, Watts C (2005) Destructive potential of the aspartyl protease cathepsin D in MHC class II-restricted antigen processing. *Eur J Immunol* 35:3442–51 [PubMed: 16259009]
- Mueller DL (2010) Mechanisms maintaining peripheral tolerance. *Nat Immunol* 11:21–7 [PubMed: 20016506]
- Muixi L, Contreras V, Collado JA, Alexandre Y, Ballingall K, Bonneau M, Jaraquemada D, Schwartz-Cornil I (2012) Unraveling features of the natural MHC class II peptidome of skin-migrated dendritic cells. *Int Immunol* 24:59–69 [PubMed: 22194283]

- Muzaki AR, Tetlak P, Sheng J, Loh SC, Setiagani YA, Poidinger M, Zolezzi F, Karjalainen K, Ruedl C (2016) Intestinal CD103(+)CD11b(−) dendritic cells restrain colitis via IFN-gamma-induced anti-inflammatory response in epithelial cells. *Mucosal Immunol* 9:336–51 [PubMed: 26174764]
- Myers JT, Tsang AW, Swanson JA (2003) Localized reactive oxygen and nitrogen intermediates inhibit escape of *Listeria monocytogenes* from vacuoles in activated macrophages. *J Immunol* 171:5447–53 [PubMed: 14607950]
- Nakagawa T, Roth W, Wong P, Nelson A, Farr A, Deussing J, Villadangos JA, Ploegh H, Peters C, Rudensky AY (1998) Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus. *Science* 280:450–3 [PubMed: 9545226]
- Nakagawa TY, Brissette WH, Lira PD, Griffiths RJ, Petrushova N, Stock J, McNeish JD, Eastman SE, Howard ED, Clarke SR, Rosloniec EF, Elliott EA, Rudensky AY (1999) Impaired invariant chain degradation and antigen presentation and diminished collagen-induced arthritis in cathepsin S null mice. *Immunity* 10:207–17 [PubMed: 10072073]
- Nakayama M (2014) Antigen Presentation by MHC-Dressed Cells. *Front Immunol* 5:672 [PubMed: 25601867]
- Nakayama M, Takeda K, Kawano M, Takai T, Ishii N, Ogasawara K (2011) Natural killer (NK)-dendritic cell interactions generate MHC class II-dressed NK cells that regulate CD4+ T cells. *Proc Natl Acad Sci U S A* 108:18360–5 [PubMed: 22042851]
- Narayan K, Su KW, Chou CL, Khoruzhenko S, Sadegh-Nasseri S (2009) HLA-DM mediates peptide exchange by interacting transiently and repeatedly with HLA-DR1. *Mol Immunol* 46:3157–62 [PubMed: 19647320]
- Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L (2008) Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455:396–400 [PubMed: 18701890]
- Nguyen H, James EA (2016) Immune recognition of citrullinated epitopes. *Immunology* 149:131–8 [PubMed: 27531825]
- Nguyen TB, Jayaraman P, Bergseng E, Madhusudhan MS, Kim CY, Sollid LM (2017) Unraveling the structural basis for the unusually rich association of human leukocyte antigen DQ2.5 with class-II-associated invariant chain peptides. *J Biol Chem* 292:9218–9228 [PubMed: 28364043]
- Nijman HW, Kleijmeer MJ, Ossevoort MA, Oorschot VM, Vierboom MP, van de Keur M, Kenemans P, Kast WM, Geuze HJ, Melief CJ (1995) Antigen capture and major histocompatibility class II compartments of freshly isolated and cultured human blood dendritic cells. *J Exp Med* 182:163–74 [PubMed: 7790816]
- Nishimura H, Nose M, Hiai H, Minato N, Honjo T (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11:141–51 [PubMed: 10485649]
- Olsson N, Schultz LM, Zhang L, Khodadoust MS, Narayan R, Czerwinski DK, Levy R, Elias JE (2018) T-Cell Immunopeptidomes Reveal Cell Subtype Surface Markers Derived From Intracellular Proteins. *Proteomics* 18:e1700410 [PubMed: 29493099]
- Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S (2008) Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc Natl Acad Sci U S A* 105:10113–8 [PubMed: 18635688]
- Painter CA, Negroni MP, Kellersberger KA, Zavala-Ruiz Z, Evans JE, Stern LJ (2011) Conformational lability in the class II MHC 310 helix and adjacent extended strand dictate HLA-DM susceptibility and peptide exchange. *Proc Natl Acad Sci U S A* 108:19329–34 [PubMed: 22084083]
- Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, Munz C (2005) Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 307:593–6 [PubMed: 15591165]
- Pathak SS, Blum JS (2000) Endocytic recycling is required for the presentation of an exogenous peptide via MHC class II molecules. *Traffic* 1:561–9 [PubMed: 11208144]
- Pathak SS, Lich JD, Blum JS (2001) Cutting edge: editing of recycling class II:peptide complexes by HLA-DM. *J Immunol* 167:632–5 [PubMed: 11441064]

- Perreraudeau M, Taylor PR, Stauss HJ, Lindstedt R, Bygrave AE, Pappin DJ, Ellmerich S, Whitten A, Rahman D, Canas B, Walport MJ, Botto M, Altmann DM (2000) Altered major histocompatibility complex class II peptide loading in H2-O-deficient mice. *Eur J Immunol* 30:2871–80 [PubMed: 11069069]
- Pierre P, Mellman I (1998) Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. *Cell* 93:1135–45 [PubMed: 9657147]
- Pierre P, Turley SJ, Gatti E, Hull M, Meltzer J, Mirza A, Inaba K, Steinman RM, Mellman I (1997) Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* 388:787–92 [PubMed: 9285592]
- Pieters J, Bakke O, Dobberstein B (1993) The MHC class II-associated invariant chain contains two endosomal targeting signals within its cytoplasmic tail. *J Cell Sci* 106 (Pt 3):831–46 [PubMed: 8308066]
- Pires D, Marques J, Pombo JP, Carmo N, Bettencourt P, Neyrolles O, Lugo-Villarino G, Anes E (2016) Role of Cathepsins in Mycobacterium tuberculosis Survival in Human Macrophages. *Sci Rep* 6:32247 [PubMed: 27572605]
- Platt CD, Ma JK, Chalouni C, Ebersold M, Bou-Reslan H, Carano RA, Mellman I, Delamarre L (2010) Mature dendritic cells use endocytic receptors to capture and present antigens. *Proc Natl Acad Sci U S A* 107:4287–92 [PubMed: 20142498]
- Pluger EB, Boes M, Alfonso C, Schroter CJ, Kalbacher H, Ploegh HL, Driessen C (2002) Specific role for cathepsin S in the generation of antigenic peptides in vivo. *Eur J Immunol* 32:467–76 [PubMed: 11813165]
- Pober JS, Collins T, Gimbrone MA, Jr., Cotran RS, Gitlin JD, Fiers W, Clayberger C, Krensky AM, Burakoff SJ, Reiss CS (1983) Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature* 305:726–9 [PubMed: 6415484]
- Pos W, Sethi DK, Call MJ, Schulze MS, Anders AK, Pyrdol J, Wucherpfennig KW (2012) Crystal structure of the HLA-DM-HLA-DR1 complex defines mechanisms for rapid peptide selection. *Cell* 151:1557–68 [PubMed: 23260142]
- Pos W, Sethi DK, Wucherpfennig KW (2013) Mechanisms of peptide repertoire selection by HLA-DM. *Trends Immunol* 34:495–501 [PubMed: 23835076]
- Raddrizzani L, Bono E, Vogt AB, Kropshofer H, Gallazzi F, Sturniolo T, Hammerling GJ, Sinigaglia F, Hammer J (1999) Identification of destabilizing residues in HLA class II-selected bacteriophage display libraries edited by HLA-DM. *Eur J Immunol* 29:660–8 [PubMed: 10064083]
- Ramsdell F, Ziegler SF (2014) FOXP3 and scurfy: how it all began. *Nat Rev Immunol* 14:343–9 [PubMed: 24722479]
- Ranella A, Vassiliadis S, Mastora C, Valentina M, Dionyssopoulou E, Athanassakis I (2005) Constitutive intracellular expression of human leukocyte antigen (HLA)-DO and HLA-DR but not HLA-DM in trophoblast cells. *Hum Immunol* 66:43–55 [PubMed: 15620461]
- Rausch MP, Irvine KR, Antony PA, Restifo NP, Cresswell P, Hastings KT (2010) GILT accelerates autoimmunity to the melanoma antigen tyrosinase-related protein 1. *J Immunol* 185:2828–35 [PubMed: 20668223]
- Reid PA, Watts C (1990) Cycling of cell-surface MHC glycoproteins through primaquine-sensitive intracellular compartments. *Nature* 346:655–7 [PubMed: 2166918]
- Reilly JJ, Jr., Chen P, Sailor LZ, Mason RW, Chapman HA, Jr. (1990) Uptake of extracellular enzyme by a novel pathway is a major determinant of cathepsin L levels in human macrophages. *J Clin Invest* 86:176–83 [PubMed: 2365815]
- Reith W, LeibundGut-Landmann S, Waldburger JM (2005) Regulation of MHC class II gene expression by the class II transactivator. *Nat Rev Immunol* 5:793–806 [PubMed: 16200082]
- Riese RJ, Mitchell RN, Villadangos JA, Shi GP, Palmer JT, Karp ER, De Sanctis GT, Ploegh HL, Chapman HA (1998) Cathepsin S activity regulates antigen presentation and immunity. *J Clin Invest* 101:2351–63 [PubMed: 9616206]
- Riese RJ, Wolf PR, Bromme D, Natkin LR, Villadangos JA, Ploegh HL, Chapman HA (1996) Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. *Immunity* 4:357–66 [PubMed: 8612130]

- Roche PA, Cresswell P (1990) Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 345:615–8 [PubMed: 2190094]
- Roche PA, Furuta K (2015) The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat Rev Immunol* 15:203–16 [PubMed: 25720354]
- Rock KL, Benacerraf B, Abbas AK (1984) Antigen presentation by hapten-specific B lymphocytes. I. Role of surface immunoglobulin receptors. *J Exp Med* 160:1102–13 [PubMed: 6207262]
- Rouse RV, Parham P, Grumet FC, Weissman IL (1982) Expression of HLA antigens by human thymic epithelial cells. *Hum Immunol* 5:21–34 [PubMed: 6956563]
- Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 155:1151–64 [PubMed: 7636184]
- Sallusto F, Cella M, Danieli C, Lanzavecchia A (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 182:389–400 [PubMed: 7629501]
- Sallusto F, Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179:1109–18 [PubMed: 8145033]
- Sant AJ, Chaves FA, Jenks SA, Richards KA, Menges P, Weaver JM, Lazarski CA (2005) The relationship between immunodominance, DM editing, and the kinetic stability of MHC class II:peptide complexes. *Immunol Rev* 207:261–78 [PubMed: 16181342]
- Sant AJ, Chaves FA, Leddon SA, Tung J (2013) The control of the specificity of CD4 T cell responses: thresholds, breakpoints, and ceilings. *Front Immunol* 4:340 [PubMed: 24167504]
- Santin AD, Hermonat PL, Ravaggi A, Chiriva-Internati M, Cannon MJ, Hiserodt JC, Pecorelli S, Parham GP (1999) Expression of surface antigens during the differentiation of human dendritic cells vs macrophages from blood monocytes in vitro. *Immunobiology* 200:187–204 [PubMed: 10416127]
- Savina A, Amigorena S (2007) Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* 219:143–56 [PubMed: 17850487]
- Savina A, Peres A, Cebrian I, Carmo N, Moita C, Hacohen N, Moita LF, Amigorena S (2009) The small GTPase Rac2 controls phagosomal alkalization and antigen crosspresentation selectively in CD8(+) dendritic cells. *Immunity* 30:544–55 [PubMed: 19328020]
- 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, Kesmir C (2015) Comprehensive Analysis of the Naturally Processed Peptide Repertoire: Differences between HLA-A and B in the Immunopeptidome. *PLoS One* 10:e0136417 [PubMed: 26375851]
- Schmid D, Pypaert M, Munz C (2007) Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 26:79–92 [PubMed: 17182262]
- Scott H, Solheim BG, Brandtzaeg P, Thorsby E (1980) HLA-DR-like antigens in the epithelium of the human small intestine. *Scand J Immunol* 12:77–82 [PubMed: 6997989]
- Sealy L, Mota F, Rayment N, Tatnell P, Kay J, Chain B (1996) Regulation of cathepsin E expression during human B cell differentiation in vitro. *Eur J Immunol* 26:1838–43 [PubMed: 8765029]
- Senior RM, Campbell EJ (1984) Cathepsin G in human mononuclear phagocytes: comparisons between monocytes and U937 monocyte-like cells. *J Immunol* 132:2547–51 [PubMed: 6562154]
- Shevach EM (2006) From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity* 25:195–201 [PubMed: 16920638]
- Shi GP, Bryant RA, Riese R, Verhelst S, Driessen C, Li Z, Bromme D, Ploegh HL, Chapman HA (2000) Role for cathepsin F in invariant chain processing and major histocompatibility complex class II peptide loading by macrophages. *J Exp Med* 191:1177–86 [PubMed: 10748235]

- Shi GP, Villadangos JA, Dranoff G, Small C, Gu L, Haley KJ, Riese R, Ploegh HL, Chapman HA (1999) Cathepsin S required for normal MHC class II peptide loading and germinal center development. *Immunity* 10:197–206 [PubMed: 10072072]
- Sipila KH, Ranga V, Rappu P, Mali M, Pirila L, Heino I, Jokinen J, Kapyla J, Johnson MS, Heino J (2017) Joint inflammation related citrullination of functional arginines in extracellular proteins. *Sci Rep* 7:8246 [PubMed: 28811641]
- Sloan VS, Cameron P, Porter G, Gammon M, Amaya M, Mellins E, Zaller DM (1995) Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature* 375:802–6 [PubMed: 7596415]
- Sofron A, Ritz D, Neri D, Fugmann T (2016) High-resolution analysis of the murine MHC class II immunopeptidome. *Eur J Immunol* 46:319–28 [PubMed: 26495903]
- Spencer CT, Dragovic SM, Conant SB, Gray JJ, Zheng M, Samir P, Niu X, Moutaftsi M, Van Kaer L, Sette A, Link AJ, Joyce S (2013) 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* 43:1162–72 [PubMed: 23386199]
- Steinman RM, Brodie SE, Cohn ZA (1976) Membrane flow during pinocytosis. A stereologic analysis. *J Cell Biol* 68:665–87 [PubMed: 1030706]
- Steinman RM, Hawiger D, Liu K, Bonifaz L, Bonnyay D, Mahnke K, Iyoda T, Ravetch J, Dhodapkar M, Inaba K, Nussenzweig M (2003) Dendritic cell function in vivo during the steady state: a role in peripheral tolerance. *Ann N Y Acad Sci* 987:15–25 [PubMed: 12727620]
- Stoeckle C, Quecke P, Ruckrich T, Burster T, Reich M, Weber E, Kalbacher H, Driessen C, Melms A, Tolosa E (2012) Cathepsin S dominates autoantigen processing in human thymic dendritic cells. *J Autoimmun* 38:332–43 [PubMed: 22424724]
- Stoeckle C, Sommandas V, Adamopoulou E, Belisle K, Schiekofer S, Melms A, Weber E, Driessen C, Boehm BO, Tolosa E, Burster T (2009) Cathepsin G is differentially expressed in primary human antigen-presenting cells. *Cell Immunol* 255:41–5 [PubMed: 19036358]
- Strug I, Calvo-Calle JM, Green KM, Cruz J, Ennis FA, Evans JE, Stern LJ (2008) Vaccinia peptides eluted from HLA-DR1 isolated from virus-infected cells are recognized by CD4+ T cells from a vaccinated donor. *J Proteome Res* 7:2703–11 [PubMed: 18507432]
- Sukseree S, Mildner M, Rossiter H, Pammer J, Zhang CF, Watanapokasin R, Tschachler E, Eckhart L (2012) Autophagy in the thymic epithelium is dispensable for the development of self-tolerance in a novel mouse model. *PLoS One* 7:e38933 [PubMed: 22719991]
- Surh CD, Gao EK, Kosaka H, Lo D, Ahn C, Murphy DB, Karlsson L, Peterson P, Sprent J (1992) Two subsets of epithelial cells in the thymic medulla. *J Exp Med* 176:495–505 [PubMed: 1500857]
- Surh CD, Lee DS, Fung-Leung WP, Karlsson L, Sprent J (1997) Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of CD4+ T cells. *Immunity* 7:209–19 [PubMed: 9285406]
- Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 119:301–11 [PubMed: 1400575]
- Tewari MK, Sinnathamby G, Rajagopal D, Eisenlohr LC (2005) A cytosolic pathway for MHC class II-restricted antigen processing that is proteasome and TAP dependent. *Nat Immunol* 6:287–94 [PubMed: 15711549]
- Thelemann C, Eren RO, Coutaz M, Brasseit J, Bouzourene H, Rosa M, Duval A, Lavanchy C, Mack V, Mueller C, Reith W, Acha-Orbea H (2014) Interferon-gamma induces expression of MHC class II on intestinal epithelial cells and protects mice from colitis. *PLoS One* 9:e86844 [PubMed: 24489792]
- Tolosa E, Li W, Yasuda Y, Wienhold W, Denzin LK, Lautwein A, Driessen C, Schnorrer P, Weber E, Stevanovic S, Kurek R, Melms A, Bromme D (2003) Cathepsin V is involved in the degradation of invariant chain in human thymus and is overexpressed in myasthenia gravis. *J Clin Invest* 112:517–26 [PubMed: 12925692]
- Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I (2003) Activation of lysosomal function during dendritic cell maturation. *Science* 299:1400–3 [PubMed: 12610307]
- Underhill DM, Goodridge HS (2012) Information processing during phagocytosis. *Nat Rev Immunol* 12:492–502 [PubMed: 22699831]

- van Ham M, van Lith M, Lillemeier B, Tjin E, Gruneberg U, Rahman D, Pastoors L, van Meijgaarden K, Roucard C, Trowsdale J, Ottenhoff T, Pappin D, Neefjes J (2000) Modulation of the major histocompatibility complex class II-associated peptide repertoire by human histocompatibility leukocyte antigen (HLA)-DO. *J Exp Med* 191:1127–36 [PubMed: 10748231]
- van Kasteren SI, Overkleef HS (2014) Endo-lysosomal proteases in antigen presentation. *Curr Opin Chem Biol* 23:8–15 [PubMed: 25213682]
- Vandenabeele S, Hochrein H, Mavaddat N, Winkel K, Shortman K (2001) Human thymus contains 2 distinct dendritic cell populations. *Blood* 97:1733–41 [PubMed: 11238115]
- Veerappan Ganesan AP, Eisenlohr LC (2017) The elucidation of non-classical MHC class II antigen processing through the study of viral antigens. *Curr Opin Virol* 22:71–76 [PubMed: 28081485]
- Vignali DA (2012) Mechanisms of T(reg) Suppression: Still a Long Way to Go. *Front Immunol* 3:191 [PubMed: 22783262]
- Villadangos JA, Bryant RA, Deussing J, Driessen C, Lennon-Dumenil AM, Riese RJ, Roth W, Saftig P, Shi GP, Chapman HA, Peters C, Ploegh HL (1999) Proteases involved in MHC class II antigen presentation. *Immunol Rev* 172:109–20 [PubMed: 10631941]
- Villadangos JA, Cardoso M, Steptoe RJ, van Berkel D, Pooley J, Carbone FR, Shortman K (2001) MHC class II expression is regulated in dendritic cells independently of invariant chain degradation. *Immunity* 14:739–49 [PubMed: 11420044]
- Villadangos JA, Driessen C, Shi GP, Chapman HA, Ploegh HL (2000) Early endosomal maturation of MHC class II molecules independently of cysteine proteases and H-2DM. *EMBO J* 19:882–91 [PubMed: 10698930]
- Villadangos JA, Ploegh HL (2000) Proteolysis in MHC class II antigen presentation: who's in charge? *Immunity* 12:233–9 [PubMed: 10755610]
- Villasenor J, Besse W, Benoist C, Mathis D (2008) Ectopic expression of peripheral-tissue antigens in the thymic epithelium: probabilistic, monoallelic, misinitiated. *Proc Natl Acad Sci U S A* 105:15854–9 [PubMed: 18836079]
- Vogt AB, Kropshofer H, Moldenhauer G, Hammerling GJ (1996) Kinetic analysis of peptide loading onto HLA-DR molecules mediated by HLA-DM. *Proc Natl Acad Sci U S A* 93:9724–9 [PubMed: 8790398]
- Walter W, Loos M, Maeurer MJ (1999) Differential expression of alternative H2-M isoforms in B cells, dendritic cells and macrophages by proinflammatory cytokines. *Mol Immunol* 36:733–43 [PubMed: 10593512]
- Weber DA, Evavold BD, Jensen PE (1996) Enhanced dissociation of HLA-DR-bound peptides in the presence of HLA-DM. *Science* 274:618–20 [PubMed: 8849454]
- West LC, Grotzke JE, Cresswell P (2013) MHC class II-restricted presentation of the major house dust mite allergen Der p 1 Is GILT-dependent: implications for allergic asthma. *PLoS One* 8:e51343 [PubMed: 23326313]
- West MA, Prescott AR, Eskelinen EL, Ridley AJ, Watts C (2000) Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation. *Curr Biol* 10:839–48 [PubMed: 10899002]
- Wiman K, Curman B, Forsum U, Klareskog L, Malmnas-Tjernlund U, Rask L, Tragardh L, Peterson PA (1978) Occurrence of Ia antigens on tissues of non-lymphoid origin. *Nature* 276:711–3 [PubMed: 366434]
- Wolf AJ, Arruda A, Reyes CN, Kaplan AT, Shimada T, Shimada K, Arditi M, Liu G, Underhill DM (2011) Phagosomal degradation increases TLR access to bacterial ligands and enhances macrophage sensitivity to bacteria. *J Immunol* 187:6002–10 [PubMed: 22031762]
- Wu C, Aichinger M, Nedjic J, Klein L (2013) Thymic epithelial cells use macroautophagy to turn their inside out for CD4 T cell tolerance. *Autophagy* 9:931–2 [PubMed: 23548947]
- Wucherpfennig KW, Strominger JL (1995) Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 80:695–705 [PubMed: 7534214]
- Xing Y, Hogquist KA (2012) T-cell tolerance: central and peripheral. *Cold Spring Harb Perspect Biol* 4

- Xiu F, Cote MH, Bourgeois-Daigneault MC, Brunet A, Gauvreau ME, Shaw A, Thibodeau J (2011) Cutting edge: HLA-DO impairs the incorporation of HLA-DM into exosomes. *J Immunol* 187:1547–51 [PubMed: 21768396]
- Yadav M, Stephan S, Bluestone JA (2013) Peripherally induced tregs - role in immune homeostasis and autoimmunity. *Front Immunol* 4:232 [PubMed: 23966994]
- Yang SJ, Ahn S, Park CS, Holmes KL, Westrup J, Chang CH, Kim MG (2006) The quantitative assessment of MHC II on thymic epithelium: implications in cortical thymocyte development. *Int Immunol* 18:729–39 [PubMed: 16569676]
- Yi W, Seth NP, Martillotti T, Wucherpfennig KW, Sant'Angelo DB, Denzin LK (2010) Targeted regulation of self-peptide presentation prevents type I diabetes in mice without disrupting general immunocompetence. *J Clin Invest* 120:1324–36 [PubMed: 20200448]
- Yin L, Calvo-Calle JM, Dominguez-Amoroch O, Stern LJ (2012) 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* 189:3983–94 [PubMed: 22966084]
- Yin L, Trenh P, Guce A, Wieczorek M, Lange S, Sticht J, Jiang W, Bylsma M, Mellins ED, Freund C, Stern LJ (2014) Susceptibility to HLA-DM protein is determined by a dynamic conformation of major histocompatibility complex class II molecule bound with peptide. *J Biol Chem* 289:23449–64 [PubMed: 25002586]
- Yoon T, Macmillan H, Mortimer SE, Jiang W, Rinderknecht CH, Stern LJ, Mellins ED (2012) Mapping the HLA-DO/HLA-DM complex by FRET and mutagenesis. *Proc Natl Acad Sci U S A* 109:11276–81 [PubMed: 22733780]
- Zarutskie JA, Busch R, Zavala-Ruiz Z, Rushe M, Mellins ED, Stern LJ (2001) The kinetic basis of peptide exchange catalysis by HLA-DM. *Proc Natl Acad Sci U S A* 98:12450–5 [PubMed: 11606721]
- Zawada AM, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, Heine GH (2011) SuperSAGE evidence for CD14⁺⁺CD16⁺ monocytes as a third monocyte subset. *Blood* 118:e50–61 [PubMed: 21803849]
- Zhou D, Li P, Lin Y, Lott JM, Hislop AD, Canaday DH, Brutkiewicz RR, Blum JS (2005) Lamp-2a facilitates MHC class II presentation of cytoplasmic antigens. *Immunity* 22:571–81 [PubMed: 15894275]
- Zhou Z, Callaway KA, Weber DA, Jensen PE (2009) Cutting edge: HLA-DM functions through a mechanism that does not require specific conserved hydrogen bonds in class II MHC-peptide complexes. *J Immunol* 183:4187–91 [PubMed: 19767569]
- Zhou Z, Reyes-Vargas E, Escobar H, Chang KY, Barker AP, Rockwood AL, Delgado JC, He X, Jensen PE (2017) Peptidomic analysis of type 1 diabetes associated HLA-DQ molecules and the impact of HLA-DM on peptide repertoire editing. *Eur J Immunol* 47:314–326 [PubMed: 27861808]
- Zhou Z, Reyes-Vargas E, Escobar H, Rudd B, Rockwood AL, Delgado JC, He X, Jensen PE (2016) 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* 46:834–45 [PubMed: 26707565]

Figure 1:

MHC Class II dimers are synthesized in the ER and loaded with invariant chain, after which they traffic to the endosome. Cathepsins cleave the invariant chain (Ii) to the residual peptide CLIP, which is then removed by the peptide exchange factor DM and exchanged for peptides derived from proteolyzed self- and foreign antigens. The MHC-II-peptide complex traffics to the cell surface for presentation to CD4 T cells. To maintain tolerance in noninflammatory conditions (left panel), the immunopeptidome in APCs is comprised of diverse self-peptide antigens acquired through extracellular uptake pathways as well as autophagy. Components of the antigen processing machinery including the DM inhibitor DO further enhance the diversity of the peptides presented. Upon activation and initiation of an inflammatory response (right panel), antigen sampling is reduced, endosomal proteases are redistributed to late endosomes, DO expression is downregulated, and MHC-II expression is substantially increased. (The effects of inflammation on the process of autophagy in APCs are incompletely understood.) During inflammation, these changes result in greater efficiency of

MHC-II antigen presentation as well as reduced diversity in the MHC-II peptidome in an immune response.

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Table 1.

Expression of antigen processing components in mouse and human APCs.

Cell type	MHC-II expression	Endosomal proteases ^b	DM	DO	Ratio of DM:DO	Refs (human)	Refs (mouse)
Immature DCs	+++	Cat S , Cat D, Cat E, Cat H, SPPL2A (mu) Cat D , Cat G , Cat S , AEP (hu)	✓	✓	Low	Burster et al. 2005; Cella et al. 1997; Hornell et al. 2006; Nijman et al. 1995; Sallusto and Lanzavecchia 1994; Stoeckle et al. 2009	Immgen ^e ; Bergmann et al. 2013; Chain et al. 2005; Chen et al. 2006; Driessen et al. 1999; Fallas et al. 2004; Moss et al. 2005; Nakagawa et al. 1998; Nakagawa et al. 1999; Pierre et al. 1997; Trombetta et al. 2003; Villadangos et al. 2001; West et al. 2013
Mature DCs	+++++	Cat D, Cat H, Cat L, AEP (ms), Cat S (hu and ms), Cat B (hu)	✓	-	High	Sallusto and Lanzavecchia 1994) (Cella et al. 1997; Fiebiger et al. 2001; Hornell et al. 2006	Immgen; Chen et al. 2006; Pierre and Mellman 1998; Pierre et al. 1997; Trombetta et al. 2003; Villadangos et al. 2001
Macrophages	++	Cat B , Cat E , Cat F , Cat L , Cat S , AEP	✓	-	High	Barascuk et al. 2010; Pires et al. 2016; Ranella et al. 2005; Reilly et al. 1990; Santin et al. 1999	Immgen; Baumgart et al. 1998; Beers et al. 2003; Driessen et al. 2001; Kakehashi et al. 2007; Manoury et al. 2002; Nakagawa et al. 1999; Shi et al. 2000; Walter et al. 1999
Monocytes	+	Cat B ^c , Cat G, Cat K ^c , Cat L ^c , Cat S ^c (hu)	✓	-	High	Gren et al. 2015; Lee et al. 2017; Nijman et al. 1995; Senior and Campbell 1984; Zawada et al. 2011	Immgen
Mature B cells	++	Cat B , Cat D , Cat L , Cat S , SPPL2A (ms), Cat E , Cat G , AEP (hu)	✓	✓	Low	Arunachalam et al. 2000; Burster et al. 2004; Burster et al. 2008; Chalouni et al. 2003; Glazier et al. 2002; Manoury et al. 1998; Riese et al. 1996	Immgen; Bergmann et al. 2013; Fallas et al. 2007; Honey et al. 2001; Nakagawa et al. 1999; Pluger et al. 2002; Rausch et al. 2010
GC B cells	+++	Cat S	✓	-	High	Chalouni et al. 2003; Chen et al. 2002; Glazier et al. 2002	Immgen; Fallas et al. 2007; Shi et al. 1999
cTECs ^a	++	Cat L , TSSP ^(ms) Cat G, Cat V (hu)	✓	-	High	Douek and Altmann 2000; Rouse et al. 1982; Stoeckle et al. 2009; Tolosa et al. 2003	Immgen; Gommeaux et al. 2009; Kasai et al. 1998; Nakagawa et al. 1998; Yang et al. 2006

Cell type	MHC-II expression	Endosomal proteases ^b	DM	DO	Ratio of DM:DO	Refs (human)	Refs (mouse)
mTECs ^a	++	Cat L ^c , Cat S ^c	✓	✓	Low	Douek and Altmann 2000; Karlsson et al. 1991; Rouse et al. 1982	Immgen; Douek and Altmann 1997; Kasai et al. 1998; Surh et al. 1992; Yang et al. 2006
Thymic DCs ^a	+++	Cat S (hu and ms), AEP (ms)	?	?	?	Stoeckle et al. 2012; Vandenabeele et al. 2001	Manoury et al. 2002; Nakagawa et al. 1998; Yang et al. 2006
Endothelium/epithelium ^d	++	Cat B, Cat D, Cat L (hu), Cat S (hu and ms)	✓	?	?	Bania et al. 2003; Barrera et al. 2001; Daar et al. 1984; Hershberg et al. 1997; Lin et al. 2005; Pober et al. 1983; Scott et al. 1980; Wiman et al. 1978	Beers et al. 2005; Kreisel et al. 2010; Muzaki et al. 2016

Abbreviations: DCs: dendritic cells; GC: germinal center; cTECs: cortical thymic epithelial cells; mTECs: medullary thymic epithelial cells; Cat: cathepsin; SPPL2A: signal peptide peptidase-like 2a; AEP: asparagine endopeptidase; TSSP: thymus-specific serine protease; hu: human; ms: mouse.

^aRelative MHC-II expression is estimated based on data in references included. For thymic DCs, no published data is available regarding DM and DO expression.

^bProteases in which enzymatic activity is reported are in bold, as are the references that report enzymatic activity (demonstrated via active site labeling or through effects on antigen presentation of specific epitopes). Of note, endosomal proteases have been shown in many instances to exhibit redundancy in their activity and specificity.

^cmRNA expression demonstrated only.

^dHuman endothelium and epithelium basally express MHC-II, while mouse MHC-II has been reported both to be expressed basally on epithelium and endothelium as well as only in response to inflammation (e.g. exposure to IFN- γ). MHC-II levels shown are in the context of inflammation. No published data are currently available on DO expression in human or mouse endothelium/epithelium in the periphery.

^eImmunological Genome Project (www.immgen.org)