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Review

Immune surveillance of intracellular pathogens via autophagy

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Abstract

MHC class II molecules are thought to present peptides derived from extracellular proteins to CD4$^+$ T cells, which are important mediators of adaptive immunity to infections. In contrast, autophagy delivers constitutively cytosolic material for lysosomal degradation and has so far been recognized as an efficient mechanism of innate immunity against bacteria and viruses. Recent studies, however, link these two pathways and suggest that intracellular cytosolic and nuclear antigens are processed for MHC class II presentation after autophagy.

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; DriPs, defective ribosomal products; TAP, transporter associated with antigen processing; APC, antigen-presenting cell; MIIC, MHC class II compartment; I_i, invariant chain; HLA, human leukocyte antigen; DC, dendritic cell; EBV, Epstein–Barr virus; EBNA1, EBV nuclear antigen 1; LCL, lymphoblastoid cell line; M1, influenza A matrix protein 1; LAMP, lysosome-associated membrane protein

Introduction

The T cells of the adaptive immune system monitor all body cells for the presence of pathogenic constituents with an elaborate detection system, involving display of microbial fragments on major histocompatibility complex (MHC) molecules at the cell surface. Proteins derived from intracellular or internalized pathogens are degraded by intracellular proteases into small protein fragments or peptides, which subsequently are loaded into the peptide-binding groove of MHC molecules. Peptide–MHC complexes are then presented on the cell surface and recognized by T cells with their specific T-cell receptor (TCR). There are two main classes of classical and polymorphic MHC molecules, MHC class I and II, that present peptides to two classes of T cells with different effector functions.[1] MHC class I molecules present peptides to cytolytic CD8$^+$ T cells and MHC class II molecules present peptides to CD4$^+$ T cells, which can have both immunoregulatory and cytolytic functions. Protein fragments for MHC class I and II presentation are in their majority generated by distinct and different proteolytic events. MHC class I ligands are primarily produced by the proteasome, whereas MHC class II ligands are generated in lysosomes.[2,3] In this review, we will discuss the classical paradigm concerning the antigen processing for MHC class I and II presentation and will describe recent developments suggesting that autophagy contributes to the processing and presentation of intracellular antigens on MHC class II molecules.

The Paradigm of Antigen Processing for MHC Presentation

The two main classes of classical and polymorphic MHC molecules are loaded with protein fragments in distinct cellular compartments and their peptide cargo reaches these compartments by different routes: Antigens for MHC class I presentation are primarily degraded by the proteasome, a large multicatalytic protease complex residing in nucleus and cytosol.[4] Targeting of these antigens for proteasomal degradation is often mediated by ubiquitinylation.[5] A large proportion of MHC class I ligands is derived from the so-called defective ribosomal products (DRiPs),[6,7] which are degraded by the ubiquitin–proteasome system immediately after misfolding or premature termination of translation[8] and provide cells with a rapid warning system against newly synthesized microbial proteins. The peptides generated by the proteasome are imported via the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER),[9] where they meet newly synthesized MHC class I molecules that have been cotranslationally inserted into the ER. With the help of the MHC class I loading complex, which includes chaperones, aminopeptidases and thiol oxidoreductases.[10–12] individual peptides of 8–9 amino acids in length are loaded into the peptide-binding groove of MHC class I molecules. Stable peptide–MHC class I complexes are then exported via the Golgi apparatus to the cell surface for recognition by CD8$^+$ T cells. Since MHC class I ligands are mainly generated in this proteasome- and TAP-dependent fashion, MHC class I antigens are thought to be primarily of cytosolic and nuclear origin.

In contrast, MHC class II ligands are thought to originate mainly from extracellular antigens, which are endocytosed by constitutively MHC class II-positive professional

antigen-presenting cells (APCs) for presentation to the immune system. These endocytosed antigens are degraded by lysosomal endo- and exoproteases and meet MHC class II molecules in the so-called MHC class II compartments (MIICs) or class II vesicles (CIIV).[13] MHC class II molecules migrate to these late endosomal compartments after cotranslational insertion into the ER, because they associate with the transmembrane protein invariant chain (I_i). I_i not only blocks the peptide-binding groove of newly synthesized MHC class II molecules,[14] but also contains an endosomal targeting signal[15] and thus targets MHC class II molecules to late endosomes, where they meet peptides generated by lysosomal proteases. In this MHC class II loading compartment, lysosomal proteases also degrade the I_i, and the remaining peptide (CLIP for class II-associated I_i peptide) is exchanged for antigenic peptides with the help of the nonclassical MHC class II molecule HLA-DM.[16] As a result of this pathway, MHC class II ligands are generated from extracellular antigens after endocytosis and degradation in lysosomes. Hence, MHC class II antigens are thought to be primarily of extracellular origin.

Nonclassical Pathways of Antigen Presentation

Until recently, MHC class I and II molecules were thought to be specialized in presenting peptides derived from distinct sources. MHC class I ligands were thought to be derived from cytosolic and nuclear proteins, whereas MHC class II ligands were believed to be solely generated from extracellular sources. Although these classical pathways of antigen presentation remain correct, it has become apparent that other pathways contribute to antigen presentation and that antigens from inside and outside the cell can be presented on both MHC class I and II.[17]

The classical paradigm of antigen processing was first challenged, when it was discovered that professional APCs, especially dendritic cells (DCs), are able to present extracellular antigen not only on MHC class II, but also on MHC class I.[18,19] This new exogenous pathway, termed 'cross-presentation' pathway, is thought to be important in both immunity and tolerance. It allows DCs to prime $CD8^+$ T-cell responses to antigens synthesized by cells other than DCs and to trigger both $CD8^+$ and $CD4^+$ T-cell responses at the same time, generating more effective and sustained T-cell responses.

The argument that the immune system should be able to survey all cell types and that antigens from all cellular compartments should be presented on both classes of MHC molecules, implies that a similar change in the antigen presentation paradigm might be necessary for MHC class II. Pathogens that replicate in the cytoplasm of professional APCs should be detectable for the immune system via both MHC class I and II presentation. Indeed, it has been shown that MHC class II molecules can present intracellular antigens, including cytosolic and nuclear proteins. This nonclassical MHC class II pathway was coined 'endogenous MHC class II pathway' and will be further discussed in the next paragraphs.

Endogenous MHC Class II Processing

The first evidence for the existence of an endogenous MHC class II pathway came from the analysis of natural MHC class II ligands. When MHC class II molecules were purified, primarily from Epstein–Barr virus (EBV)-transformed B lymphoblastoid cell lines (LCL), the majority of natural MHC class II ligands were found to be derived from intracellular proteins.[20,21] Surprisingly, more than 20% of the identified sequences came from cytosolic proteins[21,22] (Table 1). The sources of these peptides included cytoskeletal proteins (e.g. actin, tubulin, F-actin capping protein), constitutive metabolic enzymes (e.g. glyceraldehyde-3-phophate dehydrogenase (GAPDH), aspartate aminotransferase (AAT)), heat shock proteins (Hsp70)) and proteins involved in vesicular trafficking (Rab5A). In addition, a few of the identified peptides were derived from nuclear proteins, such as histones[23] (Table 1).

Further evidence for the existence of an endogenous MHC class II pathway came from the fact that $CD4^+$ T cell could recognize cytosolic and nuclear proteins after endogenous processing (Table 2). This pathway for $CD4^+$ T-cell recognition was first described by Long and colleagues, who studied presentation of cytosolic measles virus and influenza virus antigens to $CD4^+$ T cells.[24–27] These authors performed cell-mixing experiments to test whether the recognized antigens exit the cell and re-enter via endocytosis, that is, follow the classical MHC class II pathway. They observed, however, that antigen-specific $CD4^+$ T cells did not recognize mixtures of antigen-negative, HLA class II-matched B cells with antigen-expressing, HLA class II-mismatched B cells, but only antigen-expressing, HLA class II-matched B cells, thereby demonstrating that the antigen was not released and then endocytosed for MHC class II presentation.[24,25] These experiments showed for the first time that endogenous processing of cytosolic antigens could lead to MHC class II presentation.

Subsequently, presentation of endogenous proteins on MHC class II has been described for a number of other viral antigens[28–31] as well as self-antigens,[21,32–34] model antigens,[35–40] and tumor antigens[41,42] (Table 2). On the basis of these findings, four endogenous MHC class II processing pathways can be postulated[43–45] (Figure 1). Firstly, secreted/transmembrane proteins (e.g. influenza hemagglutinin (HA)[28]) can associate with newly synthesized MHC class II molecules in the ER, and then follow MHC class II–I_i complexes to endosomal compartments, where processing and peptide loading occurs. This pathway contributes the majority of endogenous MHC class II ligands, which were found to be derived from secreted/transmembrane proteins that intersect with the endocytic pathway.[21–23] Secondly, cytosolic peptides can be imported into the ER via TAP for binding to MHC class II molecules.[25] In certain APCs like DCs, this pathway is even accessed by exogenous antigens like influenza HA and neuraminidase, which leave the endosome for proteasome- and TAP-dependent processing onto MHC class II.[46] A third pathway involves processing of cytosolic or nuclear proteins (e.g. glutamate decarboxylase 65 (GAD65)[33]) by the proteasome and is TAP independent.[33,40] For this pathway, peptides seem to be imported directly into

Table 1 Cytosolic and nuclear protein sources of natural MHC class II ligands

Protein source	Localization	Cell type[a]	References
Actin	Cytosol	B, M	21,61,96
Actin-like protein	Cytosol	M	21
F-actin capping protein	Cytosol	B, M	21
Tubulin α- and β-chain	Cytosol	B, M	21,61
Microtubule-associated protein PB1	Cytosol	B	21
α-Catenin	Cytosol	B	21
Clp36	Cytosol	B	21
GAPDH	Cytosol	B, M, E	21,23,61,97
Aspartate aminotransferase	Cytosol	B, M	21
Alcohol dehydrogenase	Cytosol	M	21
Glucose-6-phosphate isomerase	Cytosol	M	21
Casein kinase 1-α	Cytosol	B	21
Rab5A	Cytosol	B	21
Cofactor D	Cytosol	B	21
pp65 macrophage protein	Cytosol	M	21
ATP citrate lyase	Cytosol	B	61
Actin-interacting protein 1	Cytosol	B	61
Triosephosphate isomerase 1	Cytosol	B	61
Peptidylprolyl isomerase A (cyclophilin A)	Cytosol	B	61
Atg8 (MAP1LC3b)	Cytosol	B	61
Annexin A2	Cytosol	B	61
Rab7	Cytosol	B	61
Acetyl-CoA acyltransferase 1	Cytosol	B	61
Dipeptidyl peptidase II	Cytosol	B	61
Phosphoglycerate kinase	Cytosol	B	23,61
Pyruvate kinase	Cytosol	B, E	23,97
Macrophage migration-inhibitory factor (MIF)	Cytosol	B	23,61
GBP-2 (IFN-induced guanylate-binding protein)	Cytosol	B	22
NADH-cytochrome b_5 reductase	Cytosol	B	22
c-Myc	Cytosol	B	22
k-Ras	Cytosol	B	22
Myosin	Cytosol	E	97
Fatty acid synthase	Cytosol	E	97
α-Enolase	Cytosol	B	61,97
Elongation factor 1	Cytosol	B	61,97
NEDD4La	Cytosol	B	61
Hsc70	Cytosol/Nucleus	B	22,23,61
Hsp90-beta	Cytosol/Nucleus	B	23
Ribosomal proteins S10, S13	Cytosol/Nucleus	B	61
Ubiquitin	Cytosol/Nucleus	B	61
EBV major capsid protein	Cytosol/Nucleus	B	22
Histone H3	Nucleus	B	23
Histone H2B	Nucleus	B	61
Rad23b	Nucleus	B	61
RAN	Nucleus	B	96

[a]Cell types: B = B cells; M = macrophages; E = epithelial cells

endosomal/lysosomal compartments, via a transporter that was recently suggested to be Lamp-2a, the transporter of chaperone-mediated autophagy.[47] In addition to these proteasome-dependent pathways, cytosolic and nuclear proteins can also be processed by a proteasome- and TAP-independent pathway: This fourth pathway involves the direct import of cytosolic/nuclear proteins (e.g. the EBV nuclear antigen 1 (EBNA1)[30,31]) into endosomes/lysosomes and is in part mediated by autophagy. The latter three pathways (processing of cytosolic or nuclear proteins by proteasome-dependent or -independent mechanisms) contribute more than 20% of endogenous MHC class II ligands.[21–23] Therefore, proteins residing in a compartment that is topologically distinct from the endocytic route and thus isolated from the classical MHC class II pathway, can gain access to MHC class II molecules and broaden the repertoire of MHC class II ligands.

Proteasome- and TAP-Independent Processing of Cytosolic and Nuclear Proteins

TAP- and proteasome-independent antigen processing of cytosolic and nuclear proteins onto MHC class II has been described for Influenza A matrix protein 1 (M1),[27,48] neomycin phosphotransferase II[35] and the nuclear antigen 1 of the Epstein–Barr virus (EBNA1).[31] Lysosomal proteases were shown to be responsible for antigen processing onto MHC class II in all three cases. For neomycin phosphotransferase II and EBNA1, autophagy was implicated in the delivery of antigens into lysosomes, while this has not been demonstrated for influenza matrix protein M1. However, when the half-life of M1 was modified with the N-end rule, only long-lived M1 ($t_{1/2} = 5$ h) was presented on MHC class II and able to stimulate $CD4^+$ T cells, while short-lived M1 ($t_{1/2} = 10$ min)

Table 2 Intracellular antigens processed endogenously onto MHC class II

Type of antigen	Protein	Localization	Cell type	References
Viral	Measles virus matrix protein	Cytosol	HLA-DR transf. fibroblasts	24
	Measles virus nucleocapsid protein	Cytosol	HLA-DR transf. fibroblasts	24
	Influenza A virus matrix protein 1	Cytosol, nucleus	B cells	26,27,48
	Influenza A virus Hemagglutinin	Cytosol, ER	HLA-DR-transf. HeLa, B cells	25,28
	Hepatitis C virus (HCV) core protein	Cytosol	B cells	29
	Epstein–Barr Virus (EBV) EBNA1	Nucleus	B cells	30,31
Self	Glutamate decarboxylase (GAD65)	Cytosol	B cells	33
	Complement C5	Cytosol	B cells, macrophages	32
	Actin, AAT, Rab5	Cytosol	B cells, DCs	21
	Igλ light chain	ER	B cells	34
Model	Hen egg lysozyme (HEL)	Cytosol, ER, mitoch., nucleus	B cells, MHC class II-transf. sarcoma cells	36,38
	Ovalbumin, conalbumin	Cytosol	B cells, macrophages	39
	Neomycin phosphotransferase II	Cytosol, nucleus	B cells, IFNγ-treated epithelial cells	35
	β-Galactosidase	Nucleus	Thymic epithelial cells	37
	I-Eα_{52-68}-GFP	Cytosol	Macrophages	40
Tumor	MUC-1	Cytosol	Dendritic cells	42
	Mutated Cdc27	Cytosol	HLA-DR-transf. 293 cells, melanoma cells	41

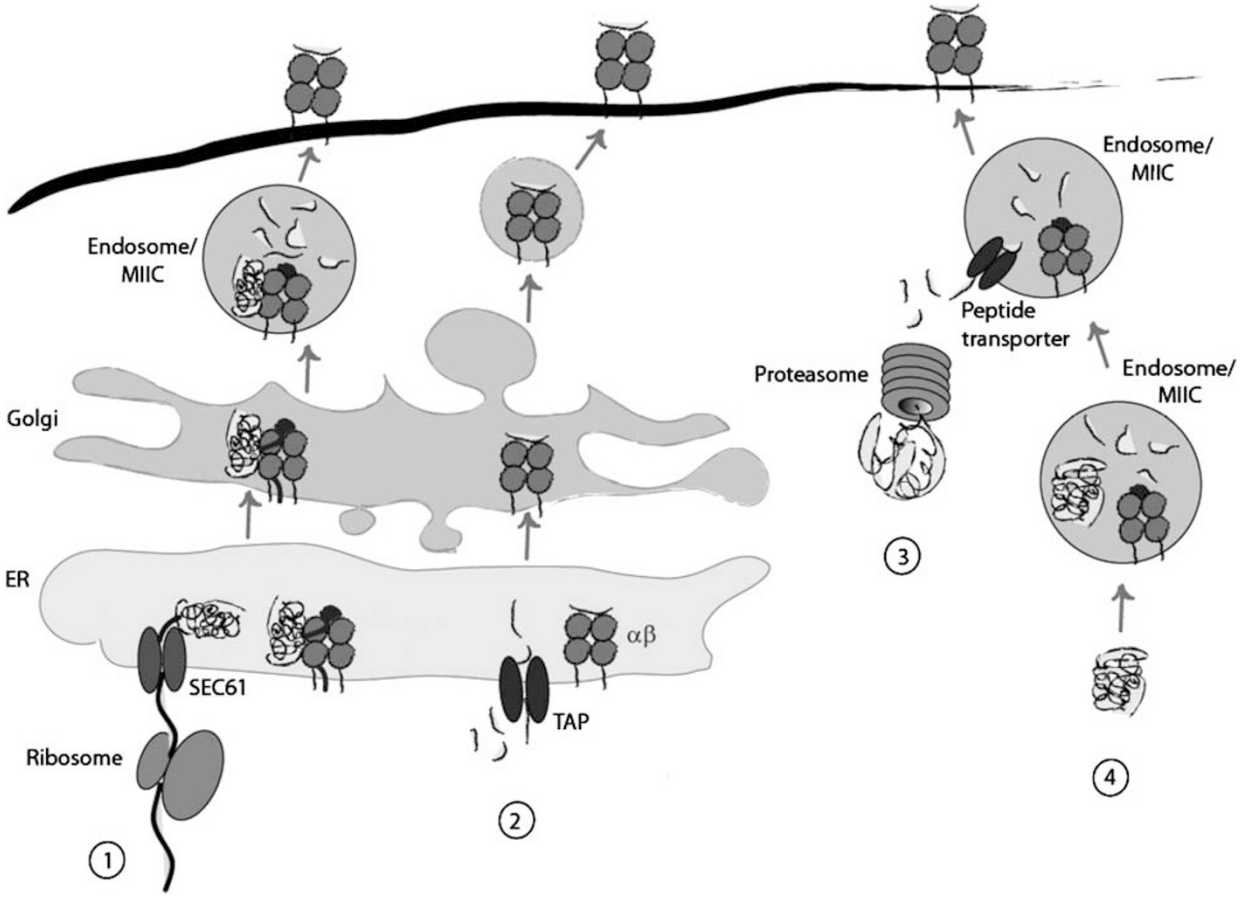

Figure 1 Proposed processing pathways for endogenous presentation of intracellular antigens on MHC class II. Four different pathways have been postulated: (1) Secreted/transmembrane proteins (e.g. influenza A hemagglutinin [28]) can associate with newly synthesized MHC class II molecules after their cotranslational synthesis into the ER via the Sec61 transporter. Complexes of antigen with MHC class II–I_i then traffic to endosomal compartments, where processing and peptide loading onto MHC class II occurs. (2) Similar to the classical MHC class I-processing pathway, cytosolic peptides (e.g. a 12-mer HA peptide [25]) can be imported via TAP into the ER and then associate with MHC class II molecules. It is thought that peptides either bind into the peptide-binding groove of MHC class II molecules that failed to associate with invariant chain (I_i) or they comigrate with MHC class II–I_i complexes and get loaded onto MHC class II in the endosomal MIIC with the help of HLA-DM. (3) Other cytosolic proteins (e.g. GAD65 [33]) are degraded by the proteasome and then follow a TAP-independent pathway onto MHC class II. It is thought that peptides are directly imported into endosomal/lysosomal compartments via a peptide transporter, possibly Lamp-2a.[47] (4) Cytosolic and nuclear proteins (e.g. the EBV nuclear antigen 1 (EBNA1)[31]) can be processed by lysosomal proteases after direct import into endosomal/lysosomal compartments

failed to be detected by M1-specific CD4$^+$ T cells, but stimulated M1-specific CD8$^+$ T cells.[48] Autophagic protein degradation in lysosomes has been suggested to mainly discard long-lived proteins ($t_{1/2} > 0.5$ h),[49] whereas many short-lived substrates are degraded by the proteasome.[5,50] Therefore, both long-lived M1 and EBNA1 ($t_{1/2} > 20$ h in B cells)[51–53] fit the long half-life criteria of autophagy substrates. In addition, the long-lived cytosolic proteins GAPDH ($t_{1/2} = 130$ h)[54] and Hsc70 ($t_{1/2} = 20$ h)[55] were frequently identified as a source of natural MHC class II, but not class I ligands (Table 1).[20] Hence, autophagic degradation might deliver long-lived endogenous proteins into the MHC class II pathway.

Delivery of Antigens to Lysosomes for MHC Class II Processing Via Autophagy

Involvement of autophagy in endogenous MHC class II processing has been demonstrated for only a few antigens. Neomycin phosphotransferase II,[35] complement C5[32] and MUC1[42] were found to be processed onto MHC class II via autophagy after transfection. All these studies employed the pharmacologic inhibitors of autophagy, wortmannin[56] and 3-methyladenine[57] to inhibit CD4$^+$ T-cell recognition of these antigens, but did not report any localization of the respective antigens to autophagosomes upon inhibition of lysosomal degradation. To date EBNA1 is the only pathogen-derived antigen for which processing onto MHC class II after autophagy has been demonstrated.[31] We could visualize EBNA1-containing autophagosomes after inhibition of lysosomal degradation by fluorescence and electron microcopy. Furthermore, MHC class II-restricted EBNA1 recognition by CD4$^+$ T cells was inhibited after RNA silencing of the essential autophagy gene *atg12*[58] as well as after 3-methyladenine treatment. Autophagic delivery of EBNA1 for MHC class II processing was demonstrated in B-cell lines, which either were transfected with EBNA1 or expressed physiological levels of EBNA1 after B-cell transformation by EBV. These studies suggest that there might be a substantial overlap between the autophagic route of degradation and MHC class II loading. This implies that autophagic destruction of other pathogens like *Mycobacterium tuberculosis*[59] and *Streptococcus pyogenes*[60] might also result in MHC class II presentation of antigens from these pathogens. Since IFNγ has been shown to upregulate autophagy,[59] activated CD4$^+$ T cells might then even further stimulate infected cells in order to clear intracellular pathogens. Therefore, autophagy might mediate innate resistance to pathogens, lead to MHC class II presentation of pathogenic determinants and be used as effector mechanism of adaptive immunity to target intracellular pathogens.

Further evidence for the involvement of autophagy in antigen processing for MHC class II presentation comes from biochemical studies on natural HLA-DR ligands.[61] In this study, the authors characterized peptides, which were eluted from immunoaffinity-purified HLA-DR molecules of an EBV-transformed B LCL. The MHC class II ligandomes of LCLs in the steady state and after induction of autophagy via starvation were compared. After 24 h of starvation the MHC class II presentation of peptides from intracellular and lysosomal proteins rose by more than 50%, while presentation of membrane and secreted proteins remained constant. The four most regulated MHC class II ligands were derived from one lysosomal (cathepsin D) and three cytosolic/nuclear proteins (eukaryotic translation elongation factor 1 alpha, ubiquitin-protein ligase NEDD4La and RAD23 homolog B nucleotide excision repair protein). In the same study, the extensive analysis of HLA-DR ligands from LCLs cultured in nutrient-rich conditions revealed a peptide, derived from the essential autophagy gene product Atg8/LC3. Atg8/LC3, which is coupled to the autophagosome membrane in an ubiquitin-like fashion, is essential for autophagosome formation.[62] These findings suggest that upregulation of autophagy leads to enhanced MHC class II presentation of cytosolic/nuclear proteins and that autophagosomes constitutively fuse with MHC class II-loading compartments.

Transport of Autophagosomes to MHC Class II Loading Compartments

It is well established that MHC class II molecules localize to endosomal compartments, where they meet processed antigen and get loaded with antigenic peptide. The nature of this MHC class II-loading compartment (termed 'MIIC' for MHC class II compartment) has been studied extensively using immunofluorescence or electron microscopy and cell fractionation.[13,63,64] These studies have characterized MIICs as late endosomal compartments containing the late endosomal/lysosomal markers LAMP1, CD63 and partially processed cathepsin D. Since no late endosomes/lysosomes devoid of MHC class II and HLA-DM are observed in MHC class II-expressing APCs, it is thought that MHC class II-loading compartments are conventional endosomal compartments that, in addition, contain the components for MHC class II loading, namely MHC class II and the peptide-loading chaperone HLA-DM.[13,65,66] This implies that classical APCs like macrophages, B cells and DCs have equipped late endosomes for MHC class II loading.

In electron microscopy, MIIC compartments have a typical multivesicular or multilaminar morphology.[65–68] The multivesicular phenotype can be explained by the transport of MHC class II molecules to multivesicular endosomes, which are abundant in the endocytic pathway.[69] The multilaminar, 'onion-like' phenotype of MIICs, however, has remained more enigmatic. It is tempting to speculated that MIICs obtain their multilaminar phenotype by fusion of MHC class II-containing endosomes with autophagosomes, given the fact that autophagosomes are delineated by a double membrane and often contain internal membrane sheets.[70,71] Indeed, electron microscopy studies have demonstrated that the endocytic pathway converges with the autophagy pathway: Endosomal compartments, labeled with colloidal gold as an endocytic tracer, were found to fuse with double-membrane bound, gold-negative autophagosomes to form endosome–autophagosome fusion compartments called 'amphisomes'.[72,73] Amphisomes contained both endocytosed gold and undegraded cytoplasm and were delimited by double or multiple membranes.[73] Often, fusion compartments also contained

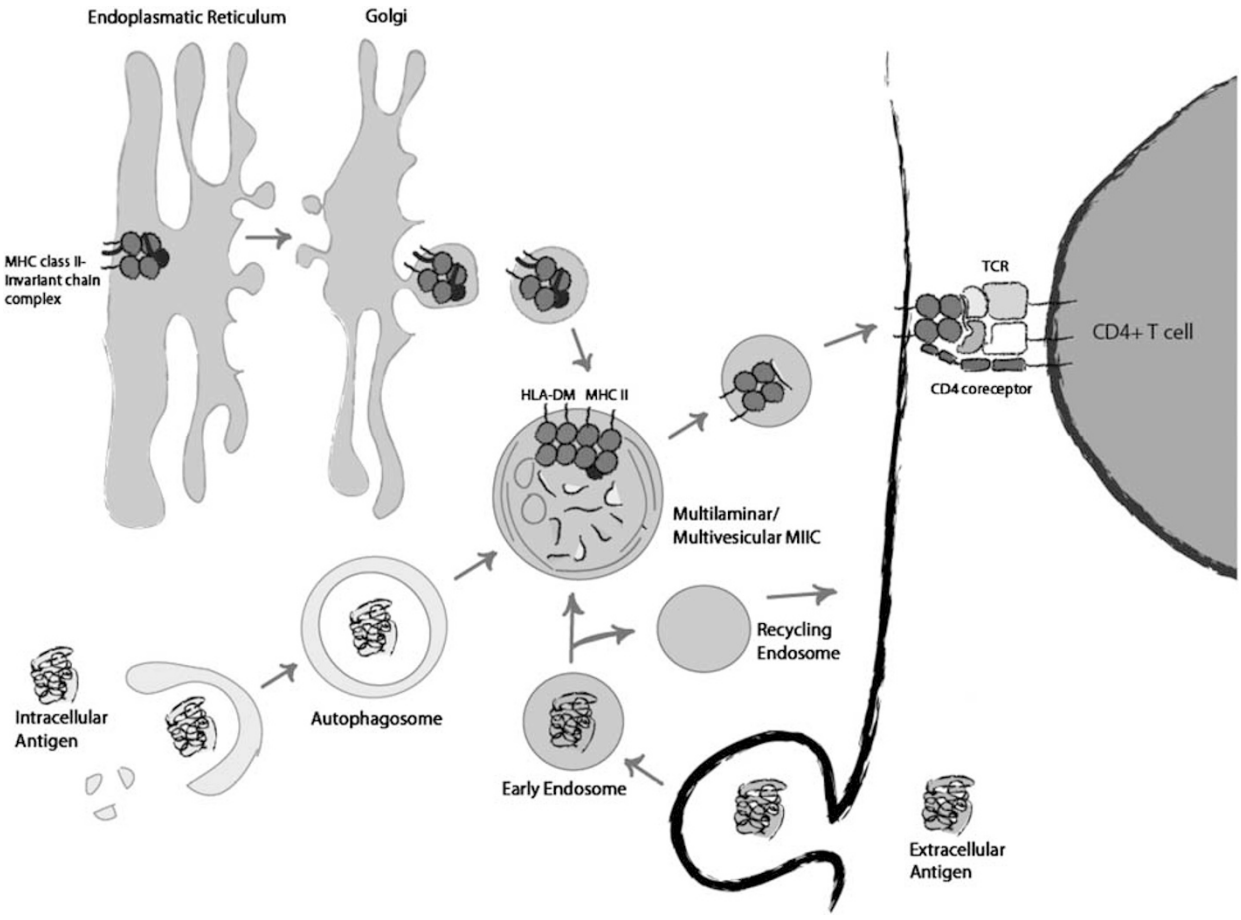

Figure 2 Autophagy as a novel pathway for endogenous MHC class II presentation. Classically, extracellular antigens were thought to be the sole source of peptides for MHC class II presentation. Extracellular antigens are taken up via endocytosis/phagocytosis into endosomal compartments and are degraded by lysosomal proteases. Antigenic peptides generated in this process get loaded onto MHC class II molecules in late endosomal MHC class II-loading compartments (MIICs) with the help of the peptide-loading chaperone HLA-DM, and MHC class II–peptide complexes are presented on the cell surface for recognition by $CD4^+$ T cells. MHC class II molecules reach the endosomal pathway after their synthesis into the ER and association with a glycoprotein called invariant chain (I_i) (shown in blue), which contains a targeting signal for endosomes. Recent evidence, discussed in this review, suggests that cytosolic and nuclear antigens can gain access to MHC class II-loading compartments via autophagy. Thus, autophagic degradation contributes to MHC class II presentation of intracellular antigens to $CD4^+$ T cells

multiple internal vesicles, presumably due to the fusion of autophagosomes with multivesicular endosomes. Hence, autophagosomes fuse with different types of endosomal compartments and thus deliver autophagy substrates into the endocytic route. In MHC class II-expressing cells, this fusion event could constitutively lead to the delivery of autophagy substrates into endosomal MIIC compartments and thus loading of processed autophagy substrates onto MHC class II molecules (Figure 2).

Possible Role for MHC Class II Processing Via Autophagy During the Development of the Immune System and during Adaptive Immune Responses

So far MHC class II presentation after autophagy has only been described in classical APCs like macrophages,[32] B cells[31,35] and DCs.[42] However, endogenous MHC class II processing might be much more relevant for MHC class II-positive tissues with little or no phagocytic capacity. Along these lines, murine cortical epithelial cells of the thymus were found to have high constitutive autophagy, especially in newborn mice.[74] These cells are believed to have low phagocytic potential, but are nevertheless involved in positive selection of $CD4^+$ T cells.[75] This implies that the MHC class II complexes involved in positive $CD4^+$ T-cell selection have to be loaded from endogenous sources, and high constitutive autophagy might deliver some of the necessary antigens into MHC class II-loading compartments.

Thymic epithelium is not the only somatic tissue with MHC class II presentation. Upon immune activation and inflammation, endothelial and epithelial cells as well as nearly all lymphocytes can upregulate HLA class II,[76] while phagocytosis is not enhanced. This suggests that MHC class II molecules on inflamed tissues might display primarily endogenous ligands for immune surveillance by $CD4^+$ T cells. The induction of $CD4^+$ T cells alongside with $CD8^+$ T cells is an important prerequisite for an effective adaptive immune response, since the development[77–79] and maintenance[80–84] of $CD8^+$ memory T cells is dependent on help from $CD4^+$ T cells. Additionally, $CD4^+$ T cells can have direct

cytotoxic effects on virus-infected cells[85–88] and contribute to the control of viral infections.[89–93] Given these important roles of $CD4^+$ T cells for adaptive immunity, it seems crucial that endogenous antigen be presented not only on MHC class I, but also on MHC class II. We suggest that part of these endogenous MHC class II ligands are generated from autophagy substrates and that MHC class II presentation of long-lived endogenous substrates complements MHC class I presentation of short-lived endogenous substrates to elicit T-cell activation during immune responses.

Conclusions

Autophagy is an innate defense mechanism against microbial pathogens.[94,95] Recent evidence suggests that autophagic degradation products are displayed on MHC class II for immune surveillance by $CD4^+$ T cells. So far autophagy has been found to deliver nuclear and cytosolic proteins for MHC class II presentation in professional APCs, namely DCs, macrophages and B cells. Since, especially in DCs, endosomes seem to be leaky and release exogenous antigen for crosspresentation on MHC class I, it is also conceivable that autophagic delivery of endogenous antigen into late endosomes contributes to MHC class I loading after protein escape into the cytosol. Thus, autophagy may contribute to immune control of infected APCs, such as EBV-transformed B cells. However, we suggest that this pathway is also used for the immune surveillance of tissues that upregulate MHC class II upon inflammation as well as for positive T-cell selection by thymic epithelial cells.

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