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Endogenous Antigen Presentation by MHC Class II Molecules

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Major histocompatibility complex class II
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Abstract

T cell recognition of antigen requires that a complex form between peptides derived from the protein antigen and cell surface glycoproteins encoded by genes within the major histocompatibility complex (MHC). MHC class II molecules present both extracellular (exogenous) and internally synthesized (endogenous) antigens to the CD4 T cell subset of lymphocytes. The mechanisms of endogenous antigen presentation are the subject of this review. Isolation and amino acid sequencing of peptides bound to the class II molecule indicate that a very high proportion (70–90%) of the total peptides presented by the class II molecule are in fact derived from the pool of proteins that are synthetized within the antigen-presenting cell (APC). This type of sequence information as well as the study of model antigens has indicated that proteins expressed in a diversity of intracellular sites, including the cell surface, endoplasmic reticulum and cytosol can gain access to the class II molecule, albeit with different efficiencies. The main questions that remain to be answered are the intracellular trafficking patterns that allow colocalization of internally synthesized antigens with the class II molecule, the site(s) within the cell where peptide:class II molecule complex formation can take place and whether presentation of 'foreign' as well as 'self' antigens takes place by mechanisms that vary from one cell type to another or that vary with the metabolic state of the APC. If such variability exists, is would imply that the array of peptides displayed by class II molecules at the cell surface has similar variability, a possibility that would impact on self tolerance and autoimmunity.

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Introduction

T cells play a critical regulatory role in the immune system. Accordingly, there has been great interest in understanding the mechanisms that are involved in T cell recognition. Work by a number of investigators has established that the fundamental event that allows T cell recognition of antigen to occur is the formation of a complex between peptides derived from the antigen and intact cell surface proteins encoded for by genes within the major histocompatibility complex (MHC). Two classes of MHC molecules, MHC class I and class II, are specialized for presentation of antigenic peptides for recognition by T cells. Crystallographic evidence reveals a peptide binding groove at the distal terminus of the MHC class I molecules [1, 2]. A similar binding pocket has been observed in MHC class II crystals [3, 4] that is constructed from the amino-terminal, genetically polymorphic domains of the class II α and β chains.

Although their structural domains are similar, the cellular compartments in which MHC class I and class II molecules function appear to differ. MHC class II molecules have the specialized capacity to present peptides generated from extracellular antigens which enter the cell by endocytosis [5, 6]. The resulting class II:peptide complexes, products of the endosomal/lysosomal processing pathway, are recognized by CD4+ T cells. CD4+ cells, in large part, function to enhance antibody production by B lymphocytes and to activate cellular immune responses. They have thus been termed helper T cells. In contrast, MHC class I molecules primarily present peptides derived from intracellular proteins with access to proteolytic machinery within the cytosol. Resulting class I:peptide complexes are important in directing the immune response of CD8+ cytolytic T lymphocytes against intracellular pathogens such as viruses.

Much has been learned in recent years regarding the intracellular events regulating MHC-restricted presentation of antigen. Although the specifics of class I antigen processing are not yet fully understood, evidence suggests that a multisubunit proteolytic complex within the cytosol may play a role in generating antigenic peptides for class I presentation [reviewed in ref. 7]. The resulting peptides are thought to be imported into an early exocytic compartment, presumably the endoplasmic reticulum (ER) by two MHC-encoded proteins (TAP-1 and TAP-2) which belong to a family of transporter proteins known as the ABC (ATP-binding cassette) superfamily [8–11]. Peptide binding to class I molecules allows for stable association with a second non-MHC-encoded protein, β_2-microglobulin [12, 13]. The assembly of the MHC class I heavy chain with β_2-microglobulin and peptide within the ER is facilitated by an 88-kD protein named calnexin [14, 15]. Only after binding to both peptide and β_2-microglobulin does the MHC class I molecule achieve a conformation suitable for exit from the ER/cis Golgi [16, 17].

Early studies investigating MHC class II-restricted antigen presentation revealed several general characteristics. It is time-dependent and blocked by agents known to inhibit the activity of lysosomal proteases. Analysis of the intracellular trafficking of the class II molecule has also implicated the importance of endosomal compartments in class II-restricted antigen presentation. During biosynthesis, MHC class II molecules, unlike MHC class I, do not appear to follow the default ER/Golgi/cell surface exocytic pathway. While transmembrane proteins within the default exocytic pathway appear at the cell surface within minutes after sialic acid addition in the trans-Golgi, expression of MHC class II molecules occurs hours after sialylation [18]. MHC class II molecules are sensitive to endocytosed

neuraminidase during this time, suggesting that these proteins intersect endocytic compartments after transport through the Golgi [19]. Interestingly, very recent subcellular fractionation studies have suggested that the class II-containing compartments may be distinct from the well-defined endosomal and lysosomal compartments [20–22]. Within these compartments or other protease containing-compartments within the endocytic pathway the invariant chain glycoprotein is released from MHC class II, an event that allows the peptide binding site within class II to become available for peptide binding [23, 24]. Thus, the currently accepted model for class II presentation of exogenous antigens involves selective trafficking of MHC class II molecules and antigen into the compartments of the endocytic pathway. Within these compartments, through the activity of resident lysosomal proteases, antigen is cleaved into peptides and invariant chain is released from the class II molecules. Peptides binding then takes place and the peptide:MHC class II complex is exported to the cell surface for recognition by CD4 T cells.

Although the class II molecule is specialized to sample extracellular antigens which enter the cell by endocytosis, it has become increasingly clear that class II molecules can also efficiently bind and present peptide-derived from proteins synthesized within the antigen-presenting cell (APC) [25–33]. Although the extent to which class II molecules display peptides derived from internally synthesized proteins is becoming increasingly apparent, the mechanisms governing their presentation remain poorly defined. In this review, I will discuss research which has provided insight into the mechanisms involved in endogenous antigen presentation and the immunological implications of endogenous antigen presentation by the MHC class II molecule.

Peptides Isolated from the MHC Class II Molecule

Recently, highly sophisticated and sensitive biochemical methods have been developed to derive sequence information from complex mixtures of peptides. Two general methods of peptide sequencing have been applied to the study of MHC-bound peptides. Both methods typically involve isolation of the MHC molecule through monoclonal antibody affinity chromatography followed by acid elution of peptides from the intact MHC molecule. Low molecular weight peptides are then separated from the larger proteins and polypeptides and the peptides are fractionated by high performance liquid chromatography (HPLC). Amino acid sequencing is then performed on individual HPLC fractions. In one method sequencing is accomplished through the use of typical Edman degradation techniques, while in the other method, sequence information is derived from tandem mass spectrometry. This latter method has the advantage of requiring less material and also has the capability to sequence individual peptides contained within complex mixtures. Both methods have provided a great deal of information on the nature of peptides naturally bound to the MHC class II molecule.

One of the early and surprising results from the analyses of peptides eluted from class II MHC molecules is that a significant fraction of the peptides isolated from MHC expressed on cells in culture is derived from internally synthesized proteins [34, 35]. Depending on the allelic product of class II molecules studied, peptides derived from internally synthetized antigens constitute from 70 to 90% of the peptides sequenced. These results suggest first that in the absence of receptor-mediated uptake of extracellular protein, internalization and/or functional utilization of

exogenous proteins may be quite inefficient [36, 37], leaving the majority of class II molecules free to bind peptides derived from internally synthesized proteins. Second, these results point out that although class II molecules are indeed specialized to present antigens from an exogenous source, they are quite capable of displaying peptides that are synthesized within the APC and in fact, in the absence of pathogenic challenge, the vast majority of peptides displayed on the cell surface of an APC likely represent the array of proteins synthesized within that APC. The quantity of peptide recovered from class II molecules is close to equimolar to the MHC class II molecule itself, suggesting that the vast majority of MHC molecules are not 'empty' but rather bound by autologous peptides [38]. This finding is in agreement with earlier studies in which isolated class II was tested for the ability to bind peptide in vitro, where it was found that only a very small fraction (5–10%) of the class II molecules were active in the binding assay. Presumably, the remaining class II molecules in these studies persisted in their association with self peptides through the isolation procedure, and were thus unavailable for binding of the test peptide. Because class II molecules isolated under these procedures were eluted from anti-class II monoclonal antibody affinity columns through the use of denaturants, the inference is that once peptide is bound to the class II molecule, it remains almost irreversibly associated, in agreement with the studies made with class II molecules tested with model antigens and peptides [39–43].

With regard to structure, peptides eluted from the class II molecule are heterogeneous in size, ranging from 13 to 25 amino acids, with the majority in the range of 14–18 amino acids [34, 44–46]. Heterogeneity in the length of peptides bound to class II molecules is not only found among peptides derived from different source proteins, but also individual peptides derived from the same source peptides. Thus, the peptides bound to class II molecules are nested sets derived from a variety of cellular proteins. Heterogeneity in peptides bound to class II from a given source protein is most commonly displayed at the carboxy-terminus although amino-terminal variability is not unusual. Microheterogeneity in the termini of peptides bound the MHC class II molecule infers the activity of exopeptidases and also of flexibility in the ability of class II molecules to accommodate peptides of different sizes. This latter conclusion is supported by in vitro binding studies that have shown a great deal of permissiveness in length of the peptides that have potent binding activity to the MHC class II molecules [44]. The size microheterogeneity in peptides bound to class II is in sharp contrast to the peptides isolated from MHC class I molecules, which are almost exclusively 8–10 amino acids in length, most commonly nonomers [reviewed in ref. 47]. The longer length of peptides bound to class II despite the structural homologies in the peptide binding site in class I and class II molecules suggests that peptides in the class II binding site likely extend out from the pocket marked by the termini of the α helices.

Perhaps one of the most intriguing findings derived from elution and sequence of MHC class II-bound peptides is that an extraordinarily high percentage of source proteins are MHC molecules themselves. One very extensive study performed by Chicz et al. [45, 46] described peptides bound by six different human DR allelic products isolated from lymphoblastoid cell lines. Depending on the allele studied, MHC-related peptides constitute from 10 to 60% of the total peptides sequenced. The peptides derived from MHC molecules do not represent one particular region of the MHC molecule. Some of the pep-

tides are derived from the helical regions in the membrane distal domains, while others were contained within the membrane-proximal external domain of either class I or class II. Preferential binding of MHC-derived peptides cannot be attributed solely to selective localization of these molecules within the endocytic pathway, as a high percentage of source proteins are derived from MHC class I molecules, which should not have preferential access to the MHC class II antigen processing compartment. There are at least two potential explanations for the predilection of MHC peptides to be present on the MHC class II molecule. First, it is possible that, contrary to conclusions from biochemical and histochemical studies, both MHC class I and class II molecules are in fact enriched in the classical endocytic compartments relative to other membrane proteins. It is conceivable that even though the intact class I molecules are not readily detected there, perhaps because of their protease sensitivity, they traffick to these compartments by additional mechanisms than those used to degrade typical cell surface proteins. Alternatively, because both MHC class I and class II molecules assemble as multicomponent complexes within the endoplasmic reticulum and because this assembly is time-dependent and limits export from the endoplasmic reticulum, a relatively high percentage of newly synthesized class I and class II molecules may actually be degraded because of their inefficient egress. Potential mechanisms for degradation of ER-localized proteins and subsequent binding to MHC class II molecules are discussed below.

Regardless of the mechanism that leads to preferential presentation of MHC-derived peptides on MHC class II molecules, the consequences of this phenomenon are interesting to consider. MHC molecules are the most genetically polymorphic molecules that exist. Based on the stability of the interaction and the evidence that peptide actually influences the conformation of the presenting molecule [48–50], peptide that is bound to an MHC molecule can be considered to be an actual component of the MHC molecule. Both MHC class I and MHC class II molecules can thus be considered to be trimeric structures. The prevalence of genetically polymorphic peptides within the peptide binding site therefore increases the diversity of the expressed MHC molecules. Thus, a given MHC molecule that is expressed on cells of divergent MHC backgrounds is essentially unique, due to the presence of divergent MHC-derived peptides within its peptide binding site. This model has particular relevance when one considers the roles of MHC polymorphism and of peptide in T cell development and in allorecognition.

Intracellular Events Involved in Presentation of Internally Synthesized Antigens by MHC Class II Molecules

Studies with Model Antigens

The intracellular mechanisms that are involved in endogenous antigen presentation by the MHC class II molecule have been probed by a number of investigatiors using model antigens. Much of the focus of these experiments has been to identifiy whether endogenous antigen presentation by class II molecules, like exogenous antigen presentation, proceeds via the conventional endosomal pathway, whether it occurs by a class I-like pathway or whether it represents a third pathway of presentation. These models have also been used to clarify the constraints of endogenous antigen presentation by class II molecules, in order to determine if all cellular antigens have equal access to the class II molecule or if presentation is restricted to proteins having identifiable characteristics or particular subcellular localizations.

Analysis on the subcellular sites of localization that allow internally synthetized antigens to gain access to class II has led to some general conclusions. First, with one exception derived using virus-infected cells [6], it has generally been found that proteins that are expressed at the cell surface can be presented by MHC class II molecules [25, 28, 30, 31, 33, 51, 52]. This has been found to be the case even when an ordinarily secreted exogenous protein (hen egg lysozyme) has been genetically manipulated so that the protein encoding the antigenic peptide was covalently linked to the transmembrane and intracytoplasmic segment of an MHC class I molecule [30]. Cell surface localization of an internally derived antigen would allow theoretical access to most sites of intracellular degradation, including that that takes place in the ER (for the newly synthetized protein), cytoplasm (for that fraction of protein translated on ribosomes that have not docked onto the ER and therefore remain in the cytoplasm) and for degradation that takes place after expression at the cell surface due to internalilzation for ultimate lysosome-mediated protein turnover. Thus, it seem likely that the majority of cell surface proteins would have the capability to provide peptides for presentation by class II molecules, provided that they contain peptides that fit the binding requirements of the particular class II molecules expressed by that individual.

In general, it has also been found that secreted proteins also have ready access to class II molecules. The options available for proteolysis are the same as that for cell surface proteins, except that access to lysosomes would be anticipated to most readily occur after secretion and reuptake. Interestingly, however, when this mechanism has been tested for the model secreted antigens that sensitize APC for recognition by CD4 T cells, it has generally been found that the mechanism used for presentation does not appear to involve secretion and reuptake [30, 33, 52]. This issue is typically addressed either by testing the ability of culture supernatant from the cell secreting the model antigen to sensitize APC for recognition, or more vigorously, by coculturing cells that secrete the antigen in question but lack the class II restriction element with cells that express the class II restriction element but lack expression of the antigen. Coculture is generally allowed to proceed for several days to mimic the conditions available to the APC that expresses both molecules. For proteins that are secreted at modest levels typical of normal cellular proteins (in the range of nanograms per milliliter), and that do not have specialized mechanisms of reuptake, secretion and endocytosis does not appear to be a viable mechanism for presentation. Nonetheless, these secreted molecules do get presented by class II molecules, raising the question of the mechanism and sites of their protein degradation and association with the class II molecule (discussed below).

To examine whether export from early biosynthetic compartments is necessary for presentation via the MHC class II molecule, as might be expected for an endosomal processing event, several strategies have been applied. For soluble proteins such as lysozyme or immunoglobulin, ER/cis Golgi retention has been accomplished by engineering an ER retention signal into the protein, encoded for by the amino acid sequence KDEL [26, 30]. To retain a transmembrane protein within the ER, our laboratory has engineered an adenovirus E19 sequence into the cytosolic tail of the model antigen we have studied (H-2L^d) [53]. Despite successful retention of the antigens tested within the ER, presentation via the class II molecules in all cases was still observed.

The site of intracellular localization that has been the most controversial is the cyto-

plasm. This site of expression is presumed to be the main source for peptides destined to be presented by the classical class I presentation pathway. When target antigens presented by MHC class II molecules have been localized to the cytoplasm, contrasting results have been obtained. One of the early models for endogenous antigen presentation by MHC class II molecules involved the normally cytoplasmic matrix protein of influenza virus. The matrix protein, encoded for by a recombinant vaccinia virus, was able to be presented by class II molecules, although at less efficiency than presented by the MHC class I molecule [32]. When proteins normally expressed at different subcellular localizations have been redirected to the cytoplasm (most typically achieved for normally secreted or cell membrane-expressed proteins by elimination of the signal peptide, which will prevent translocation across the ER membrane), many investigators have failed to find presentation via the class II molecule [26, 33, 54]. Some investigators have seen presentation of cytosolic-localized antigen, but this typically required high levels of protein expression [55]. Thus, it is likely that cytosolically localized antigens do have some access to the class II molecule, but presentation of these types of antigen may require atypically high levels of protein synthesis or ongoing viral infection to be presented by the class II molecule.

Mechanisms of Presentation of Internally Synthesized Antigens by MHC Class II Molecules

Based both on the use of model antigens and on knowledge of peptides naturally bound by the class II molecules, it is now generally accepted that many internally synthesized antigens can be presented by the MHC class II molcule. What is still uncertain, however, is the mechanism(s) involved in this presentation event. The primary mechanistic issues to be resolved are the sites and proteases involved in protein degradation and the sites involved in the final binding of the derived peptide to the MHC class II molecule. Potential sites for protein degradation include the endosomal/lysosomal compartments used to degrade exogenous antigens, the cytosolic proteosome system presumed to be the major pathway used by antigens destined to be presented by class I molecules [56, 57], or proteases localized to the ER that are involved in degradation of incompletely assembled or misfolded newly synthesized proteins [58, 59]. The potential sites of peptide binding to class II molecules would be the early sites of class II biosynthesis (ER/cis Golgi) and would be presumed to be analogous to MHC class I, or the endocytic compartments that are the likely site of binding of peptides derived from exogenous antigens.

Experimentally, it is difficult to distinguish among the preceding potential sites of protein degradation. The main limitation for these studies is a paucity of effective protease inhibitors that specifically inhibit the activity within one but not the other sites. For this reason, the major tool used to dissect the intracellular events of MHC-restricted antigen presentation has been the use of metabolic inhibitors. Early studies that first delineated differences between MHC class I- and MHC class II-restricted antigen presentation noted that two types of reagents appeared to selectively block the classical (endogenous) class I and classical (exogenous) class II pathways of presentation. Pioneering work on the mechanisms involved in presentation of exogenous antigens by MHC class II molecules showed that presentation was effectively blocked by treatment of APC with lysosomotropic amines such as ammonium chloride and chloroquine, implicating acid compartments in this presentation [60]. Later studies probing the events involved in MHC class I presentation utilized a

fungal metabolite, brefeldin A (BFA), which blocks egress of newly synthesized proteins from the ER/cis Golgi [61]. Sensitivity of the class I presentation pathway to BFA implied that newly synthesized class I molecules were those involved in presentation of the internally synthesized antigen, a finding now consistent with data showing that class I presentation involves uptake of cytosolically degraded antigens into the ER for binding to newly synthesized class I molecules. Thus, BFA and lysosomotropic reagents have been regarded in many ways as the 'fingerprint' of a class I or class II pathway or antigen presentation, respectively.

Utilization of these metabolic inhibitors to probe class II-restricted presentation of endogenous antigens is problematic for several reasons. First, BFA is a ubiquitous inhibitor of protein export, due to its generalized effect on the organization and integrity of the ER, cis Golgi and later Golgi compartments [62, 63]. Sensitivity of an antigen presentation event to BFA thus merely implicates any newly synthesized membrane associated protein in the antigen presentation event. There is increasing evidence that both the class I and class II presentation pathways primarily involve newly synthesized MHC restriction elements, rather than preexisting molecules on the plasma membrane. Thus it is likely that most class II-restricted presentation should be BFA-sensitive, regardless of the site of peptide generation or class II binding. The longer lag time between synthesis and cell surface expression of class II molecules compared to class I molecules [18] has been used BFA studies to distinguish a class I vs. class II type presentation event. Rapid inhibition of antigen presentation by BFA is most consistent with peptide binding that takes place early in biosynthesis, followed by rapid export and cell surface display of the immunogenic MHC:peptide complex.

Lysosomotropic amines such as ammonium chloride and chloroquine have been used to implicate a role for endosomes or lysosomes in the presentation event. These studies are complicated on several accounts. First, both peptide generation (by the lysosomal proteases) and peptide binding to class II molecules occur most efficiently at acid pH [64–66]. Therefore, raising the intravesicular pH will diminish both processes. Thus, sensitivity to lysosomal amines does not assign the site of peptide generation. Secondly, degradation of invariant chain from the class II molecule is mediated by lysosomal proteases. This degradation of invariant chain is required for two key events in class II-restricted presentation: peptide binding to the class II molecule [24, 67] and export from the endosomal compartments to the cell surface [68, 69]. Our laboratory has shown that invariant chain has a potent endosomal retention function [69]. Persistent invariant chain association with class II molecules, induced by lysosomal amines, leads to retention of the class II/invariant chain complex within the endosomal compartments. Finally, many intracellular trafficking events, particularly those requiring membrane fusion, are pH-dependent and are thus inhibited by lysosomal amines [70, 71]. Thus, sensitivity of an antigen presentation event to lysosomotropic reagents most simply indicates endosomal compartments in the presentation event. These compartments could be used for pH-dependent peptide generation, peptide binding to class II or pH-dependent trafficking events. Total insensitivity to lysosomotropic reagents implies a pathway that does not involve those compartments, such as the ER-to-Golgi-to-cell surface default pathway used by class I molecules and most cell surface proteins.

Given these limitations to the utilization of these reagents, I will now discuss the results obtained when these types of experiments

have been performed to dissect the events involved in class II-restricted presentation of internally synthesized antigens. One of the early studies on endogenous antigen presentation by MHC class II molecules found that presentation of internally synthesized influenza matrix protein, an exclusively cytosolic antigen, was insensitive to chloroquine but was acutely sensitive to BFA [27]. When the viral antigens were provided as an exogenous antigen, the opposite finding was made: presentatioin was sensitive to chloroquine but resistant to the effects of BFA. This suggested that two distinctive pathways could be used for presentation of the same antigen. A similar BFA sensitivity on endogenous antigen presentation was seen for an constitutively expressed class I molecule (HLA-B7) whose peptide is presented in the context of class II (DR1) [31]. This presentation was resistant to chloroquine.

Several studies have shown that internally synthesized antigens are presented by a pathway that is sensitive to lysosomotropic amines. When recombinant vaccinia virus was used to direct expression of heterologous viral proteins within human B cell lines, two cytoplasmic viral antigens (hemagglutinin matrix and a leader-minus form of the hemagglutinin), a cell surface viral protein (wild-type hemagglutinin) and even a mini-gene encoding only the peptide epitope that is synthesized within the cytoplasm were found to be presented by a chloroquine-sensitive pathway [72] within lymphoblastoid cells, as was presentation of cytosolic or ER-retained forms of hen egg lysozyme [55] within murine B cell lines. Insensitivity to lysosomotropic amines was found when measles virus proteins but not influenza proteins were presented by B cell lines or by fibroblasts [73], suggesting that sensitivity to these amines may be dependent on the mode of virus entry or to the epitope studied.

Several conclusions can be drawn from these studies. First, Malnati et al. [72] found that presentation of the viral epitope encoded by a mini-gene was sensitive to chloroquine, which is consistent with the view that chloroquine sensitivity does not identify the site of antigen processing, as the mini-gene is genetically 'preprocessed' into an immunogenic peptide. Chloroquine effects must under these conditions be due to the block in invariant chain dissociation or to disruption of endosomal trafficking events. It would be particularly informative to know whether the chloroquine effect was dependent on expression of invariant chain within the APC. Our laboratory has shown that deposition of newly synthesized class II to the plasma membrane is not blocked by lysosomotropic amines if the class II molecules are expressd in the absence of invariant chain [69], despite the fact that these molecules can be shown to traverse the endocytic pathway during biosynthesis [74]. Dependence of the chloroquine effect on invariant chain expression would implicate invariant chain dissociation as the site of chloroquine inhibition. Such a mechanism would necessitate an exploration of the mechanism by which the cytosolic peptide gains access to the endosomal compartments for binding to the MHC class II molecule.

Secondly, the finding by several groups that presentation of cytoplasmically synthesized protein is sensitive to lysosomotropic amines argues against utilization of a classical class I presentation pathway of cytoplasmic protein degradation and endoplasmic reticulum binding. Because peptide binding to the class II molecule has been shown to be blocked by the presence of invariant chain, and because invariant chain has been implicated in sorting to endosomal compartments, it has been predicted [75] that stable binding of peptide to class II molecules in the ER, as would occur in a class I-like antigen process-

ing pathway, might lead to trafficking of class II molecules by the default pathway to the cell surface. If such is the case, two phenotypes should be observed as a consequence of ER-derived peptide binding to class II molecules: independence from chloroquine, whose effects would only be detectable in endocytic events, and acute sensitivity to the effects of BFA. The fact that cytosolic antigens are inhibited by chloroquine thus implicates endosomal compartments in some aspect of the presentation event, which tends to argue against binding of the peptide in the ER, assuming the model discussed above on the consequences of early loading of class II is correct. Lack of utilization of the classical class I pathway for cytosolic antigens presented by class II molecules is also supported by the observation that, when studied, presentation of cytoplasmic antigens by the class II molecule [72], like presentation of other internal antigens by class II molecules [33], is independent of TAP gene expression, thought to be involved in import of cytoplasmically generated peptides into the ER for MHC class I binding [8, 9, 11, 76].

Thus, at the present time, a major unresolved question related to class II-restricted presentation is whether the endosomal/lysosomal compartments are the exclusive site of peptide binding to class I molecules, to be used regardless of the intracellular site of peptide generation. The endosomal compartments have been implicated as the major site of peptide class II interactions based on the ability of low pH to facilitate peptide binding to class II [64–66] and through the observation that, upon entry into endosomal compartments and release of the invariant chain, MHC class II molecules obtain a conformational state that is associated with stable peptide binding (maintenance of αβ dimer formation in SDS) [77, 78]. It is not yet clear how cytosolic or ER-localized antigens would gain access to the MHC class II molecule, especially if binding requires access to the endosomal compartments. There is some evidence that cytosolic and ER proteins can enter the classical lysosomal/endosomal compartments through the processes of autophagy and chaperone-mediated uptake [79–81], processes induced by cellular stresses such as nutritional deprivation and viral infection. Autophagy is thought to involve formation of a cytoplasmic vacuole from the ER membranes (the autophagic vacuole). This vacuole thus likely contains resident ER proteins and also soluble cytosolic proteins. The vacuole is then thought to completely separate from the ER and eventually fuse with lysosomal membranes, thus delivering its contents to this latter organelle. In contrast, heat shock protein-mediated uptake is thought to involve direct translocation of cytosolic protein into the lysosome. This process involves selective recognition of particular amino sequences (related to KFERQ) and thus is thought to be selective for particular proteins.

In addition to providing a mechanism to allow localization of ER and cytosolic antigens to the lysosome for binding to MHC class II molecules, there is another interesting consequence that would derive from such processes. If particular metabolic and/or pathogenic events increase that access of ER and cytosolic proteins to the MHC class II molecule by these two intracellular trafficking pathways, then the array of self as well as foreign peptides bound to class II molecules will change under these conditions. These processes thus might reveal new peptide:class II complexes at the cell surface. Even 'self' peptides, having never previously had access to class II molecules, might thus be regarded as 'foreign' by the immune systen and initiate an autoimmune response.

Role of Invariant Chain in the Presentation of Endogenous Antigens by the MHC Class II Molecule

The invariant chain glycoprotein assembles with the MHC class II αβ dimer soon after biosynthesis in the ER. A number of biological activities have been demonstrated for invariant chain that influences antigen presentation by the MHC class II molecule [reviewed in ref. 82]. Two of these activities would be particularly expected to influence endogenous antigen presentation by class II molecules. The first is the ability of invariant chain to antagonize peptide binding to the MHC class II molecule. This property of invariant chain has now been demonstrated by several investigators [24, 67, 75]. The ability of invariant chain to block peptide bindings has been interpreted as the mechanism that would prevent internal antigens from binding to class II molecules early in biosynthesis and thus would distinguish the site of class I antigen binding from that involved in class II peptide binding. By this model, assembly of invariant chain with the class II molecule early in biosynthesis would preclude binding of ER-available peptides, such as those that are presented by MHC class I molecules. The peptide binding site for class II would thus only be available after invariant chain is proteolytically released from the class II molecule in endosomal compartments. If this model is correct, any early peptide binding to class II molecules would be expected to be antagonized by the presence of invariant chain within the APC.

The second activity of invariant chain that would influence class II-restricted presentation of internally synthesized antigens is its ability to localize the class II molecule within endocytic compartments, a property controlled by the cytoplasmic tail of invariant chain. The amino-terminal cytosolic segment of invariant chain is one of the last proteolytic fragments to be released from the class II molecule. This segment thus might retain class II molecules in endocytic compartments after the peptide binding site has been freed of invariant chain and is made competent for peptide binding. Therefore, internal antigens that require endosomal loading of class II or long steady-state expression of the class II molecule within these compartments might be expected to be enhanced by expression of invariant chain within the APC.

Until recently, it has been difficult to demonstrate a negative influence of invariant chain on MHC class II-restricted presentation of endogenous antigens. When examined in several model systems, invariant chain has been shown to either have no effect [33] or to facilitate endogenous antigen presentation by class II molecules [83, 84], apparently arguing against one of the earliest models for invariant chain function: to block peptide binding to class II molecules in the ER. Rather, the studies showing a positive influence of invariant chain on endogenous antigen presentation argue in favor of the endosomal compartments as the site of peptide:class II molecule complex formation, even for internally synthesized antigens. However, several recent reports [84, 85] have shown that for some antigens, invariant chain expression within the APC can antagonize class II-restricted presentation. The simplest interpretation of these recent experiments is that those class II:peptide epitopes blocked by invariant chain are formed in the ER. The potential sites of antigen proteolysis would then be presumed to be either the ER or the cytosol. Interestingly, in the studies of Bodmer et al. [84], it was shown that APC lacking expression of invariant chain express unique class II:peptide epitopes that are recognized as foreign by T cells from normal mice. This finding suggests the possibility that when cells in the body are induced to become class II-positive, for instance dur-

ing an inflammatory response, but do not coinduce invariant chain, they may be able to initiate an autoimmune response, due to their display of class II:peptide complexes that the circulating T cell pool is not tolerant to. Alternatively, they may be particularly efficient at presenting internally synthesized antigens such as tumor antigens, that are expressed primarily in early biosynthetic compartments or in the cytosol.

Concluding Remarks

It is now clear that a significant fraction of peptides that are displayed by the MHC class II molecule are derived from proteins synthesized within the APC itself. The proteins that contribute to the class II peptide repertoire are likely to be those that enter the exocytic pathway and are destined to be expressed ultimately as a secreted or membrane-associated form. Nonetheless, it is also clear that even cytosolic proteins can gain access to the class II molecule, particularly in cases when they are expressed at high levels or during viral infection. Thus it is likely that the capacity of class II molecules to display peptides from pathogens or altered self proteins that are uniquely expressed in tumor cells is quite diverse, which is of course of benefit to the host.

What is less clear at the present time are the intracellular events involved in class II-restricted presentation of endogenous antigens. There remain several major issues to be resolved. The first is the potential for the ER to serve as a site of peptide generation and peptide binding to the class II molecule. Although there are now several findings that argue that this can occur, it is not yet clear whether these represent rare exceptions or if the ER is a reasonably viable option for class II molecules to associate with peptide. Aside from its importance from the point of view of the cell biology of class II molecules, one of the main consequences of peptide binding to class II molecules within the ER is that there is the potential for invariant chain expression to impact on the array of peptides presented by class II molecules. Lack of invariant chain would lead to the association of a distinctive set of peptides with the class II molecules, some of which may have the potential to serve as a target of an autoimmune response, while others may provide targets for protective immune responses to intracellular pathogens or tumors.

A second major unresolved issue is the intracellular trafficking pattern that allows cytosolic and ER-retained antigens to gain access to the class II molecule. Of particular interest are the possibilities that these proteins may gain access to endosomal compartments by such processes as autophagy or heat shock protein-mediated uptake. Both of these processes may be selective for certain proteins and might be regulated by the metabolic status of the host cell and by conditions of stress or pathogenic insult. Thus, if these mechanisms do control import of cytosolic antigens and/or ER-derived peptides into the class II molecule binding compartment, the display of peptides derived from self as well as foreign proteins on the class II molecule reflects dynamic rather than static processes. Similarly, the efficiency and conditions that induce these processes may vary from one cell type to another, leading to cell type-dependent variability in the display of self as well as foreign proteins by the MHC class II molecule. Such heterogeneity could have several potential consequences. It might lead to selective participation of certain APC in the response to pathogens, which in turn has the potential to regulate the T cell effector function elicited. In addition, dissimilarity in the array of self peptides displayed by distinctive cell types would likely influence self tolerance induction and the potential for autoimmunity.

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