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Molecular chaperones in the processing and presentation of antigen to helper T cells

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Abstract. Helper T lymphocytes recognize peptide fragments of antigen bound to Major Histocompatibility Complex (MHC) class II molecules on the surfaces of antigen presenting cells (APC). Antigen processing involves internalization of the antigen into an acidic compartment where the antigen is degraded and the resulting peptide fragments of the antigen are bound to MHC class II molecules and the complexes subsequently displayed at the APC surface. Thus, antigen processing represents a complex, intracellular assembly process which may, like many intracellular protein folding and assembly processes, require the function of molecular chaperones. This contribution focuses on the evidence which suggests that members of the heat shock protein family of molecular chaperones play a role in this pathway.
Key words. Antigen processing; MHC class II; molecular chaperones.

The recognition of foreign antigen by helper T lymphocytes requires that the antigen be processed and presented by cells which express the Major Histocompatibility Complex (MHC) class II molecules termed antigen presenting cells or APC (reviewed in 12). Antigen processing is an early critical event in the initiation of immune responses, the vast majority of which require the activation of helper T lymphocytes. Processing involves the internalization of antigen into an acidic compartment within APC, proteolysis of the antigen and binding of the resulting peptide fragments to the MHC class II molecules. The peptide-MHC complexes are subsequently expressed on the APC surface for recognition by helper T cells. Thus, antigen processing results in the molecular transformation of a foreign, native protein antigen into a peptide-MHC class II complex. Over the last several years remarkable progress has been made in delineating the molecular details of the structure of the peptide-MHC class II complex and the cellular and molecluar mechanisms underlying its assembly (reviewed in 12, 14). The production of peptide-MHC class II complexes by APC is an intricate, intracellular protein folding and assembly process. It has become abundantly clear over the last several years that cells have a large number of families of proteins which facilitate and promote the folding, assembly and disassembly of proteins and perform internal housekeeping of inappropriately folded or degraded proteins (reviewed in 15). These have been given the name molecular chaperones, an important family of which is the 70 kD heat shock protein (hsp70) family. The assembly of processed antigen-MHC class II complexes may, like most assembly processes within the cell, require the function of molecular chaperones. Such chaperones may be differentiated products of the immune system specifically tailored to facilitate antigen processing or members of the abundant, ubiquitous chaperone families such as the hsp70 family. Here, a review of the processing and presentation of antigen with the MHC class II molecules will be presented with a particular emphasis on the role of molecular chaperones in this process.

Before beginning a discussion of the assembly of class II molecules, it may be worthwhile to comment on the types of cells which express class II molecules and which process and present antigen. The expression of class II molecules is a differentiated function of a restricted number of cells (reviewed in 16). Under normal conditions, class II molecules are expressed nearly exclusively by three cell types in the immune system, namely B cells, macrophages and dendritic cells. MHC-class II molecules may be normally expressed at low levels in nonimmune cells such as epithelial cells, particularly those lining the gut. MHC-class II molecule expression can also be induced in a number of cell types under certain conditions. For example, class II molecules are expressed in the pancreas in autoimmune diabetes, in the thyroid in Graves disease and in the brain in microglial cells in a variety of encephalitic and degenerative diseases. Such class II expressing cells have the ability to process and present antigen. Indeed, cells which do not normally express class II molecules but which have been transfected with and express the class II genes have been demonstrated to process and present antigen. However, it is not clear that the processing of antigen is identical in all cases. Indeed, as will be discussed below, the processing of antigen by so-called nonprofessional APC may not be identical to that of the professional APC, namely B cells, macrophages and dendritic cells. Such observations suggest that cells which have differentiated to express MHC-class II molecules may have specialized cellular or molecular

machinery to facilitate the assembly of processed antigen-class II complexes. The molecular mechanisms which underlie antigen processing in differentiated professional APC may ensure appropriate presentation of antigen. The processing of antigen by nonprofessional cells which do not express the appropriate machinery may lead to inappropriate presentation and potentially to autoimmune disease.

MHC class II molecules are integral membrane proteins composed of an α and a β chain that exist as nondisulfide-bonded dimers (reviewed in 5). The α and β chains fold together to form a peptide-binding groove which will accommodate peptides of 8–9 amino acids[2]. However, because the ends of the binding groove are open, peptides can extend outside the groove, allowing class II molecules to bind peptides considerably longer than 8–9 residues. Current evidence indicates that the $\alpha\beta$ dimer is relatively unstable in the absence of peptide[11,13]. Class II molecules which have bound peptide can be distinguished biochemically from empty $\alpha\beta$ dimers by their relative mobility in SDS-PAGE[10]. Under appropriate conditions, the $\alpha\beta$ dimers which contain peptide migrate more rapidly than the empty $\alpha\beta$ dimers in what has been termed the compact and floppy forms, respectively. The α and β chains associate to form dimers upon synthesis in the endoplasmic reticulum (ER). The $\alpha\beta$ heterodimers rapidly associate with a nonpolymorphic chain called the invariant chain (Ii)[19]. The $\alpha\beta$Ii complexes exist as trimers which are assembled in a step-wise fashion in the ER by three $\alpha\beta$ dimers associating with an Ii trimer[17]. The $\alpha\beta$Ii complexes are transported through the Golgi to a post-Golgi, endosomal compartment where the complexes dissociate and the $\alpha\beta$ dimers bind processed antigen which has entered the cell through the endocytic route.

At present there is no firm evidence that the initial assembly of $\alpha\beta$Ii complexes requires the function of ER resident chaperone BiP/GRP78. Thus, although many newly synthesized proteins in the ER interact with BiP (BiP = immunoglobulin heavy chain binding protein) which facilitates their proper folding and oligomerization there is no evidence for a role for BiP in early class II synthesis. Nor is there any evidence that APC express novel chaperones which may play a BiP-like function. However, recent indirect evidence indicates that the class II molecules may interact with two resident ER chaperones, GRP94, an hsp90 family member, and ERp72, a stress protein with homology to another ER resident protein, PDI, a protein disulfide isomerase[32]. Thus, when class II molecules are expressed in the absence of Ii, many class II molecules fail to exit the ER and are found associated with GRP94 and ERp72. This finding suggests that class II may normally, transiently associate with these stress proteins during biosynthesis, and in the absence of Ii fail to dissociate. Class II molecules have also recently been shown to associate in the ER with calnexin, a calcium-binding membrane-bound chaperone unrelated to the HSP families[33]. In addition, the Ii itself may be viewed as playing a chaperone-like function in class II molecule assembly.

There are two predominant forms of Ii, p31 and p41 (reviewed in 21). The initial Ii trimer in the ER can contain both, while individual $\alpha\beta$ dimers will contain either p31 or p41. The Ii chains have at least two important functions in the biosynthesis of class II molecules[21]. The first is to prevent the $\alpha\beta$ dimers from binding peptides which may be present in the ER or Golgi compartments, thus, avoiding the saturation of the MHC class II molecules with self peptides. The second is to direct transport of the $\alpha\beta$ dimers to the correct post-Golgi compartment where processed antigen is present for binding. In addition, during biosynthesis in the ER, the Ii appears to provide a chaperone-like function in ensuring appropriate folding of the $\alpha\beta$ chain dimer. Thus, although $\alpha\beta$ chain dimers assemble in cells which do not express Ii, the dimers are often not folded properly, as evidenced by their failure to express certain antigenic epitopes recognized monoclonal antibodies[26]. In the absence of Ii, the movement of the $\alpha\beta$ chain dimers out of the ER is less efficient[1,26] and many dimers remain in the ER associated with the ER resident hsp GRP94 and ERp72 (ref. 32). Thus, Ii appears to play an important role in ensuring the proper folding and transport of $\alpha\beta$ dimers from the ER.

Ii also functions to block peptide binding to the class II molecule until the class II molecule has reached the appropriate site in the APC where processed antigen is available. Indeed, class II-Ii complexes are not able to bind to peptide in vitro[29]. Recently, an overlapping set of peptides derived from Ii representing residues 80–104 have been shown to be stably associated with class II molecules[4,24,31]. These peptides termed CLIP bind with high affinity to the class II $\alpha\beta$ dimer and compete with antigenic peptides for binding[4]. It is not clear if the competition is due to a direct competition for binding to the class II peptide binding groove or to an allosteric effect on the class II peptide-binding groove induced by CLIP binding elsewhere on the class II molecule. In the former case CLIP may be functioning as a surrogate peptide for class II molecules. As stated above, in the absence of bound peptide, the class II $\alpha\beta$ dimers are relatively unstable. Thus, Ii may function as a molecular chaperone by contributing a peptide to the binding groove of the class II molecule in the ER. The binding of peptide would promote the folding of the class II molecule into a stable intermediate in the ER which would later exchange the CLIP peptide for an antigenic peptide in an endocytic compartment resulting in a further stabilization of the final mature $\alpha\beta$ dimer. In this model, Ii is not functioning as a classic chaperone, namely by binding to an unfolded protein and facilitating its folding, but rather by acting as a ligand for the binding site of the class II dimer.

Once the class II-Ii complexes exit the ER they transverse the Golgi and enter the trans-Golgi network. At present it is somewhat controversial whether the class II-Ii complexes move directly to a post-Golgi processing compartment[25] or whether they move first to the plasma membrane by a default pathway and are then internalized into an endosomal processing compartment[30]. Both Ii and the β chain of the class II molecule contain information which allows targeting to the processing compartments. In the absence of Ii the class II molecules do enter endosomal compartments. However, expression of Ii dramatically enhances that localization[18]. The signal required for localization to endocytic compartments is in the cytosolic tail of Ii and deletion of the signal results in transport of Ii alone or $\alpha\beta$Ii complexes directly to the plasma membrane. There is also evidence that the class II β chain cytosolic tail contains endosomal targeting signals, which may account for the ability of some class II molecules to be targeted to the endosomes in the absence of Ii[21]. The Ii chain may also function to retain the class II molecules in endosomes until processed antigen is bound[23]. Whether the retention and targeting sequences are the same is not clear at present.

Both the p31 and p41 forms of Ii chain are able to function in the biosynthesis of class II molecules, as described[21]. However, recently the p41 form of Ii has been shown to have a greater impact on the ability of an APC to process and present antigen. Indeed, in transfection experiments p41 but not p31 was shown to dramatically enhance antigen processing[27]. It has been suggested that p41 may have a unique function in directing the class II molecules to the endosomal compartments where processed antigen-class II dimers are assembled. The notion that class II $\alpha\beta$ dimers may be transported to different endosomal compartments depending on which form of Ii is associated has important implications for antigen processing. It suggests that there may be two classes of class II molecules whose peptides are derived from two distinct subcellular pools. What function such classes of molecules would play is not clear.

Once the $\alpha\beta$Ii complex reaches the appropriate processing compartment, the nine chain complex must dissociate and the Ii must be removed from the $\alpha\beta$ dimers. At present there is little molecular detail concerning this deoligomerization process. Current evidence indicates that the Ii is proteolytically cleaved while bound to the class II molecule and the resulting fragments dissociate from class II[5]. As discussed above the CLIP peptide of Ii may remain bound, functioning to stabilizing the $\alpha\beta$ dimer prior to antigenic peptide binding.

At present, there is little information concerning the cellular or molecular machinery, including chaperones, which may be involved in this complex process of deoligomerization and peptide binding in the endosomal processing compartments. In an attempt to better characterize the assembly of processed antigen-class II complexes we undertook the purification of the subcellular compartments in which assembly occurred. We reasoned that the assembly process was likely to be a dynamic one, involving very few of the MHC class II molecules present in the APC at any given time. The interactions of class II and Ii with the APC's assembly machinery was predicted to be transient. Thus, to study the assembly process, it would be necessary to purify class II molecules in the assembly process from the bulk of class II molecules in the cell. We carried out subcellular fractionation of APC which had processed a model protein antigen[28]. The processed antigen-class II complexes were detected by the ability of purified, freeze-fractured vesicles to stimulate an antigen-specific T cell hybrid in vitro. We determined that functional, processed antigen class II complexes are first formed in dense vesicles which cosediment with lysosomes. The complexes are subsequently detected in late and early endosomes and later accumulate at the plasma membrane. The vesicles which contain functional processed antigen-class II complexes do not contain intact Ii chain and thus the majority Ii must have been removed before class II entered this compartment. The majority of the class II molecules in this compartment are in the SDS-stable compact form and thus presumably contain peptides. We also identified a second class II containing compartment which was physically separable from the assembly compartment. This compartment has no known markers including those for the ER, plasma membrane, Golgi, lysosomes or early and late endosomes. It has no access to antigen entering the cell by fluid phase pinocytosis. Interestingly, the class II $\alpha\beta$ dimers in this compartment readily present antigenic peptide in vitro at neutral pH and are in an SDS-unstable or floppy form indicating that they are either empty or have not yet bound a peptide which will stabilize the $\alpha\beta$ dimer. Ii is present in this compartment, however the ability of class II molecules to present antigenic peptides at neutral pH suggests that at least a portion of class II is not bound to Ii. We propose that this compartment represents a site where the $\alpha\beta$Ii complexes dissociate, freeing the $\alpha\beta$ dimer to move into the assembly compartment. The ability to isolate these discrete compartments provides a powerful tool to characterize the cellular and molecular machinery required for the assembly of processed antigen-class II complexes. Indeed, as predicted, we found that only a very small fraction of the MHC class II molecules in an APC at any given time are present in either of the two compartments we identified. The vast majority are in the ER and plasma membranes. Thus, the identification of proteins, such as molecular chaperones, which may transiently associate with the MHC molecules during assembly would be difficult if one analyzed the bulk of class II rather than those in the assembly compartments.

What molecular chaperones if any might be involved in the dissociation of Ii from class II, the movement of the class II $\alpha\beta$ dimer into the assembly compartment and the binding of processed antigen to the $\alpha\beta$ dimer? One interesting candidate has recently been suggested by the identification of a gene encoded in the MHC gene complex which strongly influences the ability to APC to process antigen. The phenotype of cells which contain a homologous deletion of this gene is a dramatically reduced ability to process antigen[6]. However, mutant cells are able to present an antigenic peptide which does not require processing. The vast majority of the class II molecules isolated from mutant cells contain CLIP peptides and thus, have not exchanged CLIP for antigenic peptides. It was recently determined that the affected gene is one encoding a class II like molecule called DM (ref. 22). How DM functions is a matter of speculation. One clue as to its function might be found in the recently solved crystal structure of the class II molecule which showed it to be a dimer of $\alpha\beta$ dimers[2]. If the dimer of $\alpha\beta$ dimers represents a stable preferred conformation it may be that the DM molecule functions as a surrogate dimer partner stabilizing the $\alpha\beta$ dimer and chaperoning it to the assembly compartment. Among other alternatives is the possibility that DM serves to facilitate the removal of Ii peptides. Such models make certain predictions which are testable given current abilities to isolate the assembly compartments and to analyze the class II associated proteins.

Over the last several years we have proposed the hsp70 family members as potential candidates in antigen processing (reviewed in 7,8). We began our search for proteins which might facilitate the assembly of processed antigen with the MHC class II molecules prior to the discovery that the mammalian hsp70s were molecular chaperones. We were struck by the early in vitro binding studies of class II to peptides which showed that the binding was extraordinarily slow, in the order of days, and once bound the peptide essentially did not dissociate[3]. However, there was ample evidence that in APC, processed antigen peptide complexes rapidly form, in 15–30 minutes after antigen enters the cell and are relatively rapidly disassembled[20]. One simple explanation was that the APC contained proteins which facilitated the assembly of processed antigen-class II complexes. We envisioned that these might be peptide-binding proteins which would function as scavengers of peptides and facilitate their binding to the class II molecules. Peptide-affinity purification of the APC proteins yielded a mixture of three to four predominant 70 kD proteins. We were encouraged that this mixture of peptide-binding proteins was relevant to antigen processing as antisera raised against the mixture blocked processing. Moreover, the peptide-binding proteins isolated from APC, which had processed radiolabeled antigen, contained radiolabeled peptide indicating that these proteins had access to processed antigen in the cell. In immunoelectron microscopy antisera raised in rabbits to the mixture of proteins stained the plasma membrane and cytoplasmic vesicular structures. Encouraged that the mixture contained proteins involved in processing, we began a biochemical characterization of the individual proteins. Thus far, we have not identified the protein within the mixture which accounts for the antigen presentation phenomenon. We have determined that the three most abundant proteins are unlikely to play a role in antigen processing. These were the already described mammalian resident ER hsp70, BiP and the resident cytoplasmic hsp70, hsc70. The third protein which we had termed PBP74, is the previously unidentified mammalian mitochondrial hsp70[9]. However, currently available serological reagents identify a 72–74 kD protein in the subcellular compartments described above in which processed antigen-class II assembly occurs. This protein is in a protease-protected site. Thus, there appears to be a protein, serologically related to the hsp70 family, in the processing compartment. Further work will be required to establish its identity and its role in antigen processing.

In summary, the assembly of processed antigen-class II complexes in APC is an intricate process which may require the function of molecular chaperones at several stages. The Ii which is coexpressed in class II expressing cells may provide chaperone functions, ensuring the proper folding of class II molecules. In addition, recently identified class II-like gene products may also provide a chaperone function during peptide binding. Lastly, resident ER chaperones and proteins related to the hsp70 family residing in APC assembly compartments may play a role in assembly. Thus, APC may use both highly specialized chaperones as well as common cellular chaperones to ensure the appropriate assembly of processed antigen-class II complexes. At present, there is little molecular detail as to how chaperones function in class II assembly and future work is likely to reveal the function of chaperones implicated in class II biosynthesis as well as the involvement of additional molecular chaperones.

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