A revised model for invariant chain-mediated assembly of MHC class II peptide receptors

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The enormous number of allelic MHC class II glycoproteins provides the immune system with a large set of heterodimeric receptors for the binding of pathogen-derived peptides. How do inherited allo- or isotypic subunits of MHC class II combine to produce such a variety of functional peptide receptors? We propose a new mechanism in which pairing of matched MHC class II α- and β-subunits is coordinated by the invariant chain chaperone. The assembly is proposed to occur in a sequential fashion, with a matched β-chain being selected by the α-chain–invariant chain ‘scaffold’ complex that is formed first. This sequential assembly is a prerequisite for subsequent intracellular transport of the α-chain–invariant chain–β-oligomer and its maturation into a functional peptide receptor.

Inherited polymorphisms result in novel protein–protein interactions

It is estimated that ~60,000 single nucleotide polymorphisms of the human genome are contained within genes [1]. This diversity generates a large number of genes that occur in several alleles. Due to the random combination of parental alleles in every generation of offspring, polymorphic gene products are essentially expressed in a novel environment. In molecular terms, this means that the functional life span of a polymorphic protein is accompanied by numerous novel interactions with other proteins. An extremely variable region of the genome, the MHC region, is an example of a highly regulated assembly of polymorphic proteins [2]. The MHC class II (MHCII) region encodes a great variety of proteins that serve the immune system by presenting antigenic peptides to CD4+ helper T cells [3]. Evolutionary selection pressure by pathogenic microorganisms has ensured that MHCII genes display an extremely high level of polymorphism [4]. To date, more than 870 MHCII alleles have been identified in the human population (European Bioinformatics Institute; www.ebi.ac.uk), from which ~600 proteins were expressed. This high polymorphism produces a great diversity of peptide receptors and enables the species to survey an amazing variety of peptide sequences derived from the multitude of pathogenic organisms.

The polymorphic MHCII α- and β-subunits combine with the non-polymorphic invariant chain (II) to form a premature peptide receptor. The present model of MHCII subunit assembly suggests that α- and β-chains form a heterodimer, which binds to the trimer of II [5]. This model, however, does not explain how mismatched pairing, which might occur between some of the polymorphic α- and β-chains, is prevented. Here, we introduce a revised concept of how the enormous variety of MHCII subunits is matched by interaction with II to form functional peptide receptors.

Isotypic and haplotypic pairing of MHCII molecules

The human MHC consists of two regions, one containing the class IA, B and C genes and one containing the class II D genes. The class II region is divided into three subregions that encode α- and β-subunits of the DP, DQ and DR isotypes (note, in the mouse H2 region, two class II subregions encode α- and β-subunits of isotypes I-A and I-E; I denotes ‘immune response genes’). The duplication of genes, resulting in MHCII isotypes, has created additional diversity to the codominantly expressed alleles, at the level of individuals. In the sequence of MHCII, most polymorphisms are concentrated in the first domain of β-chains. DP and DQ α-chains show some moderate allelic variations at the N-terminus of the α1 domain, whereas the DRα-encoding gene exhibits unremarkable genetic diversity. The mouse orthologs for DR (I-E) and DQ (I-A) exhibit a comparable polymorphism.

The MHCII αβ heterodimers present antigens (which have been harvested in endocytic compartments and delivered to the cell surface) for recognition by CD4+ T cells. During antigen loading, the cohort of MHCII receptors binds to a large array of peptides that contain certain motifs. An antigenic sequence of eight to ten amino acid residues contained within a peptide of 13 to 18 residues in length is lodged in a groove formed by the association of MHCII α- and β-glycoproteins. One to four (anchor) residues of the peptide sequence enable binding of the peptide into the polymorphic pockets of the β-sheet of the MHCII groove. The peptide-binding groove protects the core peptide from further proteolysis.

MHCII α-subunits show a distinct preference for combining with their matched isotype β-subunit (i.e. DQα prefers to combine with DQβ). However, in transfected cells, some of the MHCII isotype subunits might
combine to give rise to mixed heterodimeric complexes, creating a genetically unpredicted diversity [6], but it is unclear whether the mixed isotype complexes exist under normal conditions within the cell. Expression of mixed isotype combinations of the MHCII peptide receptors would be expected to increase the repertoire of antigens presented by a single antigen-presenting cell (APC). However, this is not necessarily the case because formation of misfolded MHCII complexes by aberrant association of subunits could severely impair antigen presentation. Furthermore, inter-isotypic MHCII pairing in human APCs would strongly reduce the amount of the less abundantly expressed DP and DQ molecules (compared with DR) and possibly abolish the unique role of these two isotypes. Thus, regulation of matched isotype α- and β-chain pairing could be required to ensure optimal immune responsiveness (Figure 1).

Similar to isotype matching, allotypic class II subunit pairs have to be examined to determine if they match to form peptide receptors. Allotypic MHCII variants differ in their assembly efficiency. For assembly of some allotypic combinations, coexpression of Ii is strictly required, whereas other combinations assemble efficiently even if expression of Ii is omitted [7]. Mouse H2-A α- and β-chains that are encoded on a single chromosome seem to be evolutionarily selected for heterodimer formation [8]. Mixed haplotype pairing occurs when α- and β-chains of the same isotype, but encoded from genes expressed on the allelic chromosome, combine to form functional units. In inbred mouse strains, these combinations of polymorphic MHCII subunits are not found on parental cells and are unique in APCs from F1 hybrid mice. Indeed, in mice it has been shown that H2-A subunits encoded by some mixed haplotypes show an absolute dependence on the Ii chaperone for optimal folding and transport of the resultant heterodimer [8].

The chaperone role of Ii in MHCII subunit assembly
Upon biosynthesis, proteins are translocated into the endoplasmic reticulum (ER) and there pass a quality control system that involves interaction with chaperones. Most known ER-resident chaperones transiently bind to a wide array of proteins, which appear after translation. Discrete transport competent multiprotein complexes are formed upon selective folding and assembly of protein subunits. Newly synthesized MHCII glycoproteins pass through a transiently aggregated phase [9,10]. These aberrantly mixed MHCII subunits are temporarily complexed with chaperones, such as the ER-resident membrane protein calnexin, or the soluble immunoglobulin heavy chain binding protein (BiP) and possibly other, not yet defined, chaperones [11–13]. Immediately following the aggregated phase, Ii binds to the MHCII chains, promoting folding and subsequent export of the class II complex out of the ER [14]. An additional role of Ii is to sort MHCII molecules into endocytic compartments, where Ii is degraded. An MHCII-attached fragment of Ii, class II-associated Ii peptide (CLIP), is released upon interaction with the class II-like DM molecules [HLA-DM (human), H2-DM (mouse)] and replaced by antigenic peptide [15]. Although Ii is essential for the optimal expression of surface MHCII, α- and β-chains can form dimers in the absence of Ii [9]. For example, in vitro translated α- and β-chains form dimers in the presence of microsomes, which are essential for the assembly of the MHCII membrane-bound subunits [16]. Therefore, it was suggested that a preformed α–β heterodimer binds to Ii [16,17]. Because an interaction of Ii with individual MHCII subunits has also been reported [18–20], this issue remained controversial. Although Ii is not required for the presentation of all antigens [21], the absence of Ii often causes defective MHCII export [22] and aberrant association of DM with MHCII subunits in the ER (DM catalyses the acquisition of peptides by the MHCII heterodimer) [23]. Ii-dependency of MHCII function is explained by the role of Ii to promote the assembly of MHCII subunits and by targeting the MHCII–Ii complex to peptide-loading compartments.

The Ii chain occupies the MHCII groove and prevents the binding of proteins that appear after biosynthesis in the ER. In MHCII-transfected Ii-free cells, newly

Figure 1. A model for matched isotype MHCII subunit assembly. MHCII isotypes derive from duplicated genes, which encode α- and β-chains. Assembly of the α- and β-subunits to MHCII peptide receptors is regulated in an APC yielding matched intra-isotype, rather than inter-isotype, combinations. The matched isotypes are subsequently expressed on the cell surface as peptide receptors (middle arrow). (a) DR, DP and DQ α-chains (green, red and blue, respectively) assemble in the presence of the invariant chain, Ii (yellow), with matched isotype DP, DQ and DR β-chains (green, red and blue, respectively) to form functional peptide receptors. Ii regulates matched isotype pairing. Following the maturation of MHCII oligomers, Ii is degraded, and the Ii-derived fragment CLIP (grey) is replaced by an antigenic peptide (light brown). (b) In the absence of Ii, the α- and β-isotype subunits form mixed complexes. The fate of the mixed-isotype MHCII heterodimers is unclear. The excess of DR subunits might result in the formation of matched isotype heterodimers, which are exposed as peptide receptors on the cell surface.
synthesized MHCII molecules are not significantly loaded with peptide but associate with misfolded polypeptides in the ER [24]. Presumably, the loading conditions in the ER are not optimal for MHCII peptide binding [25].

Ii binds to the enormous number of polymorphic class II glycoproteins without any reported exception. Interaction of Ii with the MHC isotypes DP, DQ and DR is strong, whereas binding to the nonclassical MHCII molecules DM and DO and to the class Ib molecule CD1d can only be detected in lysates using mild detergent [26,27].

**Conserved Ii-binding sites on MHCII chains**

Three Ii chains form a trimer [28], to which MHCII subunits are attached. The multicomponent framework of the MHCII–Ii structure suggests several interaction sites [29]. It has been reported that the transmembrane domains of MHCII and Ii are capable of self-association [30]. However, this interaction is only stable in mild detergent and is complemented by association of other parts of the molecules [31,32]. Ii utilizes a sequence encompassing residues 91 to 99 to bind to the groove of MHCII peptide receptors. The peptide backbone of this Ii sequence provides an additional promiscuous binding site to conserved amino acid residues in the peptide-binding groove of MHCII heterodimers [33].

Interaction of Ii with distinct non-polymorphic sequences on MHCII subunits is required for the promiscuous binding of Ii to the large family of MHCII glycoproteins. Individual MHCII α-chains efficiently coisolate with Ii, whereas individual β-chains exhibit only a low-affinity interaction with Ii [34]. Ii residues Met91, Ala94 and Met99 bind to pockets 1, 4, and 9 of the MHCII (DR3 allotype) groove [33]. The first pocket (P1) is predominantly formed by conserved DRα residues (Figure 2a). A Met91-Gly mutant (which is the anchor for P1) of Ii completely abolished co-isolation of Ii with individual DRα chains [34]. This result suggests that Ii is docked to conserved residues of DRα. Binding of the α-subunit to Ii might suggest interaction with non-polymorphic residues, which explains the promiscuous binding of Ii to all MHCII allo- and isotypes. It is important that the single β-chain does not bind efficiently to Ii because the large molar excess of Ii, which is expressed in APCs, would impair assembly of α–Ii–β complexes and preferentially produce α–Ii and β–Ii pairs. The detection of a preformed α–Ii complex suggests a sequential binding of α- and β-subunits to the Ii trimer. Consistent with this finding, α–β dimer intermediates were not observed [5].

How does the α–Ii complex promiscuously interact with matched MHCII β-chains? The extra-cytoplasmic part of DRβ consists of a highly polymorphic β1 and a more conserved β2 domain. One study identified a novel motif in the sequence of the DRβ chain, which is defined by two tryptophan residues separated by 22 amino acids [35]. This ‘WW MHC class II’ (WWCII) sequence is conserved in the MHCIIβ2 domains of vertebrate species. In the DRβ chain, Trp153 and Tyr123, which are located within the WWCII sequence, form a pocket-like structure and, in conjunction with Asp152, mediate interaction with a proline-rich region of Ii found adjacent to the groove-binding sequence of Ii (Figure 2b).

**Editing of matched MHCII subunits by Ii**

Unlike MHC-I, MHCII heterodimers do not acquire peptides in the ER. Therefore, at the stage of assembly,
the α- and β-subunits are not examined by antigenic peptides to form functional peptide receptors. Based on novel data [34,35], we propose a new model for MHCII subunit assembly whereby the MHCIIα–Ii complex forms a scaffold to which a matched β-glycoprotein is selectively bound (Figure 3a). The α–Ii matrix can bind to any MHCII β-chain. However, in the presence of the isotype-matched β-subunit, a transport-competent α–Ii–β oligomer is formed. This α–Ii–β complex is qualified to mature into a α–β peptide receptor.

Within the transient α–Ii–β complex, the β-chain is examined as a potential subunit of the MHCII peptide receptor complex. The groove-binding sequence of Ii has the role of a master peptide. Interaction of some polymorphic residues of the MHCII β-chain with the Ii master peptide sequence and with the α1 domain might test the ability of the β-chain to fit into the complex. The polymorphic residues of the MHCII β-chain might determine the conversion of transient to stable MHCII complexes. In addition, a recently described hydrogen bond between the conserved His81 of the β-chain and a carbonyl group of the peptide backbone stabilizes the complex [36]. If interaction of the β-chain to the master sequence is inefficient, it is replaced by one of the various MHCII β-chains expressed in APCs. Interaction of the β-chain with α–Ii is accomplished by binding of a proline-rich region of Ii to WWCIID. In contrast to the previously described model (outlined in Figure 3), our new model suggests that Ii is required and subsequently expressed on the cell surface [35]. In the presence of wild-type DRβ, however, association of the WWCIID mutant to DRα and Ii was abolished. It was therefore suggested that the chaperone role of Ii is regulated by interaction of Ii with the WWCIID domain on the DRβ chain.

The conformation of the MHCII peptide receptor is influenced by its interaction with Ii. A conformational change of the α–β heterodimer, observed in the presence of Ii, is maintained following Ii degradation in endosomes and MHCII expression on the cell surface [37]. The structure of the α–Ii–β complex could be different from a complex that would be produced by preformed α–β that associates with the Ii trimer [Figure 3b(i)]. The ‘classic’ model suggests that α–β heterodimers are required to insert into a loop contained in the Ii trimer [38]. By contrast, our revised model suggests that the α- and β-chains form a sandwich-like structure with the Ii trimer [Figure 3b(ii)]. An advantage of this sandwich model is that a mismatched β-chain can still dissociate from the α–Ii matrix and be replaced by a matched β-chain. In contrast to α–Ii–β [Figure 3b(iii)], an exchange of the β-chain in the α–β–Ii complex [Figure 3b(iii)] containing preformed α–β dimers requires several steps of dissociation and reassociation.

Concluding remarks

In contrast to the previously described model (outlined in Figure 3), our new model suggests that Ii is required...
to select polymorphic β-chains in the formation of peptide-binding units. Given the numerous α- and β-allo-
types encoded by the human genome, not all α–β dimers will form functional peptide receptors. This is particularly
important for DQ and DP subunits because both α- and β-chains of these isoforms are polymorphic. A novel com-
bination of polymorphic MHCII α- and β-subunits in every offspring needs to be examined as a functional peptide-
binding unit. The ‘classic’ model of a preformed α–β heterodimer does not explain how matching subunits are
efficiently selected out of the various MHCII glycoproteins expressed in a given APC. The requirement for selective
assembly is evident when one considers the number of potential combinations of isotype subunits and the resulting
intra-isotype heterodimers [39]. We suggest that in this selection process, Ii functions as a unique chaperone for
MHCII isotype subunit assembly. Ii promotes the formation of DR, DP and DQ α–β heterodimers and deters inter-isotypic MHCII pairing. At present, there is no evidence for MHCII peptide receptors other than DR, DP or DQ heterodimers. It is, however, conceivable that, out of the great variety of MHCII molecules, some mixed isotype α–β heterodimers are assembled, which form functional peptide receptors.

Matched or aberrantly assembled MHCII subunits can be distinguished by their ability (or inability) to enter the
MHCII processing pathway. It is still unclear which structural requirement enables export of the MHCII molecules
from the ER.

An additional question concerns the stoichiometry of MHCII oligomers. Are there several MHCII isoforms con-
tained in one complex with the Ii trimer? For mouse MHCII, it has been demonstrated that I-A and I-E hetero-
dimers form distinct complexes with Ii [40,41]. This question has not been resolved for human MHCII isoforms.

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