

MHC-GUIDED PROCESSING: BINDING OF LARGE ANTIGEN FRAGMENTS

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Ever since the emergence of models for the processing and presentation of antigenic determinants by MHC class II molecules, the main view has been that proteins are unfolded, enzymatically cleaved into peptide lengths of about 12–25 amino acids and then loaded onto MHC class II molecules. There is, however, an alternative model stating that partially intact unfolding antigens are first bound by MHC class II molecules and then trimmed to fragments of a smaller size while remaining bound to the MHC class II molecule. In this analysis, we make the case that a considerable portion of the elutable peptide cargo belongs to this latter class.

Although the crystal structure of MHC class II molecules is less than 10 years old, it was appreciated early on that the strategies of ligand disposition in the MHC class I and class II systems were distinctive. In contrast to the closed end peptide-binding cleft of MHC class I molecules, the MHC class II cleft allows the binding of long peptides or even whole protein molecules¹, as long as there exists a relatively available stretch of peptide, 9–15 residues in length, which can settle into the MHC class II peptide-binding groove. The crystal structure of the MHC class II molecule, indicating its open-ended binding groove, provided direct structural evidence for the observed ability of MHC class II molecules to bind long peptides. Even in the light of such data, however, it was widely assumed that MHC class I and class II molecules have a common mechanism of binding, involving short pre-processed peptides. For MHC class II molecules, these were the products of various proteolytic events that start in the nearly neutral early endosomes and culminate in the more acidic, vesicular compartments of the antigen-presenting cell (APC). This could be termed the ‘cut/trim first, bind later’ model. In this article, we review some of the evidence for an alternative model, which could be termed the ‘bind first, cut/trim later’ model of MHC class II binding, and point out that this leads to various functional consequences that are important for thinking about immunodominance and autoimmunity.

The cut first, bind later model

The cut first, bind later model became the paradigm for the binding of peptides to MHC class II molecules, largely because of the early parallel studies on the structure and binding characteristics of MHC class I molecules. Short peptides of 8–10 amino acids fit tightly into the MHC class I peptide-binding grooves of different mouse alleles. The closed ends of MHC class I molecules do not allow long peptides to bind, and the charges at the ends of the MHC class I peptide-binding groove bind the complementary amino and carboxyl groups at the ends of a peptide. The enzyme activity of the proteasome produces short peptides that, in a highly orchestrated and chaperoned series of steps, enter the endoplasmic reticulum (ER) and eventually bind to the MHC class I molecule^{2,3}.

Measurements of the size of peptide fragments that were eluted from MHC class II molecules after overnight exposure to exogenous or endogenous sources of native antigen indicated that the average size of these peptides was from 13–22 amino acids, with the modal size being 17–19 amino acids. However, smaller-sized core peptides, from 10–12 amino acids in length could also bind to MHC class II molecules and in many cases had immunogenicity that was similar to the longer peptides that were eluted. Peptides that interact with the same MHC class II

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molecule shared a binding motif and could inhibit interactions with the binding groove of the other peptide, on the basis of their relative affinities⁴. Short peptides of 10–15 amino acids were adequate for the induction of proliferation and other functions of T cells, which could be recalled by the same peptide. Furthermore, peptides with the highest affinity for MHC class II molecules were often the most immunogenic.

These experiments led to the conclusion that this was the common size of MHC CLASS II DETERMINANTS, and it was confirmed by the structural evidence that, similar to MHC class I molecules, the essential specific core pockets in MHC class II molecules also fit a nonamer⁵. When a set of MHC-bound peptides (from a single protein antigen), which were generated overnight by the processing and presentation machinery of an APC, were eluted and sequenced, a partially overlapping set of amino-acid sequences were found, with a common core nonamer present in each of them, and ragged amino and carboxyl termini of differing lengths⁶. (Actually, such data were equally consistent with a bind first scenario.)

These findings gave rise to the common conception that the pools of peptides that bind to MHC class II molecules are late products of proteolytic cleavage in the more acidic parts of the endosomal/lysosomal system, which then exchange with the class II-associated invariant-chain (Ii) peptide (CLIP)^{7–9}. CLIP (Ii residues 81–104) is the residual portion of Ii, the core of which is bound in the MHC class II peptide-binding groove and its displacement by an antigenic element is promoted by the HLA-DM/HLA-DO chaperone molecules. HLA-DM/HLA-DO are MHC-encoded molecules, the function of which is to catalyse the removal of CLIP from the MHC class II peptide-binding groove and to help in editing the selection of tightly binding peptides.

Bind first model: accessibility of determinants

It has been known for many years that despite the impossibility of binding a tightly folded globular molecule, such as hen-egg lysozyme (HEL), to MHC class II molecules, a molecule as large as fibrinogen could apparently bind in the absence of processing¹⁰. This indicated to the authors that there must be a relatively open and available portion of fibrinogen that could gain access to MHC class II peptide-binding grooves, and this was identified. Similarly, Sette *et al.*¹ showed that full length, reduced versions of four different antigens — bovine albumin, HEL, ovalbumin and ribonuclease, could be bound to the appropriate MHC class II molecule, but the unreduced versions could not. This was an early hint of the importance of determinant accessibility for binding to MHC class II molecules. A second feature of these results was that the reduced antigen generally bound to only one of the MHC class II molecules that were present — a sign that the MHC class II molecules might have to compete for a limited number of available determinants on these reduced antigens.

High-affinity, but invisible, determinants

In the cut first model, one would expect that many determinants on the antigen would make an impact on the system and few, if any, determinants of high affinity for MHC would remain invisible or poorly presented. Indeed, this is not the case. In fact, for many antigens, there is only a single dominant determinant that is presented in a haplotype, despite the existence of other determinants that can bind adequately to MHC class II molecules. Furthermore, reports exist in which high-affinity binders, such as HEL (87–96)⁴ with I–E^K, fail to induce a response when provided in the context of a whole protein antigen. Such a finding would not be expected from a cut first model, unless it is presumed that the assortment of available processing enzymes cannot generate certain determinants. Clearly, high affinity alone does not guarantee dominant presentation of a potential MHC class II determinant.

Protection of the bound peptide

Werdelin¹¹ made the suggestion that the basic function of I–A molecules was to protect small peptides, actually rescuing them from degradation by the proteolytic machinery in the APC. Later experiments by this group¹² clearly showed that the portion of a peptide in the MHC class II peptide-binding groove remained protected from enzyme attack. Interestingly, a mutually beneficial relationship exists between the bound peptide and the MHC class II molecule. When bound to a peptide ligand, the MHC class II molecule adopts a stable structure that prevents its aggregation and degradation in the lysosomal compartment¹³. As the authors point out, there are two possible fates for the MHC class II molecule after the loss of CLIP: productive binding of a local peptide and eventual presentation of the complex on the cell surface, or aggregation of the empty dimers and their destruction. Initial interactions of low-affinity peptides with the binding cleft might be short-lived, but they render the MHC molecule particularly receptive for binding. Generation of the receptive phase seems to be the rate-limiting step before the rapid step of association with a high-affinity ligand¹⁴.

Probably the best test of the protection hypothesis was carried out by Donermeyer and Allen¹⁵, who prepared a peptide that contained the good I–A^K binder, HEL (52–61), which was flanked by unnatural D- rather than the natural L-amino acid 12-mers at both the N- and C-termini, so that only the central portion would lie in the groove and supposedly be protected from chymotryptic activity. In fact, the extended peptide, HEL (40–73), when first bound to I–A^K, was 1,000 times more resistant to chymotrypsin than in the absence of MHC class II molecules. This experiment fully validated the concept of determinant protection and also showed that large peptides can bind and not obstruct presentation of the determinant that is safely ensconced in the MHC peptide-binding groove.

Longer peptides are better

In early published work from Geffer's¹⁶ and Schwartz's¹⁷ laboratories, peptides of 27–39 amino acids had been shown to be presented by fixed APC. Actually, rather than

MHC CLASS II DETERMINANT
A region on an antigen comprised of the same core residues that contact the MHC peptide-binding groove with various flanking residues. A determinant, in the strict sense, is the sequence that is required for recognition by a particular T cell.

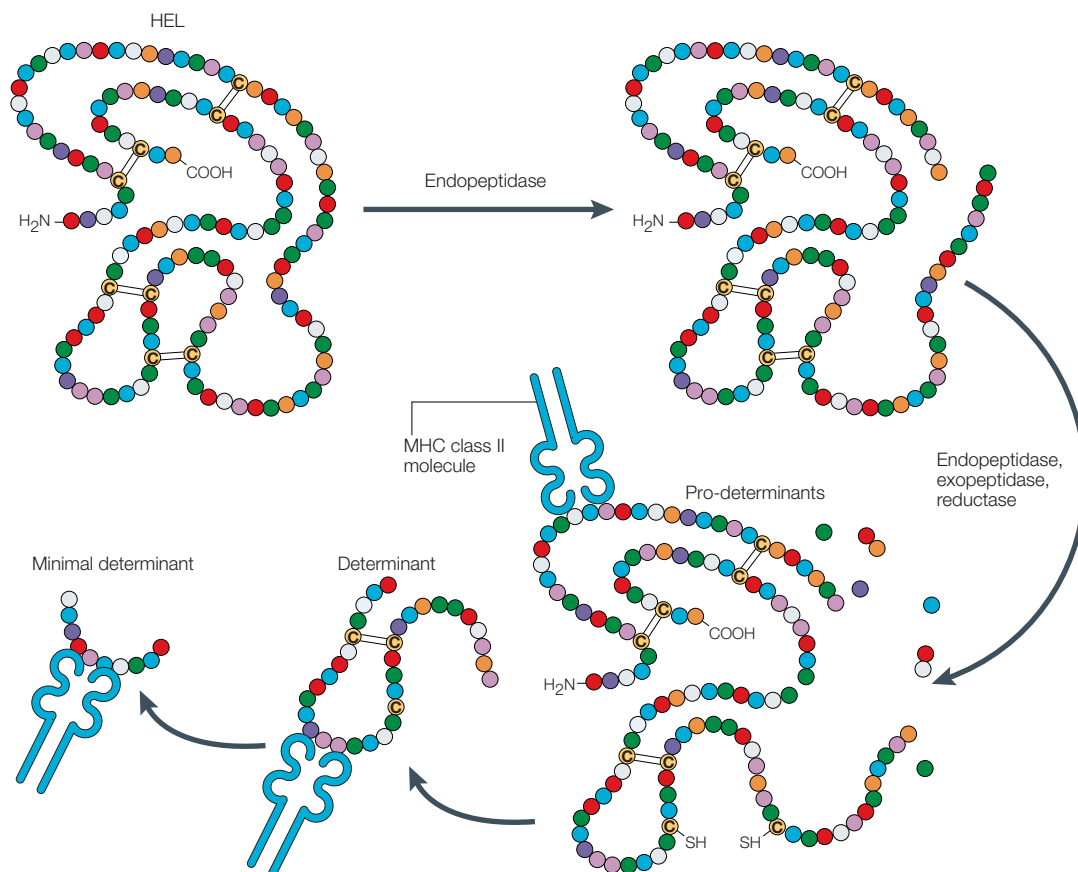


Figure 1 | MHC-guided processing and the role of proteolytic enzymes. Definitions of sequential stages are shown in the processing of an antigen such as hen-egg lysozyme (HEL). HEL is first cleaved by an endopeptidase at its most susceptible site. Subsequent cleavage by exopeptidases and endopeptidases, as well as the reduction of certain disulphide bonds, continues the degradation of the molecule. During this process, the various 'pro-determinants' are produced that can bind to MHC class II molecules at many locations in the acidic vesicular endosomal system, after removal of the class II-associated invariant chain (Ii) peptide (CLIP). Determinants are the polypeptides or short peptides that can be eluted from the MHC class II molecule, and these include the minimal core determinant with or without peptide-flanking residues.

the shorter MHC class II peptides being optimal for binding, inducing or recalling responses to them, the opposite was true and the longer peptides were much better. In a study of cytochrome *c* peptides and hybrid peptides constructed with them, it was shown by Pierce's group¹⁸ that a peptide of 51 amino acids not only required no processing, but also was preferable to a peptide that was less than half its size. There was a steady improvement in the immunogenicity of the core-peptide region as the peptide was lengthened. The 23-mer peptide was more than 32 times better than the 10-mer cytochrome *c* peptide.

MHC-guided processing

'MHC-guided processing'^{19,20} is a consequence of the bind first, trim later model. In this model, the entity that initially makes contact with the MHC class II peptide-binding groove lies in a large fragment of the original antigen (FIG. 1). Once bound, its optimal binding core is protected by the MHC class II peptide-binding groove, but its protruding ends remain available for trimming by exopeptidases. MHC-guided processing is closely connected to immunodominance, because the

IMMUNODOMINANT DETERMINANT of the antigen will be a direct function of the availability and affinity of such determinants of the original antigen and their early selection at the first stages of processing by MHC class II molecules. For self antigens, these are the determinants that, by carrying out negative selection (and possibly also, positive selection), have a role in the subsequent qualitative establishment of the T-cell repertoire.

It is of interest that the recent evidence on the processing of determinants for MHC class I binding fits the pattern of MHC-guided processing outlined earlier^{21,22}, which had been indicated for MHC class I molecules by Falk *et al.*²³. Although the final peptides that are bound in the MHC class I peptide-binding groove are 8–10 amino acids in length, most antigenic products that are released by cytosolic proteasomes are longer, although not as long as the extended peptides that bind to MHC class II molecules. For example, in the well-studied ovalbumin model of the SIINFEKL peptide binding to H-2K^b, more than twice the number of SIINFEKL peptides have N-terminal extensions of 1–7 extra amino acids²⁴, and N-terminal trimming follows after secure binding of the

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A determinant on a multi-determinant antigen that induces a response in antigen-primed cells, challenged *in vitro* with a peptide that contains the determinant.

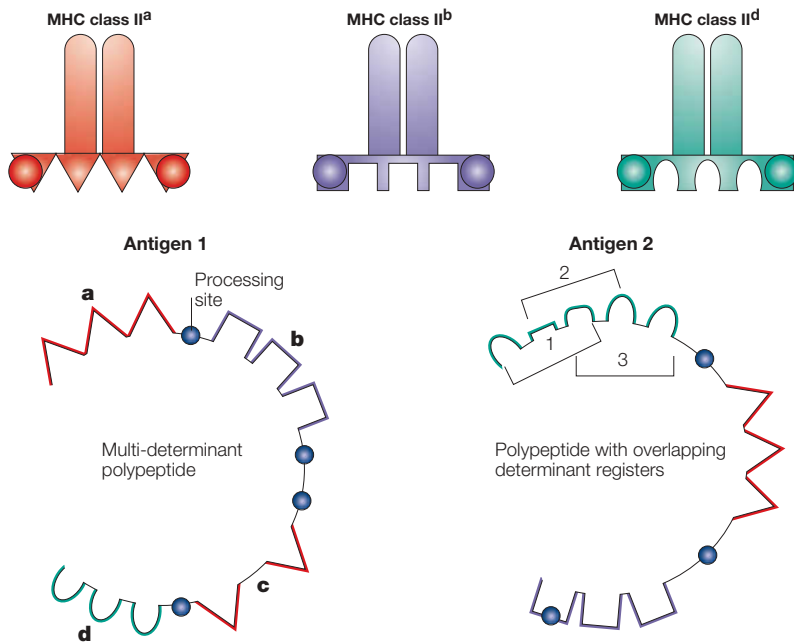


Figure 2 | Competition between MHC class II molecules for antigen and vice versa. Antigen 1 (with determinants denoted as a, b or d) and several scattered processing sites, can bind to MHC class II allotypes a, b or d. During determinant capture, two different MHC class II molecules non-competitively compete for well-fitting determinants on local antigens. In particular, determinants a and c can bind to MHC class II^a, whereas determinants b and d can bind to MHC class II^b and MHC class II^c, respectively. Each of the MHC class II molecules can, therefore, compete for initial binding to the most available of the determinants on antigen 1, which mostly depends on the local endopeptidase activity and the position of relevant processing sites. In the competition between overlapping or adjacent determinants, antigen 2 has a region with overlapping determinant registers, which can bind with differing affinities to MHC class II^d. Registers 3>2>1 are arranged in their order of affinity for the MHC class II^d peptide-binding groove; accordingly, determinant 3 will win the competition, and if the disparity between the binding affinities is great, 3 might prevent 2 or 1 from any attachment to the MHC class II^d molecule.

C-terminal anchor residue at the right edge of the MHC class I peptide-binding groove. Removal of the flanking N-terminal residues requires the presence of the MHC class I molecule²²; the ER aminopeptidase that concludes the processing has recently been identified²⁵. So, both MHC class I and class II molecules can guide the processing of their bound antigenic peptides.

Pro-determinants and the first cut

A crucial initial step in the processing of a compact globular antigen is frequently required before bind first, trim later events can be initiated. The identity of endopeptidase or reductase enzymes that make the first cut of the antigen and allow the earliest binding to MHC class II molecules by a structure known as the 'PRO-DETERMINANT'¹⁹ has been a mystery, until recently, when three different approaches have yielded three possible candidates. Manoury, Watts and their colleagues^{26,27} isolated an asparagine-specific cysteine endopeptidase. In studying the antigen processing of a domain of the microbial tetanus toxin antigen (TTCF), it was discovered that cleavage by this enzyme was required for the successful presentation of TTCF to a panel of TTCF-specific T-cell clones. Using another approach, Schneider *et al.*²⁸ found that by mutating HEL to introduce a dibasic processing

PRO-DETERMINANT
The largest derivative of the whole antigen that can bind directly to a MHC class II molecule.

site adjacent to a subdominant determinant, presentation of the determinant was enhanced 10–40 fold. It was believed that a member of the family of pro-protein convertases²⁹ was involved. Another crucial enzyme, γ -interferon-inducible lysosomal thiol reductase (GILT) was recently implicated in processing determinants of proteins with disulphide bonds^{30,31}. GILT-knockout mice could respond to HEL (20–35) and (30–53), each sharing a cysteine at position 30, but the tightly folded double disulphide-bond region that encloses HEL (74–88) failed to elicit a response. Interestingly, the presentation of a determinant in HEL (46–61), without a cysteine but close to Cys64, was affected by GILT, presumably because reduction of the nearby disulphide bond, Cys64–Cys80, is GILT dependent and its reduction is required before any further processing can occur.

Competition among MHC class II molecules

The production of such partially open antigenic structures should lead to the display of newly available sites of attachment for MHC class II molecules that require a binding peptide. The competition that arises among different MHC class II molecules for the newly available determinants would presumably be won by those with the greatest affinity for an available stretch of a large, unfolding fragment of the original antigen. After the initial binding event, further action by endopeptidases would render other processing sites available for enzyme attack. Later, exopeptidic processing events would trim the bound antigen. Thereby, most antigen-processing events occur flanking the protected portion of the unfolding antigen, which lies in the MHC peptide-binding groove. Provided that the early peptide–MHC interaction is stable enough, the combination might then be dominant in responsiveness to the whole antigen. This type of competition is known as determinant capture, because the local MHC class II molecules compete to capture a single pro-determinant (FIG. 2).

It is also possible that a second form of competition exists, between the different regions of the unfolding antigen for binding to a single MHC class II molecule. Within the large pro-determinant there might be more than one region that is available for binding to MHC class II molecules. In such a case, the most available determinant with the strongest affinity for the MHC class II molecule will predominate. Note that the affinity of a region for the MHC class II molecule is not the only factor that determines the outcome of this competition. Availability also has a crucial role. This type of competition has been termed competitive capture, because different determinants of the unfolding antigen compete for binding to a single MHC class II molecule (FIG. 2).

Indirect evidence for determinant capture

Studies in the non-obese diabetic (NOD) mouse — a strain that develops spontaneous diabetes and has a single MHC class II molecule, I–A⁸⁷ — were carried out to test whether competition existed among different MHC class II molecules for binding to a multi-determinant polypeptide. It was known that provision of another MHC class II

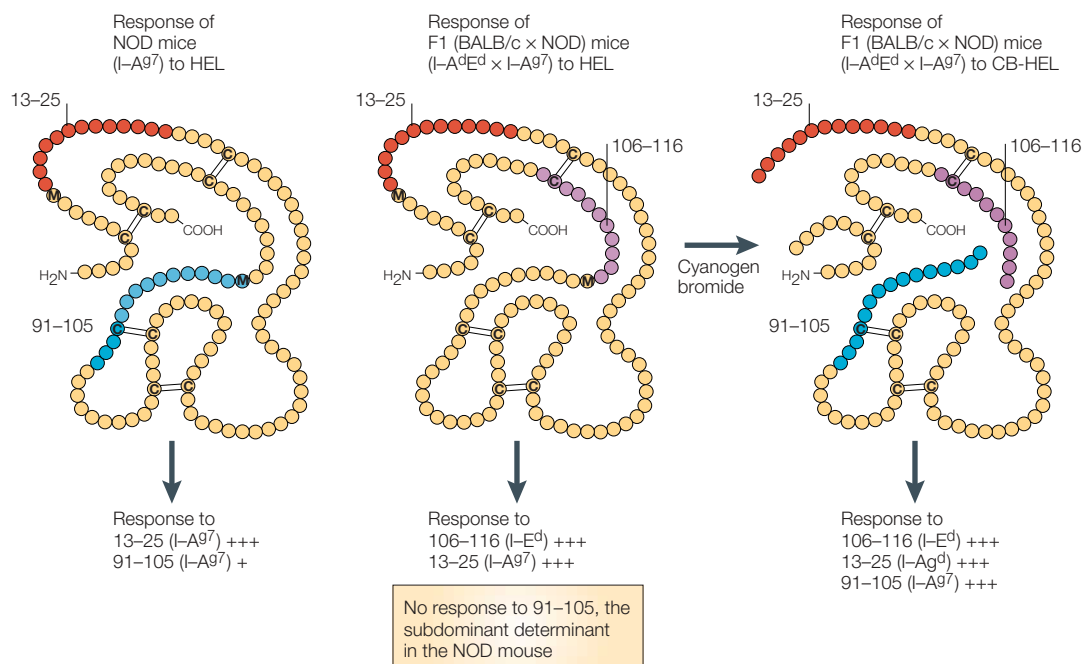


Figure 3 | Determinant capture in the response to HEL by NOD mice. The response to hen-egg lysozyme (HEL)-derived determinant 13–25 is dominant in non-obese diabetic (NOD) mice, whereas peptide 91–105 gives a subdominant response. To study possible determinant capture by the dominant 106–116–(I-E^d) determinant, the F1 generation between BALB/c and NOD was produced and its response to HEL studied. As predicted, there was no response to the subdominant determinant 91–105 in the F1 mice, presumably owing to the existence of the neighbouring 106–116 determinant and its capture by I-E^d. To test this notion, treatment of HEL with cyanogen bromide (CB) was used as it cleaves HEL at the carboxy-terminal side of methionine residues at positions 12 and 105. The latter cleavage separates 106–116 from 91–105, so they can be independently bound to their corresponding MHC class II molecule. Indeed, when the molecule, CB-HEL, was used as the immunogen, not only was the response of 91–105 detected but also, because of its enhanced access, this response became a co-dominant one. Accordingly, the lack of response to 91–105 after immunization of the F1 mouse with HEL is not due to a regulatory effect or the lack of T cells, or a defect in the function of antigen-presenting cells. Instead, it is due to the determinant-capture effect by I-E^d, which binds to HEL (106–116) strongly, preventing access by I-A^{g7} to the neighbouring HEL (91–105) determinant. See the text for further details of this experiment.

molecule by transgenesis could prevent diabetes³². But why? We sought an answer by exploring the mechanism of determinant capture in the context of the NOD mouse, using HEL as a model antigen³³. The response in NOD mice to HEL is focused on a dominant determinant in peptide 11–25 and a subdominant determinant at residues 91–105 that are both presented by I-A^{g7}. To create a competitive environment, we provided the NOD mice with extra MHC class II molecules, I-E^d (and I-A^d), by crossing NOD with BALB/c mice. Specifically, we asked whether competition between I-E^d for its high-affinity binding determinant (amino acids 108–116) and I-A^{g7} and its ligand (amino acids 91–105) would eliminate the weaker response to the 91–105 bound by I-A^{g7}. In fact, this was the case (FIG. 3). We hypothesized that the lack of response to 91–105–(I-A^{g7}) in the F1 animal was due to the capture of a pro-determinant of HEL — containing both the 108–116–(I-E^d) determinant and the flanking 91–105–(I-A^{g7}) determinant — by I-E^d. In other words, binding of 108–116 to I-E^d captured a long polypeptide that is derived from early degradation of HEL and rendered the neighbouring 91–105 region inaccessible to I-A^{g7}. To test this proposition and to assess whether there were any T cells in the F1 animal that could respond to the 91–105 peptide, we produced

a derivative of HEL that is cleaved after methionine at position 105 using cyanogen bromide. This cleaved protein remained enzymatically active; however, the 91–105 determinant that binds to I-A^{g7} was separated from the I-E^d-binding determinant 106–116. It was hypothesized that if the lack of response to the I-A^{g7}-binding determinant 91–105 in the F1 animal were due to the binding of 106–116 by I-E^d, then the separation of these two determinants should allow for the binding and presentation of 91–105 by I-A^{g7}, which seemed to be true. When the cyanogen bromide-treated HEL was used to prime the (NOD × BALB/c) F1 animals, the 91–105 determinant gave an enhanced response in the mouse. In summary, cleavage of the 91–105 determinant from 106–116 by cyanogen bromide prevented the I-E^d molecule from capturing the 91–105 region during the process of binding the 106–116 peptide in its MHC peptide-binding groove. As a result, the 91–105 determinant gave an enhanced response in the F1 mouse, which was comparable to a dominant determinant, attributable to its extra freedom at the newly created peptide-chain terminus (FIG. 3). Various similar situations exist^{34,35} that could be interpreted to involve successful competition between MHC molecules for a portion of an unfolded molecule.

Direct evidence of partial degradation

Castellino *et al.*^{36,37} showed that, when care was taken not to exclude the longer peptides during the elution and separation of bound peptides from the MHC class II molecules, a considerable number of larger HEL peptides — with a molecular weight between 3,000 and 7,000 — could be eluted from either I–A or I–E MHC class II molecules. Nevertheless, these larger species were still a minority of the peptides eluted. Similarly, experiments by the Germain group³⁶ with a model antigen further supported the notion of MHC-guided processing. Using the HEL model, they reported that large complexes of 120 kDa could be found in the endocytic pathway that were comprised of a single HEL polypeptide chain of about 70 amino acids, bound to two different MHC class II isotypes, I–A^k and I–E^k. Surprisingly, these complexes were long lived; this was attributed to the coexistence of the two bulky MHC class II molecules that interfered with the access of some proteases, preventing further cleavages. In this example, there is no single MHC molecule that predominates among the different MHC molecules. Nevertheless, it clearly shows that binding of MHC class II molecules can occur before excessive antigen processing.

Comparison to invariant-chain processing

The bind first, trim later model of antigen processing can be envisioned as comparable to the stepwise processing of the invariant chain, Iip31, (31 denotes the size in kDa of its predominant form) (FIG. 4). Initial contact with the MHC peptide-binding groove seems to be restricted to the CLIP portion of Ii, although there is evidence that additional weaker sites of interaction that are distant from the groove-interaction site exist, in particular at residues 91–99 of Ii^{37–42}. The non-CLIP interactions with MHC class II molecules (at A and B in FIG. 4) might be as important as those that involve CLIP, in helping to align or stabilize the pro-determinant in the peptide-binding groove. There are other non-CLIP sites on Ii, which interact at different stages with MHC class II molecules for trafficking and antigen loading. Then, a succession of proteolytic events by non-cysteine and cysteine proteases remove portions of Ii sequentially, through Iip22 to Iip10 followed by the particular cleavage of Iip10 by **cathepsin S**. Many proteins might be required to be unfolded at a preferential location with a particular protease. Ii provides such a prototype with cathepsin S (in B cells and dendritic cells⁴³) or **cathepsin L** (in thymic epithelium)⁴⁴ carrying out the last trimming of Ii to CLIP. Finally, CLIP is removed in the presence of the Ii ‘chaperone system’ (HLA-DM/HLA-DO), to be replaced by polypeptides of varying length and increasingly tight fit before transiting to the cell surface for exposure of the bound peptide–MHC class II complex to CD4⁺ T cells.

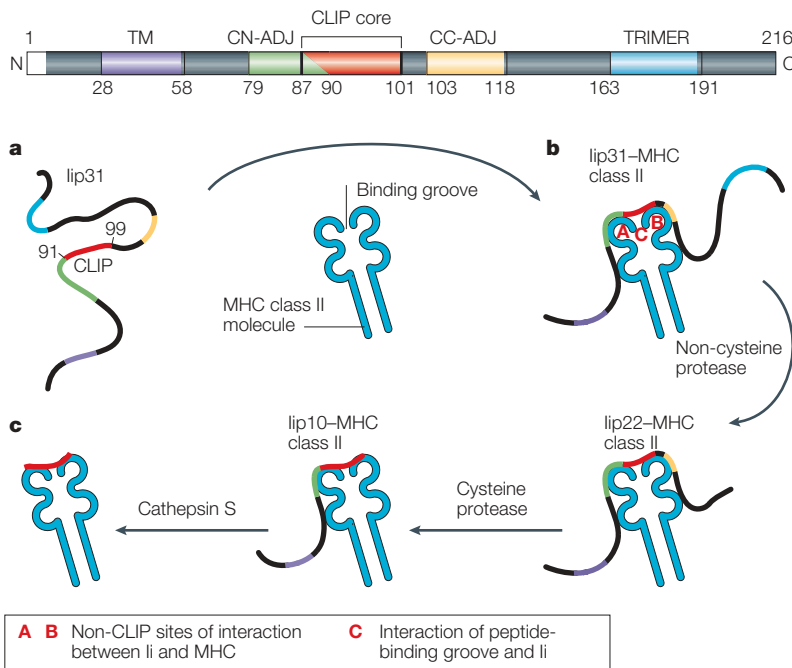


Figure 4 | Invariant chain processing: is it comparable to antigen processing? The stepwise processing of the invariant chain (Ii) is shown, with various of its functional sites of interaction with MHC class II molecules indicated by different colours — TM, transmembrane; CLIP, class II-associated Ii peptide; CN-ADJ, CLIP amino-terminal adjacent peptide; CC-ADJ, CLIP carboxy-terminal adjacent peptide; TRIMER, trimerization domain. A rough analogy is intended between the processing of Ii and of HEL, for example, as shown in FIG. 1. Ii is shown at the top of the figure in an extended form with five of its binding sites to the MHC class II molecule in their relative positions. The 216 residue Ii is eventually cleaved to CLIP, which is usually considered as comprising amino acids 81–104 — with its core binding residues, 91–99, shown in red at stage (a). At stage (b), sites A and B on the MHC class II molecule represent areas that have a binding affinity for regions on Ii; site C is the MHC class II peptide-binding groove, which binds to the CLIP segment of Ii. After arrival in the vesicular compartments, a reduction in the flanking ends of Ii proceeds stepwise until the final cleavage with cathepsin S produces a CLIP–MHC class II complex (c). Subsequently, HLA-DM molecules catalyse the removal of CLIP and its exchange with antigenic entities.

Flanking determinants and flanking residues

Competitive interactions between different determinants on a single long polypeptide for binding to the same MHC class II molecule have been studied. Such competition has often been shown to involve neighbouring or overlapping, but distinct registers on a multi-determinant region of a peptide^{34,45}. In this competitive environment, a determinant-display hierarchy can result with the winner assuming the role of a dominant determinant. For self-antigens, determinant hierarchies in the thymus have a direct impact on the development of the T-cell repertoire. In the case of thymic presentation, the best-presented determinant in a multi-determinant region will provide the strongest tolerogenic pressure. T cells that are specific for these well-presented determinants will, in most cases, undergo negative selection in the thymus. We have recently proposed this type of competition as a mechanism of protecting autoreactive T cells from negative selection. T cells that are specific for poorly presented self-determinants — those outcompeted by better binding flanking registers — will be subjected to little, if any, tolerogenic pressures. If this hierarchy is altered in the periphery so that a self-determinant that was originally presented poorly can now gain better access to the MHC class II peptide-binding groove; the autoreactive T cell, which originally escaped tolerance owing to poor determinant presentation, might become activated to induce autoimmunity. The increased presentation of the poorly expressed ‘loser’ determinant could result from various mechanisms

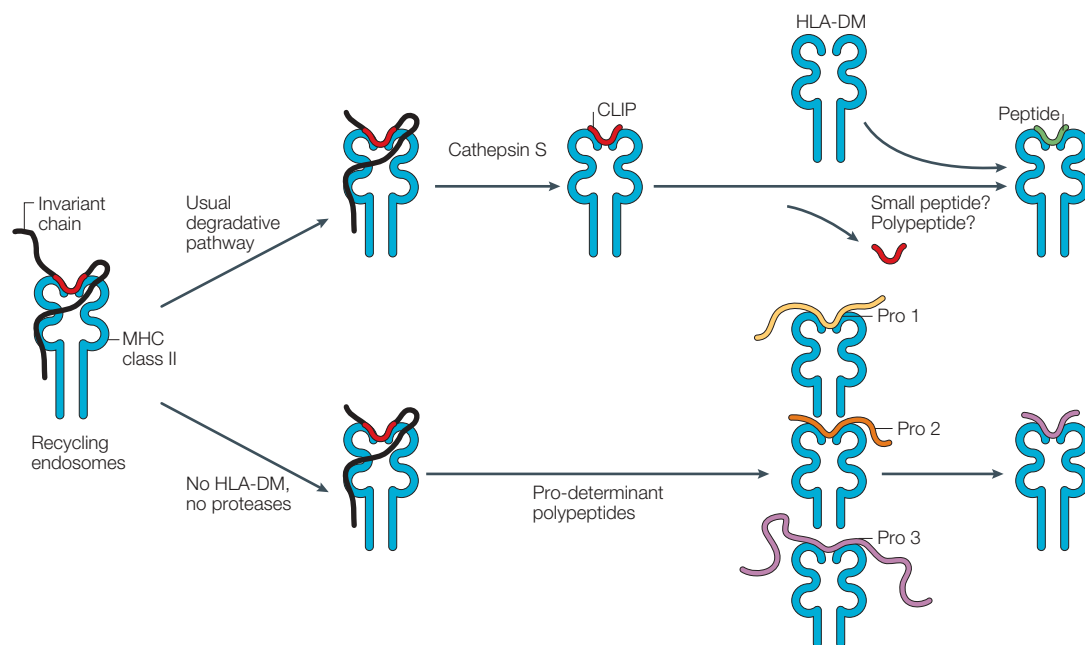


Figure 5 | **Two postulated pathways to final antigen presentation by MHC class II molecules.** The conversion of the invariant chain (Ii)-MHC class II complex to a peptide-MHC class II complex, can proceed with or without protease cleavage of Ii and HLA-DM dependence. The classic pathway (at the top) repeats the sequence shown in FIG. 3, showing the final step as a replacement of class II-associated Ii peptide (CLIP) by a local peptide or longer polypeptide. (There might be few long polypeptides available in the later endosomal compartments). The second pathway does not involve HLA-DM or proteases, but involves the removal of Ii from MHC class II molecules in an early endosome followed by the binding of various pro-determinants (Pro). (The second pathway includes ideas from REFS 51 and 54).

including: first, differential processing machinery in the periphery^{46–48}, second, the upregulation of antigen processing and presentation in the setting of inflammatory cytokines, or third, alternative splicing of RNA (or in our case, transcription from a different promoter site) that results in the loss of a competitive flanking register^{49–51}. So, in the thymus, competition at the level of thymic processing and presentation can have dangerous implications for autoimmunity.

Flanking residues near the determinant can also have an important role in hindering access of particular T cells to the determinant in the MHC class II peptide-binding groove⁵². In this situation, a bound peptide would seem to be non-immunogenic in the context of the immunogen or FUNCTIONALLY CRYPTIC, although it would have established a firm connection to the MHC class II peptide-binding groove. Alternatively, many peptide-flanking residues, particularly at T-cell receptor (TCR)-interaction positions –1 and –11 have an important participatory role in MHC class II-bound determinants for most CD4⁺ T cells⁵³. The clever studies by Unanue's group⁵⁴ on extensions of a core HEL determinant, 52–60, were in accord with the binding of an entity that was longer than the core determinant, followed by trimming.

Finally, we propose that flanking residues at some distance along the antigen might — as in the Ii model — interact closely with non-groove, adventitious binding sites on the MHC class II molecule, increasing the apparent binding affinity of the long polypeptide.

By analogy with the many interactions between Ii and MHC, and granting that certain of these have evolved to satisfy specific requirements for the optimal function of Ii, it is probable that non-groove interaction sites between large antigenic fragments and MHC class II molecules account for enhanced binding by longer peptides¹⁸.

Sites of peptide-MHC class II association

Castellino and Germain found that MHC class II molecules interact with antigen in many endosomal organelles; each compartment has a different pH and unique proteolytic capacities⁵⁵. Although we have not discussed the sites at which the unfolding antigen or derived peptides contact MHC class II molecules, it is relevant to the question of the order of events during processing. Several related issues have not yet been resolved: first, where do the Ii-MHC class II complexes enter the endocytic pathway and is it at one site or many; second, do all APCs function the same way in these respects; third, are the HLA-DM/HLA-DO chaperones and Ii always involved? It can be agreed that in the early endosomal, less acidic vesicular compartment, encounters will involve longer, and less reduced, antigenic polypeptides. Even a partially folded full-length HEL molecule can form complexes with I-A^k molecules⁵⁶. There is also accumulating evidence that entry of the Ii-MHC class II complex into the endosomal processing area requires passage through early endosomes^{57,58}.

FUNCTIONALLY CRYPTIC DETERMINANT

Unlike the dominant determinant, the functionally cryptic determinant fails to induce a response in antigen-primed cells when challenged *in vitro* with peptides that contain the determinant.

If acidification of endosomes is prevented by treatment with concanamycin B, protein trafficking among early and late endosomal compartments is disrupted⁵⁹. Concomitantly, Ii can be removed from MHC class II molecules without involvement of cathepsin S in the early endosomes, allowing direct engagement with long polypeptide pro-determinants from initial antigen processing. However, it is possible that through the classical cathepsin S–HLA-DM/HLA-DO pathway, high-affinity cores in long polypeptides could also gain access to MHC class II molecules (FIG. 5). Until the resolution of these views, the most inclusive way to think of antigen–MHC class II association is that however the delivery from the trans-Golgi network occurs, interaction of MHC class II molecules and pro-determinants will take place at various sites, such as early and late endosomes and the MHC class II compartment (MIIC), allowing the greatest access to antigen in all its forms — as long, partially processed polypeptides or as short, highly processed peptides. It has been indicated previously^{60,61} that sampling from many processing compartments, with their somewhat different mixture of proteases and their differing pHs, will maximize the chances for the organism to present the highest and most varied yield of determinants to the T-cell population.

Concluding statement

The bind first, trim later sequence agrees with all data at present and leads directly to the distinction between immunodominance and crypticity. Recent evidence of the trimer with two MHC class II molecules attached to a large lysozyme fragment³⁶ provides strong evidence for this model of MHC-guided processing^{19,20}. Several

enzymatic first steps have been shown recently^{27,28,31}, which are required for the initial unfurling and cleavage of tightly folded antigen molecules by local endopeptidases, even before the first binding events. Another insight that emerges from the realization of binding by long antigenic polypeptides is the frequency of intramolecular competitive events, and the importance of the accessibility and affinity of these interactions. The determinants that will become competitive winners are highly dependent on a series of chance events, starting with the form of the antigen, the presence of particular proteolytic enzymes in the compartment in which they are present, and the competition between proteolytic enzymes and different MHC class II molecules for binding to processing sites on the antigen.

Thoughtful consideration of MHC-guided processing would provide important insights for vaccine strategies, and for therapeutic tactics for cancer and autoimmunity. For example, in planning multiple vaccinogen studies, 'beads-on-a-string' ideas would give way to separate molecule immunizations, or at least, plans to place processing sites between each vaccinogen. In understanding the features of autoimmunity, such as the escape from negative selection by some high-affinity T-cell clones⁵¹, the views discussed here rationalize their escape, owing to the exclusion of their specific ligand from MHC binding by flanking determinants that compete successfully to prevent the binding of the auto-aggressive inducer determinant. Finally, these same types of inter-determinant molecular competition that lead to the escape from negative selection of high-affinity, self-directed T cells could be identified and used in cancer therapy.

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Online links

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ERRATUM**MHC-GUIDED PROCESSING: BINDING OF LARGE ANTIGEN FRAGMENTS**

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In the legend to Figure 5 on page 627, the incorrect references are cited. The legend should state that the second pathway includes ideas from REFS 56 and 59. These references are as follows:

56. Lindner, R. & Unanue, E. R. Distinct antigen MHC class II complexes generated by separate processing pathways. *EMBO J.* **15**, 6910–6920 (1996).
59. Villadangos, J. A., Driessen, C., Shi, G. P., Chapman, H. A. & Ploegh, H. L. Early endosomal maturation of MHC class II molecules independently of cysteine proteases and H-2DM. *EMBO J.* **19**, 882–891 (2000).

Also, on page 627 (right-hand column, second paragraph, line 10), the text should read '(TCR)-interaction positions –1 and 11' not '(TCR)-interaction positions –1 and –11'.

The online version of this article has been corrected.