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Determinants of Immunodominance for CD4 T cells

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Abstract

The term immunodominance was originally defined as a restricted T cell response to a short peptide sequence derived from a given protein [1]. The question of what determines immunodominance has been a longstanding battle for the past two decades. Hundreds of papers have been written on different aspects of epitope selection during antigen processing documenting the complexity of the process. Antigen processing machinery involves several accessory molecules and chaperons coevolved with proteins of Major Histocompatibility Complex (MHC) molecules that each plays its part in epitope selection. These molecules are targeted to specialized vesicular compartments that also accommodate antigen processing enzymes called cathepsins. Within the antigen processing compartments, highly regulated pH gradient and reducing conditions and enzymes necessary for denaturation of the antigens are available and function to optimize processing of antigen and selection of the fittest for transport to the cell membrane and presentation to T cells. Despite the complexity, a cell free reductionist antigen processing system was recently reported that included only few purified proteins, but was shown to process and select physiologically relevant epitopes from full length protein antigens [2]. Because of its minimalist nature the system has been quite helpful in dissecting the factors that contribute to epitope selection during antigen processing. In this review, we would summarize and highlight models that may explain how the dominant epitope may be selected for presentation to CD4⁺ helper T cells.

Introduction

Antigen presentation to CD4⁺ T cells by APCs begins by the uptake of exogenous antigens and their processing that involves transfer through a series of endosomal compartments containing suitable denaturing environment, accessory molecules and molecular chaperons, as well as proteolytic enzymes cathepsins [3]. Newly synthesized MHC class II (MHC II) molecule associates with the class II invariant chain (Ii), which targets it to specialized endosomal compartments called MIIC where the Ii is proteolysed until only a fragment known as the class II-associated invariant chain peptide (CLIP) remains bound in the MHC II peptide-binding groove. Efficient displacement of CLIP from the MHC groove requires

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the accessory molecule HLA-DM in human or H2-M in mice (DM) [4]. DM functions by inducing conformational changes in pMHC II complexes resulting in the release of the bound peptide inducing a peptide-receptive MHC II [5]. A peptide-receptive MHC II can quickly sample a large pool of peptides derived from exogenously acquired proteins. Hence, in addition to removal of CLIP, DM helps in shaping epitope selection (more details to follow). Cathepsins present in processing compartments contribute by cutting and trimming of the protein antigens. Somehow during these processes few peptides from many peptides that can bind to MHC II are selected to represent the antigen to T cells. In this review, we would focus of the factors that influence this selection process.

Why should there be a selection for immunodominant epitopes?

To recognize many potential pathogenic antigens, naive T cells are evolved to express a large variety of unique receptors and be contained in the limited space of lymph nodes. Because of space limitations, despite continuous output of native T cells from the thymus and the exponential increase in number of specific T cell during infections, the T cell number in the periphery remains relatively stable throughout life [6]. Cell death during the contraction phase of the immune response is a major contributor to the maintenance of cell numbers, but some memory T cells and B cells remain in our lymphatic system for future pathogen attacks, causing a slight change in lymph node size after each infection [7]. Having to keep memory T cells specific for each antigen for life necessitates that their overall numbers to remain within numbers that can be accommodated by the limited space of lymph nodes. Thus, too many memory T cells specific for the same antigen might create accommodation issues, hence there is a need for the immune system for restricting the total number of the T cells specific for each antigen.

Epitope accessibility

Multiple factors can contribute to immunodominance and one of which is '*epitope accessibility*' [8], meaning that the stretch of epitope selected as immunodominant must be accessible to the groove of MHC molecule, and/or the cathepsins that cut and trim the epitopes. Evidence in support of accessibility notion comes from localization of many known immunodominant epitopes to the more exposed C- or N-termini of antigens [9–16], or to the flexible strands of proteins [8]. Alternatively, to make epitopes accessible, in addition to denaturing environment in the antigen processing compartments, a specialized enzyme, gamma-interferon-inducible lysosomal thiol reductase (GILT) releases disulphide bonds in proteins. A strong support for the role of GILT in release of dominant epitopes was shown in GILT deficient mice, where presentation of some buried Hen Egg Lysozyme (HEL) determinants was inhibited [17]. Two paths for the capture of the selected epitopes by the MHC molecules have been described [18]. Epitopes can first be captured by the MHC groove followed by fragmentation by the cathepsins (*bind first, trim later*), or protein can be first be cut by the cathepsins and then be sorted out for optimal binding for the MHC II groove (*cut first, bind later*). While evidence for both models exist in literature, a recent report applying the reductionist, cell free antigen processing system, discussed earlier, to several antigens derived from pathogens or autoantigens, and provided convincing data that antigen processing of pathogen-derived proteins or autoantigens follow distinct paths.

Autoantigen-derived immunodominant epitopes were shown to be resistant to digestion by cathepsins, whereas pathogen-derived epitopes were sensitive [19]. Accompanied by direct evidence for epitope capture to precede cathepsin digestion for pathogen derived epitopes, authors favored the epitope capture model; and by showing that autoantigen-derived core dominant epitopes were resistant to further proteolysis by the cathepsins they strengthened the ‘*cut first, bind later*’ model [19].

Cathepsins in antigen processing

Of the most significant contributors to processing antigens are endocytic proteases or cathepsins (Cat). Cysteine proteases, aspartyl proteases and serine proteases are the three types of cathepsins studied for their roles in antigen processing [20,21]. The significance of cathepsins in antigen processing and the selection of immunodominant epitopes lie in their regulated expression levels and activity in different cell types and activation state, as well as occurrence of specific inhibitors of cathepsin activities in antigen presenting cells [22–24]. Two main roles attributed to cathepsins in antigen processing are to cleave off invariant chain, and to process antigens. Among the most extensively studied cathepsins are CatB, CatD, CatL, and CatS [25–27]. CatS was reported to be involved in Ii cleavage and antigen processing [28–31]. Mice deficient in CatL and CatS showed impairment of late stage invariant chain degradation in thymus and periphery respectively [32,33]. Asparagine endopeptidase (AEP) has been shown to have some role in the initial invariant chain cleavage [34], and it can either generate or destroy antigenic epitopes [35]. CatB and CatD knockout mice showed some but not complete processing defect, hence their role in antigen processing has been considered as dispensable [36].

Recent studies by Kim et al [19] using their cell free processing system showed that inclusion of only three cathepsins, CatB, CatH, and CatS was sufficient to mimic the processing conditions necessary to produce the immunodominant epitopes from several antigens. While CatB and CatH are mainly exoproteases, they also have endopeptidase activities, although the pH requirement might be different [27]. The team also evaluated the need for CatB in processing of two antigens in cells and observed a complete blockage of processing in the presence of a cell-permeable CatB inhibitor, CA-074ME. Importantly, authors showed that having only CatB and CatH was sufficient to select for the dominant epitope of influenza HA1 epitopes, but CatS failed to produce the dominant epitope in the absence of CatB and CatH (Table 1). Besides the cathepsins mentioned above, it has been suggested that other groups of cathepsins such as cat G and E might also play some role in regulating antigen processing [37,38]. However, when serine protease inhibitor that inhibits Cat G, or aspartic protease inhibitors, pepstatin A and pepstatin A-penetratin, were used during the processing and presentation of type II collagen and H5N1-HA proteins results indicated that CatB has a major role in epitope selections whereas CatG, CatD, and CatE influence the generation of epitopes, but may not be critically important as CatB and CatS (Figure 1) [19]. However, for a more comprehensive cell free system it would be desirable to include CatG and CatD in addition to CatS and CatB and CatH.

Not to dismiss the role for pH, cathepsins involved in antigen processing generally are activated at acidic pH in APC, which itself is highly regulated. Trombetta et al demonstrated

that efficient formation of peptide-MHC class II complexes is enhanced by activation of the vacuolar proton pump upon DC maturation that enhanced lysosomal acidification and antigen proteolysis [22]. These findings emphasize the critical role that cathepsins play in antigen presentation by establishing that strict regulatory mechanisms are in place to allow for the generation of antigenic epitopes when needed.

Role of HLA-DM in immunodominance

'Kinetic stability', or affinity model for MHC class II/peptide has been a popular model as determinant of immunodominance [39]. According to this model, immunodominant determinants form highly stable complexes with MHC class II. Hence much emphasis has been placed on screening of antigens for epitopes that bind MHC molecules stably. The model was in part built on studies reporting that the affinity of pMHC II complex correlated with susceptibility to DM, i.e., fast dissociating pMHC II complexes were susceptible to DM-mediated peptide exchange, but slow dissociating pMHC II were relatively resistant to DM [40–42]. This viewpoint changed by the demonstration of structural properties of pMHC II complexes. It was reported that empty or partially filled P1 pocket of the MHC II molecule, HLA-DR1 (DR1) generated a favorable substrate for interaction with DM, whereas compact conformation induced by a filled P1 pocket was an unfavorable substrate [43–45]. An important contribution to the DM mechanism was provided by Pos et al [46], who solved a high-resolution crystal structure of DM/DR1 complex. The structure revealed that the P1 pocket was the primary target for interaction with DM and that by filling the P1 pocket the interaction of DM with DR was disrupted [47]. Another interesting finding was recently reported by Yin et al who demonstrated that in the absence of poor P1 anchor, peptides that had strong pocket 9 binding residues were resistant to HLA-DM-mediated peptide exchange [48]. A substantial study by Yin et al compared multiple methods commonly used for the determination of immunodominance, such as intrinsic dissociation $t(1/2)$, IC(50), DM-mediated dissociation $t(1/2)$, and two epitope prediction algorithms for a large set of peptides from vaccinia virus and compared these properties to peptide-specific CD4(+) T cell responses. They found that MHC II-peptide complex kinetic stability in the presence of DM distinguished T cell epitopes from nonrecognized peptides. Taken together, these analyses demonstrated that DM-mediated dissociation $t(1/2)$ is the major factor governing peptide immunogenicity [21]. All these reports document the importance of DM in screening for immunodominant epitope selection to peptide selection. It is noteworthy however, that majority of our understanding of DM effects in epitope selection comes from the studies that utilize DR1 molecules while some MHC class II alleles are rather refractory to DM effects as they do not have a conformation recognized by DM [5,43,49,50]. For those alleles, perhaps kinetic stability may be the determinant of epitope selection.

A fresh understanding for dominant epitope selection was recently provided by Kim et al, who showed that peptides derived from pathogens, or autoantigens, behaved differently in response to DM[19]. Authors showed that for autoantigens DM susceptibility was not a required criterion, whereas for pathogen-derived dominant epitopes, DM resistance was a crucial factor. Immunodominance emerged as a result of the combined effects of DM and the antigen processing cathepsins. Autoantigen-derived immunodominant epitopes were resistant to digestion by cathepsins, whereas pathogen-derived epitopes were sensitive. As

such, sensitivity to cathepsins enforced capture of pathogen-derived epitopes by MHC II before processing, and resistance to DM-mediated-dissociation preserved the longevity of those epitopes. The overall findings established that immunodominance is established by higher relative abundance of the selected epitopes that survive cathepsin digestion, either by binding to MHC II and resisting DM-mediated-dissociation, or by being chemically resistant to cathepsins degradation. Non-dominant epitopes were found to be sensitive to both DM and cathepsins [19] (Figure 2).

Role of HLA-DO in immunodominance

In addition to DM, another non-classical MHC class II accessory molecule, HLA-DO (H2-O in mice), is known to play a role in peptide exchange [51–53]. Unlike DM, the expression of HLA-DO or H2-O (DO) is restricted to B cells, thymic medulla and certain subsets of DCs, and its cellular trafficking depends on DM. Understanding the role of DO in antigen processing has been a challenge for decades. Recently, the crystal structure of DM in complex with DO was solved, which showed that DO binds to HLA-DM at the same sites implicated in MHCII interaction [54]. Hence, the findings were interpreted as DO competing with DM in interaction with DR leading to the inhibition of DM function. While this model has some support in literature [53], kinetic peptide binding and dissociation experiments performed in the presence of DM and/or DO suggested a different mechanism [51,52]. It was reported that DO reduced binding of DR1 to some peptides, and enhanced the binding of some other peptides to DR1. Peptides that were negatively affected by DO were DM-sensitive, whereas peptides that were enhanced by DO were DM-resistant. The positive and negative effects of DO could only be measured on binding kinetics as peptide dissociation kinetics were not affected by DO. Direct binding of DO to a peptide-receptive, but not a closed conformation of DR1 was a strong indication of direct role of DO in epitope selection rather than through inhibition of DM. Thus, DO and DM work in synergy for optimizing peptide exchange and the selection of DM-resistant peptides. In light of these findings, inclusion of DO in our cell free system can better assess the dominant epitope selection.

What about T cells?

We have discussed different factors that contribute to the dominant epitope selection by the MHC II molecules. Once the immunodominant epitope is selected, another layer of complexity to consider is the availability of the specific T cells that would recognize the selected epitopes. Not only the presence of the right T cells is important, T cells bearing TCR with different affinity and/or avidity might recognize and respond to the same pMHC pair differently [55–57]. In addition, Unanue and colleagues have long studied an interesting phenomenon that reveals different clonal T cells recognize the same pMHC pair differently; Type A and Type B T cells recognize two conformations of pMHC that differ by their sensitivity to DM-mediated dissociation [58]. Another variable that was recently introduced by Jenkins and colleagues was differences in precursor frequency for naïve T cells recognizing different pMHC specificities. Authors used pMHC II tetramer based enrichment assays to show that there were up to ten folds variation in precursor populations. The study further correlated the magnitude of the T cell responses to their initial frequency [59].

Therefore, while T cells may have the final say in determination of the immunodominance, they would fail to respond if their specific pMHC is missing.

Concluding remarks

Here we have discussed a variety of contributing factors to immunodominance for CD4 helper T cells. Our own studies combined with our colleagues have led to the conclusions that it is necessary for the immune system to select for few dominant epitopes. The process involves many factors, but the success of our cell free reductionist system in identifying immunodominant epitopes from many different protein antigens has shed some light in the most critical contributors [2,19]. In combination with DM and cathepsins, immunodominant epitopes are carefully selected and appear as the most abundant peptides bound to the MHC II molecules. This gain in abundance is due to the combined effects of resistance to DM and/or cathepsins in the system as well as the elimination of the nondominant epitopes, which are susceptible to both DM and cathepsins. Once the dominant epitopes are selected, they are recognized by their cognate T cells, which hopefully do exist and hence initiate an adaptive immune response.

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Highlights

- Immunodominant epitope processing and selection requires DM, MHC II, and cathepsins
- Immunodominance is determined by epitope abundance
- Antigens from different source might be processed differently
- Pathogen derived epitopes are captured on intact antigens and are insensitive to DM
- Autoantigens are captured as peptides and are insensitive to cathepsins

Figure 1. CatB, but not CatG is a critical endosomal protease for generating H5N1-HA(259-274) immunodominant epitope

ELISPOT assay measuring IFN- γ production of T cells isolated from DR1-transgenic mice immunized with H5N1-HA(259-274) peptide in CFA. Cells from draining lymph nodes were stimulated with HA(259-274) or proteins in vitro for 48h in the presence, or absence of CA-074ME, which is a cell-permeable CatB inhibitor, or a CatG specific inhibitor. Error bars represent SD of spots from triplicate cultures shown. Peptide stimulation in vitro serves as controls to assess the toxicity of the enzyme inhibitors. Processing of HA1 protein by APC in vitro yields the dominant epitopes identified by the cell free processing system. Roles of CatB, CatD, and CatE in generation of the H5N1-HA(259-274) immunodominant epitope upon processing of HA1 protein by the APC in 48 h culture in ELISPOT assay using specific inhibitors has been described in ref 19.

Differential Sensitivity of antigens to HLA-DM and Cathepsins

Figure 2. Two processing pathways for antigens derived from pathogens or self

Pathogen-derived protein antigens may be captured by MHC II first, and then processed by cathepsins. Pathogen-derived dominant epitopes are highly sensitive to destruction by the cathepsins, are generally DM-insensitive, and are protected by the groove of MHC II. On the contrary, auto antigen-derived epitopes are resistant to cathepsins, and may, or may not be sensitive to DM-mediated peptide exchange (from Ref 19). Cathepsins are shown as scissors, peptides and epitopes are depicted as part of the denatured proteins, or in short stretches of sequences that carry a MHC II P1 fitting anchor or no anchor. The selected pMHC complexes are transported to the APC cell surface waiting for T cell stimulation. Small dots represent degraded peptides.

CatB and CatH are necessary and sometimes sufficient for the selection of the immunodominant epitope of HA1

Table 1

Summary of rHA1 derived peptides identified in the cell-free reductionist antigen processing system [2]. DR1 and DM were incubated with rHA1 protein followed by addition of CatB and H, CatB, CatH and CatS, or CatS alone. Peptide/DR1 complexes were isolated and peptides were eluted from DR1. Immunodominant epitope from influenza strain A/Texas/1/77 HA(306-318) is shown in Red color. Recombinant chimeric HA1 protein used here is a fusion product of influenza Puerto Rico strain A/PR/8/34 and the HA(306-318) from A/Texas/1/77 attached at C-terminal end. Blue and magenta fonts show other nondominant epitopes.

Recombinant Influenza hemagglutinin (rHA1) MRGSHHHHHHTDPSSRSADADTICIGYHANNSTDITVDITVLEKNVTVTHSVNLLSDSHNGKLCRLKGIAPIQLKCNIAAGWLLGNPECDPLLPVRSWSYVIVETPNSENGICYPGDFDYEELREQLSSVSSFEFEIFPKESSWPNNHNTNGVTAACSHEGKSSFYRNLLWLTEKEGSYPKLKNSYVNNKGKEVLVLWGIIHPPNSKEQQNLYQENAYVSVVTSNYNRRFTPEIAERPKYRDQAGRMNYYWTLTKP*GDTIIFEANGNLIAPMYAFALSRGFGSGITTSNASMHECNTKQTPLAGAINSSLPYQNIHPVTITGECPKYVYRSAKLRMVTGLRNIPSIQSRGACPKYVYKQNTLKLATGMRKLIHHHHH			Identified Peptide Sequences	
Cathepsins included in cell-free system	rHA1 derived peptides			
CatB and CatH Or	HA ₃₀₆₋₃₁₈ from A/Texas/1/77			GAC _{C_{ant}} PKYVYKQNTLKLATGM _{ox} R GAC _{C_{ant}} PKYVYKQNTLKLATGM _{ox} RK GAC _{C_{ant}} PKYVYKQNTLKLATGM _{ox} R SRGAC _{C_{ant}} PKYVYKQNTLKLATGM _{ox} RK
				INSSLPYQNIHPVTITGEC _{C_{ant}} PK
CatB, CatH, and CatS	HA ₂₉₈₋₃₁₇ from A/PR/8/34			AINSSLPYQNIHPVTITGEC _{C_{ant}} PK
				NSYVNNKKGKEVLVL
				HEGKSSFYRNLI