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GETTING INTO THE GROOVE: UNUSUAL FEATURES OF PEPTIDE BINDING TO MHC CLASS I MOLECULES AND IMPLICATIONS IN VACCINE DESIGN

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1. ABSTRACT

The major histocompatibility complex presents antigenic peptides on the surface of antigen presenting cells to T cell receptors. Recognition of peptide-MHC by T cells initiates a cascade of signals in T cells which maintains a T cell dependent immune response. An understanding of the how peptides bind to MHC class I molecules is an important prerequisite in the design of vaccines. Herein, we will discuss, with special emphasis on MUC1, unusual features of MUC1 peptide binding to MHC class I, obtained from vaccine studies including a MUC1 peptide mimic and the crystal structures of low and high affinity peptides lacking canonical anchor motifs in complex with H-2K^b.

2. INTRODUCTION

MUC1 is a high molecular weight glycoprotein overexpressed by adenocarcinomas. We have used mannan-MUC1 for the selective delivery of MUC1 peptides to the MHC class I pathway (1-3). MUC1 peptides can be presented by a variety of class I molecules and can generate MUC1 specific CTLs reacting with various 9-mer epitopes (from the variable number of tandem repeats region; VNTR) that can be presented by different murine H-2 molecules and by human HLA-A2 (4, 5). For the most part, these 9-mer peptides have unusual features compared to high affinity peptides in that they lack defined anchors and bind with low affinity. Nonetheless, high avidity CTLs are produced after immunization with mannan-MUC1 (4, 5). It was also noted that 5-8-mer MUC1 peptides could bind to H-2K^b by successive deletion of amino acids at the C-terminus of the 9-mer peptide (SAPDTRPAP; MUC1-9). In addition, several MUC1 mutants have been generated as well as a MUC1 mimic peptide. The MUC1 peptides have other novel features. They bind in an unusual fashion in that the mid and C-terminal regions loop out of the groove more than other MHC class I binding peptides and are accessible to MUC1 antibodies. Indeed, MUC1 peptides are the only known peptides which are accessible to peptide-specific antibodies whilst still in the groove of class I molecules, although other antibodies can react with peptide/MHC complexes or with peptides that have an MHC-dependent configuration (6-9).

We have recently determined the X-ray structure of an 8-mer MUC1 peptide (SAPDTRPA) in complex with MHC class I H-2K^b, and demonstrated that the anchor side chains pointing into the groove are small - the anchor

pockets are not completely filled compared with high affinity peptides. These findings account for the low affinity binding of SAPDTRPA peptide to class I. We also have determined the structure of a yeast peptide SRDHSRTPM, also lacking canonical anchor motifs, but which binds with high affinity.

Herein we will review the presentation of peptides by MHC class I molecules and unusual features of their binding including a discussion of the binding of longer and shorter peptides, glycopeptides, low affinity peptides, peptides with non-canonical anchor motifs and a MUC1 mimic peptide. All of this structural and immunological information can be harnessed towards new approaches to tumor immunotherapy and in the design of new vaccines for many microbial diseases.

3. MHC CLASS I MOLECULES

In 1987 the first X-ray crystallography structure of an MHC class I molecule, HLA-A2, was determined (10). MHC class I consists of a polymorphic transmembrane heavy chain (43 kDa) which is non-covalently associated with β2-microglobulin (12 kDa). The X-ray crystal structure demonstrated that the α1 and α2 domains combine to form a platform of eight antiparallel β strands that were straddled by two long antiparallel α helices (10). A groove is formed between the helices that identified the binding site for processed peptide antigens. Later, when the first single peptide complexes for murine and human MHC class I were determined (11-14), the side chains of certain peptides, called anchor residues (15), were shown to fit into specificity pockets on the floor of the groove. The amino acid residues that line the peptide-binding groove determine the individual specificity of the peptide-MHC interaction. The peptide binding groove can be subdivided into various pockets (A-F) (16).

For MHC class I, the peptide length varies from 8-9-mers (H-2K^b, HLA-A2) and up to 13-mers (HLA-Aw68) (15). The non-anchor peptide residues that point upwards can interact with the TCR. Crystallographic studies of many human and murine MHC class I molecules have now shown that the N and C termini of the peptides are fixed and anchored in the groove by conserved interactions of MHC side chains with the peptide backbone. Anchors are generally in positions P2 and P9, but for H-2K^b are P6 and P9 for 9-mers or P5 and P8 for 8-mers. For H-2K^b, the preferred anchors are Phe/Tyr for the central P5/6 residues and Leu/Met at the C-terminus (P8/9); in some instances, Tyr at P3. Other positions may also contribute to MHC binding specificity and have been termed secondary anchors (17). Although, these anchor residues are required for stabilization and high affinity binding of peptide to MHC, some peptides which do not contain the canonical anchor residues can still bind and be presented by MHC class I and be targets for CTL (4, 5, 18-21). Until now, it has not been clear how such non-standard or low affinity peptides are presented by MHC class I molecules in order to serve as targets for CTL. Thus, we have recently determined the crystal structures of two peptides lacking canonical anchor motifs (one with low affinity and the other with high affinity binding) presented by H-2K^b which describes their mode of binding.

4. UNUSUAL FEATURES OF PEPTIDE BINDING TO MHC CLASS I

4.1. Binding of peptides lacking canonical anchor motifs

MHC class I molecules have pockets in which bind hydrophobic amino acid side chains of the bound peptide. Despite this, a number of peptides have been demonstrated to bind to MHC class I (H-2K^b and HLA-B27), which lack 'anchor motifs', yet cell lysis could be induced (4, 5, 18-21). A high affinity peptide lacking canonical anchor motifs (YEA9), derived from yeast, has also been identified to bind to H-2K^b molecules (22).

Multiple epitopes presented by five different H-2 alleles (K^b, D^b, D^d, L^d and K^k) and by HLA-A2 and HLA-A11 have been described for the human MUC1 VNTR region (4, 5, 23). H-2K^b molecules present the 9-mer peptides APDTRPAPG, SAPDTRPAP and TSAPDTRPA Table 1, but we noted that these sequences do not have the appropriate anchors for H-2K^b molecules, (Phe/Tyr at P6 and Leu/Met at P9). The 9-mer peptide, SAPDTRPAP (MUC1-9) can also be presented more efficiently as an 8-mer (SAPDTRPA; MUC1-8) which also does not contain the anchor motifs. Three peptides APGSTAPPA, PAPGSTAPP and RPAPGSTAP could be presented by H-2D^b Table 1, although none of the H-2D^b binding MUC1 peptides could stabilize RMA-S cells for flow cytometric detection of class I molecules, indicating their low affinity; nevertheless, peptide-loaded RMA-S cells could readily be recognized by CTL (4). Using recombinant mice and L cells transfected with D^d or L^d as targets, it was demonstrated that for H-2D^d the binding peptide was SAPDTRPAP, for H-2L^d APDTRPAPG and for H-2K^k was PDTRPAPGS Table 1. Furthermore, peptides SAPDTRPAP, STAPPAHGV, STAPPVHNV and LLLLTVLTV were presented by HLA-A2 and HLA-A11 Table 1 (5, 23, 24). It was of interest that seven MHC class I binding epitopes could be found in a single 20-mer sequence. This finding is unusual but not unique; similar examples of promiscuous epitopes have been previously described for HIV-gp120 and Epstein-Barr virus nuclear antigens (25, 26). Recently, peptides lacking canonical anchor motifs, SAPDTRPAP and APGSTAPPA, presented by H-2K^b and H-2D^b respectively, were identified in MUC1 transgenic mice immunized with dendritic-carcinoma fusion cells (27). Similarly, these epitopes were recognized by CTL generated in MET transgenic mice which are MUC1 transgenic and develop spontaneous tumors of the pancreas and develop MUC1- specific CTL Table 1 (28). Although most of the work in mucins so far has been on VNTR peptides, there is good evidence that CTL epitopes may lie outside the VNTR and could lead to effective CTL and anti-tumor responses. Indeed, we have found that canonical binding and non-canonical anchor motif CTL peptide epitopes from outside the VNTR region are presented by H-2^b, H-2^d and HLA-A2 table 1 (29).

Table 1. Protein epitopes detected by anti-MUC1 CTLs

MHC Allele	MUC1 VNTR	Method of detection	Reference
H-2K^b	APDTRPAPG	CTL (C57BL/6 mice)	4
	TSAPDTRPA	CTL (C57BL/6, MUC1 Tg & MET mice)	4, 27, 28
	SAPDTRPAP	CTL (C57BL/6, MUC1 Tg & MET mice)	4, 27, 28
	SAPDTRPAPGSTAP	Flow cytometry	not published
	SAPDTRPA	CTL (C57BL/6 & MUC1 Tg mice)	4, 27, 36
	SAPDTRP	CTL (C57BL/6 mice)	36
	SAPDTR	CTL (C57BL/6 mice)	36
	SAPDT	CTL (C57BL/6 mice)	36
H-2D^b	APGSTAPPA	CTL (C57BL/6 & MUC1 Tg mice)	4, 27
	PAPGSTAPP	CTL (C57BL/6 & MUC1 Tg mice)	4, 27
	RPAPGSTAP	CTL (C57BL/6 & MUC1 Tg mice)	4, 27
H-2K^b/H-2D^b	STAPPAHGV	CTL (MET mice)	28
H-2D^d	SAPDTRPAP	CTL (DBA/2, BALB/c mice)	4
H-2L^d	APDTRPAPG	CTL (DBA/2, BALB/c mice)	4
H-2K^k	PDTRPAPGS	CTL (CBA mice)	4
HLA-A2	SAPDTRPAP	CTL (HLA-A2 Tg mice)	5
	TSAPDTRPA	CTL (HLA-A2 Tg & Elution from tumors)	5, 31
	STAPPAHGV	CTL (HLA-A2 Tg & T2 cell binding)	5, 23
HLA-A11	STAPPAHGV	CTL (T2 cell binding)	5, 23
	NON MUC1 VNTR		
H-2K^b	CRRKNYGQL	Prediction algorithm program and CTL	29
	AVSMTSSVL	Prediction algorithm program and CTL	29
H-2D^b	CRRKNYGQL	Prediction algorithm program and CTL	29
	STEKNAVSM	Prediction algorithm program and CTL	29
	AVSMTSSVL	Prediction algorithm program and CTL	29
	SAPDNRPAL	Prediction algorithm program and CTL	29
H-2K^d	AVSMTSSVL	Prediction algorithm program and CTL	29
	NAVSMTSSV	Prediction algorithm program and CTL	29
	YYQELQRDI	Prediction algorithm program and CTL	29
H-2L^d	VPSSTEKNA	Prediction algorithm program and CTL	29
HLA-A2	STAPPVHNV	Prediction algorithm program and CTL	24
	LLLLTVLTV	Prediction algorithm program and CTL	24
	MUC1 VNTR MIMIC		
H-2^d	DAHWESWL	Phage display, CTL (DBA/2 mice)	59
HLA-A2	DLHWASWV	Mutation, CTL (HLA-A2 Tg mice)	59

Thus, MUC1 VNTR peptides bound to MHC class I molecules have some unusual features, which were further exemplified by antibody binding and modelling studies. We previously demonstrated that MUC1-9 (SAPDTRPAP) could loop out sufficiently at the mid and C-terminus to be detected by anti-MUC1 peptide monoclonal antibodies when bound to H-2K^b (21). Monoclonal antibodies, VA2 (epitope, DTRPA), BCP8 (DTR; middle of the peptide), BCP10 (RPAP; C-terminal residues) were able to bind to the peptide when presented by MHC class I on RMA-S cells, as measured by FACS staining of peptide pulsed RMA-S target cells (21). Interestingly, the monoclonal antibody, BC2 (APDTR; N-terminal residues) was non-reactive, suggesting that the N-terminal region was not exposed and buried in the MHC groove. The antibodies to the mid and C-terminus of the peptide blocked CTL killing but not antibodies to the N terminal sequence. From molecular modelling, it was suggestive that the C-terminus looped out and that the central Arg pointed down into the peptide binding groove (21). It was also noted that at the predicted position of this Arg in the peptide, there was an Arg-155 closely in the α2 domain of the MHC, and it was proposed that the monoclonal antibodies could substitute binding of Arg-P6 of MUC1 by binding to Arg-155 of the MHC. In fact, Arg-155 has been found to be an important TCR contact residue for the 2C TCR (30); thus, it is possible that the monoclonal antibodies are recognizing a subset of residues as those of the TCR. Similarly, the MUC1 peptide (APGSTAPPA) bound to H-2D^b, but does not contain the canonical anchor residue Asn-P5. It was predicted that the central region looped out as it was accessible to the monoclonal antibody, BCP9 (GSTAP); BCP9 could also block CTL killing (21). Furthermore, HLA-A2 bound peptides SAPDTRPAP and STAPPAHGV (5). Similarly, monoclonal antibodies to the mid and C-terminal end were able to bind to SAPDTRPAP when bound in the groove and inhibited CTL lysis of peptide pulsed target cells, although no antibody binding (epitope, APPAH) was noted for the canonical anchor motif peptide STAPPAHGV (21). All of these MUC1 peptides lacking canonical anchor motifs are presented by MHC class I molecules on tumor cells, as CTLs lyse MUC1$^+$ tumor cell lines (1-5). Furthermore, HLA-A2 peptides eluted from human breast and pancreatic tumor cells by acid elution and purified by immunoaffinity using the anti-class I mononclonal antibody (W6/32), and after separation by HPLC bound the anti-peptide monoclonal antibody BCP8 (DTR) Table 1 (31).

The 9-mer peptide (T)SAPDTRPAP was able to upregulate class I in T2-cells as well as serving as an epitope for CTL generated from primary *in vitro* cultures (31). In addition, canonical anchor motif peptides have been found to be presented by H-2D^d, H-2L^d and H-2K^k Table 1 (4).

4.2. Binding of longer peptides

It is currently considered that MHC class I molecules preferentially bind peptides 8-10 amino acids long, the size restriction being due to peptide-MHC interactions. Crystal structures of peptide-MHC complexes have clearly shown that peptides are held at their N and C termini by a network of hydrogen bonds between conserved MHC residues at either end of the class I binding site and the peptide. MHC class I, H-2K^b, can bind peptides 8 or 9 amino acids long, and the mechanism of binding of longer peptide involves creating a bulge at the center of the peptide, leaving the N and C termini interactions unchanged (13, 14). However, longer peptides may be accommodated by MHC class I by protrusion, primarily at the C-terminus. The crystal structure of a 10-mer peptide bound to HLA-A2 showed a single glycine C-terminal extension extending from the binding groove (32). Peptides with the addition, at the N- or C-termini, of two amino acids of the VSV8 and OVA8 peptides bind to H-2K^b (14); four residues could be added at the C- but not at the N-terminus of a VSV8 peptide bound to H-2K^b (33). A peptide extended at the C-terminus by 10 amino acids (an 18-mer) binds to H-2K^k as it could be eluted from the MHC class I molecule (34). Recently, it was demonstrated that extensions could occur at either the N- or C-terminus for H-2K^k, H-2K^b and H-2D^b molecules (35). T cells were able to identify the extension; thus, peptide extensions may play a role in the specificity of the T cells (35). In addition, we have demonstrated that H-2K^b can bind SAPDTRPAP peptides that are extended by up to 5 residues at the C-terminus Table 1 [Apostolopoulos et al., manuscript in preparation]. It is clear that MHC class I molecules can bind longer peptides and such peptides may be of importance in the design of peptide based vaccines.

4.3. Binding of shorter peptides

Immunization of mice with mannan-MUC1 generates CTL that recognize both peptide-pulsed and MUC1-transfected target cells and protects mice against a MUC1$^+$ tumor challenge (1, 2). MUC1-9 presented by H-2K^b, can also be presented and recognized by CTL as a 5-mer to 8-mer (deletion at the C-terminus) when RMA-S cells are pulsed Table 1 (36). The 4-mer, SAPD, is not presented, nor lysed, by CTL. Other short immunogenic peptides for class I have been demonstrated to bind to H-2L^d and to be recognized by CTL, including, a 3-mer (QNH), 4-mers (QNHR, ALDL, PFDL) and 5-mers (RALDL, HFMPT) (37-39). These observations are clearly outside the normal structural guidelines for tight binding of class I peptides, deduced from crystal structures of many peptide-MHC complexes.

4.4. Binding of low affinity peptides

Studies of T cell epitopes have focussed on peptides with high affinity binding to MHC class I molecules. Low affinity peptides are not usually detected by elution methods and prediction algorithms, and are thought to not sufficiently activate T cells due to their low stability which usually correlates with overall immunogenicity. However, low affinity tumor peptides have been identified for Lewis lung carcinoma (MUT1 and MUT2), and for MUC1 (4, 5, 36, 40). The affinity of MUC1-9 binding to H-2K^b at 4°C, 23°C and 37°C is in the10^{-5}M range but for MUC1-8 is higher in the 10^{-7}M range at 4°C and 23°C, although the complex is not stable at 37°C (36). Both of these peptides are much lower in affinity than high affinity binding peptides (10^{-9}M range from 4-37°C) such as OVA8, VSV8 and SEV9. It is of interest to speculate that low affinity binding of peptides could be less tolerogenic *in vitro* and, therefore, more immunogenic.

4.5. Binding of glycopeptides

Antigens presented by MHC class I molecules for recognition by CTL are primarily 8-10-mer cytosolic peptides. Until recently, it was not known whether post-translationally modified peptides could be presented by MHC class I molecules *in vivo*. Cytoplasmic proteins undergo a number of different post-translational modifications, such as cytosolic O-β-linked glycosylation of serine and threonine residues with N-actetylglucosamine (GlcNAc). Synthetic glycopeptides with O-β-GlcNAc substitution on serine residues can be efficiently transported by TAP into the ER and bind MHC class I and elicit glycopeptide-specific CTL in mice (41). Glycopeptide-specific T cells can also be generated after immunization of mice with a synthetic peptide conjugated to GlcNAc (42); such peptides presented by human class I MHC molecules *in vivo* comprise a significant amount (0.1%) of peptide with GlcNAc. In addition, peptides eluted from HLA-A2 molecules contained GlcNAc glycopeptides, providing further evidence that glycopeptides can naturally be presented by human class I MHC *in vivo* (43). The H-2K^b presented peptide, VSV8, with P6 linked to galabiose disaccharide is able to activate T cells (44) and the crystal structure of H-2K^b-VSV8 glycopeptide was determined (45). The crystal structure of a synthetic O-GlcNAc bearing peptide in complex with MHC class I H-2D^b has also been determined (46). This MHC peptide complex can generate H-2D^b glycopeptide-specific CTL in mice. In addition, glycopeptides have been demonstrated to bind MHC class II molecules, I-A^k and I-E^k (47, 48). The HEL peptide, 52-61, can be presented as a glycopeptide by I-A^k and recognized by CD4$^+$ T cells (48). By making changes in the saccharide molecule and in the peptide, it was concluded that the TCR made specific contacts with both the sugar and the exposed side chains of the peptide.

MUC1 is highly glycosylated and, as result of aberrant glycosylation during cancer, MUC1 exposes certain carbohydrate, peptide and possibly glycopeptides that are not normally exposed on the normal mucin. In addition, due to the overexpression of MUC1 on cancer cells as compared to the normal cells, it is possible that such glycopeptides are presented by MHC class I or class II molecules for recognition by T cells. A number of studies have demonstrated potential glycosylation sites within the

MUC1 VNTR region (49-51) and studies are being pursued to identify specific glycopeptides presented by MHC class I or class II molecules. The identification of such glycopeptides which are capable of stimulating an immune response, may be of potential value for immunotherapy studies in cancer.

5. LOW AFFINITY PEPTIDES BINDING TO MHC CLASS I: USE IN TUMOR IMMUNOTHERAPY

Vaccination with peptides derived from tumor antigens has been successfully achieved in experimental mouse models with the induction of CTL and tumor protection. The efficacy of the vaccination depends on the ability of peptides to induce and activate high avidity CTL. High affinity peptides which bind to MHC class I molecules usually induce such high avidity CTLs. However, if the tumor antigen is tumor-associated rather than tumor-specific, the vaccination may not be effective. MUC1, like most tumor antigens, is a tumor-associated antigen, in that it is expressed in normal tissues and overexpressed on cancer cells. Since most tumor antigens are self antigens, their specific CTL repertoire would most likely be deleted, as demonstrated for p53 (52, 53) leading to tolerance. This tolerance particularly concerns the high affinity MHC-associated immunodominant epitopes, but not low affinity epitopes (54). Thus, for tumor immunotherapy, it is conceivable that the best candidate peptides for immunization are the low affinity binding peptides. A major problem in the use of low affinity peptide epitopes for immunotherapy is the difficulty of their identification due to their poor immunogenicity. The affinity of peptide binding to MHC and the stability of the peptide-MHC complex has been shown to correlate with overall immunogenicity (55, 56). Furthermore, low affinity peptides cannot be detected by elution studies and prediction algorithms. Thus, peptide identification by systematic binding studies and recognition of peptide-MHC by TCR can be used for their identification. However, enhancement of the immunogenicity of low affinity MHC binding peptides is desirable where mutations could be made to the peptide anchor side chains to increase their affinity.

Low affinity MUC1 peptides (SAPDTRPAP and APGSTAPPA), presented by H-2K^b and H-2D^b respectively, were not able to stabilize RMA-S cells as determined by FACS; however, they were recognized by peptide-specific CTL (4). Mutations of SAPDTRPAP to SAPDTYPAL and APGSTAPPA to APGSNAPPA could stabilize class I on RMA-S cells (4). Furthermore, the H-2K^b low affinity peptide, MUT1 (a peptide from Lewis lung carcinoma), when mutated at P3, P5 and P8, has increased stability and peptide affinity for RMA-S cells (57). In addition, the low affinity MUC1 peptide (SAPDTRPAP) that binds to HLA-A2 has been converted by mutation to high affinity and is currently in a clinical trial (5, Apostolopoulos et al., unpublished data). We have also mutated the high affinity peptide STAPPAHGV which binds to HLA-A2, to even higher affinity; these peptides are also in clinical trial (5, Apostolopoulos et al., unpublished data). Recently, low affinity binding peptides to HLA-A2 were converted to high affinity by replacing the amino acid at P1 with tyrosine (58). In that study, thirty two peptides derived from tumor and viral antigens (HIV, HBV, melanoma antigens, Her-2/neu) were mutated and higher binding affinity and stabilization to HLA-A2 was noted.

6. PEPTIDE MIMIC OF MUC1

Molecular mimicry has entered a new and promising stage for tumor immunotherapy. Immunization with "non-self" peptides may have the potential to generate greater immune responses than those occurring with self peptides. We previously reported that, by immunizing mice with a peptide that mimics MUC1, a strong cellular and protective response is generated against MUC1, as well as lysis of MUC1$^+$ tumor cells (59). This peptide (DAHWESWL) was isolated from a phage display library as it inhibited the binding of anti-Galalpha(1,3)Gal antibodies to Galalpha(1,3)Gal expressed on pig red blood cells, endothelial cells and peripheral blood lymphocytes; these studies were of relevance to xenotransplantation in another laboratory in our Institute (60). It was also noted that Galalpha(1,3)Gal antibodies not only bound to the mimic peptide but also to MUC1 peptides Table 1 (61). These initial cross reactivity observations were taken a step further, and mice were immunized with the mimic peptide. DBA/2 (H-2^d), but not C57BL/6 (H-2^b) or HLA-A2 transgenic mice, made CTL to DAHWESWL (59). The CTL not only recognized DAHWESWL in the context of H-2^d, but also SAPDTRPAP and APDTRPAPG presented by H-2D^d and H-2L^d respectively Table 1 and were able to lyse MUC1$^+$ tumors (59). Since DAHWESWL peptide was not presented by HLA-A2, we mutated the peptide to become a more compatible structure which included appropriate A2 binding motifs (DLHWASWV) Table 1. Immunization of HLA-A2 transgenic mice with this peptide lead to the production of CTL which were able to lyse MUC1$^+$ human breast cancer cells (MCF7) *in vitro* (59). Other studies involving identification of peptide mimics, mainly rely upon more conventional amino-acid substitutions in the basic framework of a peptide epitope. We have, thus, demonstrated for the first time an example of how a completely different and unrelated peptide can elicit specific CTLs to a tumor antigen.

There has been great success in developing vaccines against many infectious diseases; however, a vaccine for cancer has been very difficult. Antigens from infectious diseases are foreign, hence, induction of an immune response to "non self" antigens is easier than inducing immunity to "self antigens". Most tumor antigens are also expressed on normal tissues, thus, making it difficult to induce strong immune responses against "self antigens". Immunizing humans with an unrelated, mimic peptide (which could be recognized by the body as foreign), may generate greater immune responses than those induced to self peptides. The MUC1 mimic peptide (DLHWASWV) is currently in a clinical trial, and it would be of interest to see in other systems whether mimicking peptides are more potent for inducing immunity than self peptides. Such attempts to find other mimicking peptides may introduce alternative, new and effective modes of immunotherapy for cancer in the future.

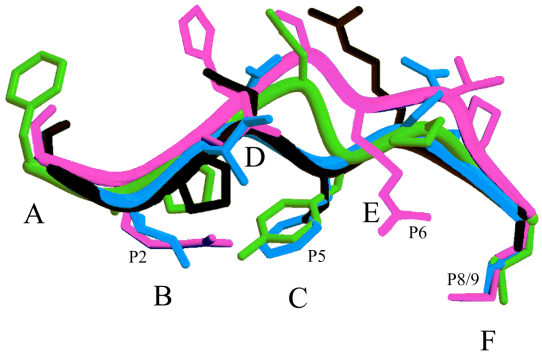

Figure 1. Structure comparisons of selected H-2K^b-bound peptides. Comparison of 8-mer peptides; non-canonical low affinity MUC1-8 (black) and canonical high affinity OVA8 peptide (blue). P2 occupies the B pocket, P5 the C pocket and P8 the F pocket. OVA8 clearly fills the B, C and F pockets whereas MUC1-8 leaves the pockets virtually empty which accounts for low affinity binding. Comparison of 9-mer peptides; non-canonical high affinity YEA9 (pink) and canonical high affinity SEV9 peptide (green). P2 occupies the B pocket, P6 the C pocket and P9 the F pocket. P6 in YEA9 does not point into the C pocket but into the E pocket, due to steric hindrance of Arg-P2. As a result there is a bulge in the peptide backbone between P5-P8. A new mode of peptide binding is demonstrated with YEA9. Figure was drawn using programs MOLSCRIPT and Raster3D.

7. CRYSTAL STRUCTURES OF PEPTIDES LACKING CANONICAL ANCHOR MOTIFS BOUND TO H-2K^b

The mode of binding of high affinity, canonical-anchor motif peptides to murine and human MHC class I molecules have been described in detail (reviewed in 62) (and see below for OVA8 and SEV9). However, no structural information has been available on the binding of non-standard or low affinity peptides to MHC class I molecules, and whether they differed substantially in their interaction with the MHC and, therefore, with the T cell receptor. We recently determined the crystal structure of two peptides lacking canonical anchor motifs bound to MHC class I, H-2K^b; one bound with low affinity (MUC1 peptide) and the other bound with high affinity (Yeast peptide) (36).

7.1. Crystal structure of low affinity MUC1 peptide

MUC1-8 (SAPDTRPA) does not contain Phe/Tyr at P5 or Leu/Met at P8 which are usually associated with high affinity binding to H-2K^b (15, 63). MUC1-8 has small residues at P2 (Ala), P5 (Thr) and P8 (Ala) (4, 36) and has a relatively low affinity of 10^{-7}M and is not stable at 37°C, compared to high affinity binding peptides which are in the 10^{-9}M range. Other H-2K^b 8-mer non canonical anchor motif containing peptides for which specific CTLs have been generated include MUT1 (FEQNTAQP) and MUT2 (FEQNTAQA) (20). We recently determined the crystal structure of MUC1-8 in complex with H-2K^b at 1.6Å resolution (36). The side chains of residues that point into the C and F pockets are small; hence, these anchor pockets are not completely filled as compared to high affinity OVA8 peptide (SIINFEKL) Figure 1. A large cavity is formed between the side chain of Thr-P5 and the α2-domain and the B-value for the side chain of Thr-P5 is high, indicating flexibility in this region; there are no cavities in this region for high affinity 8-mer peptide-MHC complexes, VSV8 and OVA8 (13, 22, 36). In addition, a significant sidewards deviation (1.7Å) of the MUC1-8 peptide backbone towards the α1 helix is present from P5-P8, as compared to high affinity binding peptides. Surprisingly, all of the hydrogen bonds which are present in the high affinity peptide-H-2K^b complexes (VSV8, OVA8, SEV9) are retained in the MUC1-8 complex with the addition of a novel hydrogen bond between Thr-P5 and Asnα70. The addition of a hydrogen bond would be expected to contribute to a higher affinity of peptide binding; however, this is not the case with MUC1-8. Peptide-MHC structures which do not have optimally-filled pockets often have additional water molecules to help occupy the vacated pockets. MUC1-8 has no additional water molecules; in fact, two water molecules (between the C and E pockets) are absent, compared to OVA8 and VSV8, leaving a large cavity at the side of the C pocket.

This low affinity peptide binds with the same overall features to MHC class I as high affinty peptides, in that, the peptide starts and finishes at the same position at the N- and C- termini; deviations occur within the central region of the peptide. The small peptide anchoring side chains at P2, P5 and P8, the absence of water molecules between the C and E pockets and the large cavity at the side of the C pocket, appear then to contribute to the low affinity and stability of MUC1-8 with H-2K^b.

7.2. Crystal structure of high affinity Yeast peptide

During expression of MHC class I, H-2K^b, in *Drosophila melanogaster* fly cells, yeastolate peptides which have been are added by the manufacturers to the culture medium, stabilize H-2K^b and fortuitously aid in its purification (64). These peptides bind to H-2K^b and the predominant peptide found in the peptide-binding groove is SRDHSRTPM, or YEA9 (22). YEA9 contains large residues at Arg-P2, Arg-P6 and Met-P9 at the anchor sites as compared to the preferred Ala/Gly/Ile-P2, Phe/Tyr-P6 and Val/Met-P9. The low affinity, MUC1-9 (SAPDTRPAP) and tumor-associated E6 human papillomavirus type-16 (RFHNIRGRW) peptides also contain an Arg-P6 (4, 19).

We recently determined the crystal structure of YEA9 in complex with H-2K^b at 1.5Å resolution (36). The non-canonical anchor motif peptide, YEA9, binds with relatively high affinity (10^{-8}M range) by insertion of long non-canonical anchor residues into the B and E pockets Figure 1. Due to charge repulsion and steric hindrance, Arg-P2 in the B pocket prevents Arg-P6 from occupying the C pocket, causing a prominent bulge between P5-P7; as a consequence, Arg-P6 points into the E pocket displacing a conserved water molecule Figure 1. Arg-P2 forms a novel buried salt bridge with Gluα24 and novel hydrogen bonds are formed between Asp-P3/Argα155, Arg-P6/Aspα77 and Thr-P7/Gluα152. An MHC class I mutant, H-2K^{bw9}, (whereby Valα9 of the MHC is replaced by Trp in order to

eliminate the C pocket), was shown to preferably bind 9-mer peptides with Pro-P3 and Arg-P5 (65). The authors predicted that Arg-P5 would most likely contact the E rather than C pocket, as is found in the YEA9 structure. Interestingly, MUC1-9, has Pro-P3 and Arg-P6 and it is possible that MUC1-9 binds similarly to YEA9 with a prominent bulge in the peptide backbone between P5-P9, and thus, may explain how anti-MUC1 monoclonal antibodies can bind to peptide in the groove (21). As a result of Arg-P6 now occupying the E pocket, the C pocket is left vacant forming a significantly large cavity, which is not found in the other high affinity peptide-H-2K^b complexes. In addition, Arg-P6 has a very high B-value, indicating flexibility within this region; despite this, YEA9 binds with relatively high affinity and is stable at 37°C.

It is clear that peptides lacking canonical anchor motifs can bind to MHC class I with relatively high affinity, and that all P2, P6 and P9 residues contribute in anchoring the peptide into the peptide-binding groove. These studies are important as they show that binding of peptide with high affinity can occur in two ways: (i) by canonical hydrophobic anchors binding in the C and F pockets and (ii) by non-canonical anchors binding in the B, E and F pockets. Non-canonical anchor motif peptides could be of value in immunotherapy studies and should be taken into account when predicting potential T cell epitopes from a protein sequence.

8. PERSPECTIVE

It is evident that MHC class I as well as class II molecules (not discussed here) are able to bind peptides which do not fit the accepted structural guidelines for high affinity peptide binding. High affinity peptides usually bind to MHC class I molecules by anchors that fit into discrete MHC class I pockets. However, it is apparent that peptides lacking canonical anchor motifs can bind and induce CTL. In addition, short peptides (3-5-mers), long peptides (up to 18-mers), and glycopeptides, have been demonstrated to bind to MHC class I. The identification of low affinity peptides, and their subsequent mutations to higher affinity, may be of greater importance for tumor immunotherapy than standard high affinity binding peptides, as T cells against low affinity self peptides would most likely not be tolerized. In addition, mimic peptides may be seen as non-self, which may also prove useful in immunization protocols. These findings broaden our understanding of the requirements and limitations of peptide binding and of epitope selection criteria for MHC class I molecules. All this information has given new insights in peptides binding MHC and future promise for the design of new vaccine candidates against many diseases.

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10. REFERENCES

1. Apostolopoulos V, G. A. Pietersz, B. A. Loveland, B. E. Sandrin & I. F. C. McKenzie: Oxidative/reductive conjugation of mannan to antigen selects for T_1 or T_2 immune responses. *Proc Natl Acad Sci USA* 92, 10128-10132 (1995)

2. Apostolopoulos V, G. A. Pietersz & I. F. C. McKenzie: Cell-mediated immune responses to MUC1 fusion protein coupled to mannan. *Vaccine* 14, 930-938 (1996)

3. Apostolopoulos V, G. A. Pietersz, S. Gordon, L. Martinez-Pomares & I. F. C. McKenzie: Aldehyde-mannan antigen complexes target the MHC class I antigen presentation pathway. *Eur J Immunol* 30, 1714-1723 (2000)

4. Apostolopoulos V, J. S. Haurum & I. F. C. McKenzie: MUC1 peptide epitopes associated with 5 different H2 Class I molecules. *Eur J Immunol* 27, 2579-2587 (1997)

5. Apostolopoulos V, V. Karanikas, J. Haurum & I. F. C. McKenzie: Induction of HLA-A2 Restricted Cytotoxic T Lymphocytes to the MUC1 Human Breast Cancer Antigen. *J Immunol* 159, 5211-5218 (1997)

6. Catipovic B, J. Dal Porto, M. Mage, T. E. Johansen & J. P. Schneck: MHC-conformational epitopes are peptide specific. *J Exp Med* 176, 1611-1616 (1992).

7. Stryhn A, P. S. Andersen, L.O. Pedersen, L. Svejgaard, A. Holm, C. J. Thorpe, L. Fugger, L. Buus & J. Engberg: Shared fine specificity between T-cell receptors and an antibody recognizing a peptide/major histocompatibility class I complex. *Proc Natl Acad Sci USA* 93, 10338-10342 (1996)

8. Porgador A, J. W. Yewdell, Y. Deng, J. R. Bennink & R. N. Germain: Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes. *Immunity* 6, 715-726 (1997)

9. Dadaglio G, C. A. Nelson, M. B. Deck, S. J. Petzold & E. R. Unanue: Characterization and quantitation of peptide-MHC complexes produced from hen egg lysozyme using a monoclonal antibody. *Immunity* 6, 727-737 (1997)

10. Bjorkman P. J, M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger & D. C. Wiley: Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329, 506-512 (1987)

11. Zhang W, A. C. Young, M. Imarai, S. G. Nathenson & J. C. Sacchettini: Crystal structure of the major histocompatibility complex class I H-2K^b molecule containing a single viral peptide: implications for peptide binding and T-cell receptor recognition. *Proc Natl Acad Sci USA* 89, 8403-8407 (1992)

12. Madden D. R, J. C. Gorza, J. L. Strominger & D. C. Wiley: The three-dimensional structure of HLA-B27 at

2.1Å resolution suggests a general mechanism for tight peptide binding to MHC. *Cell* 70, 1035-1048 (1992)

13. Fremont D. H, M. Matsumura , E. A. Stura, P. A. Peterson & I. A. Wilson: Crystal structure of two viral peptides in complex with MHC Class I H-2K^b. *Science* 257, 919-927 (1992)

14. Matsumura M, Y. Saito, M. Jackson, E. Song & P. A. Peterson: *In vitro* peptide binding to soluble empty class I major histocompatibility complex molecules isolated from transfected *Drosophila melanogaster* cells. *J Biol Chem* 267, 23589-23595 (1992)

15. Rammensee H. G, T. Friede & S. Stevanoviic: MHC ligands and peptide motifs: first listing. *Immunogenetics* 41, 178-228 (1995)

16. Saper M. A, P. Bjorkman & D. C. Wiley: Refined structure of the human histocompatibility antigen HLA-A2 at 2.6Å resolution. *J Mol Biol* 219, 277-319 (1991)

17. Kast W. M, R. Brandt, J. Sidney, J. Drijfhout, R. Kubo, H. Grey, C. J. Melief & A. Sette: Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins. *J Immunol* 152, 3904-3912 (1994)

18. Daser A, H. Urlaub & P. Henklein: HLA-B27 binding peptides derived from the 57 kD heat shock protein of Chlamydia trachomatis: novel insights into the peptide binding rules. *Mol Immunol* 31, 331-336 (1994)

19. Gao L, J. Walter, P. Travers, P. Stauss & B. Chain: Tumor-associated E6 protein of human papillomavirus type 16 contains an unusual H-2K^b-restricted cytotoxic T cell epitope. *J Immunol* 155, 5519-5526 (1995)

20. Mandelboim O, G. Berke, M. Fridkin, M. Feldman, M. Eisenstein & L. Eisenbach: CTL induction by a tumor-associated antigen octapeptide derived from a murine lung carcinoma. *Nature* 369, 67-71 (1994)

21. Apostolopoulos V, G. Chelvanayagam & I. F. C. McKenzie: Anti-MUC1 antibodies react directly with MUC1 peptides presented by class I H2 and HLA molecules. *J Immunol* 161, 767-775 (1998)

22. Fremont D. H, E. Stura, M. Matsumura, P. A. Peterson, & I. A. Wilson: Crystal structure of an H-2K^b-ovalbumin peptide complex reveals the interplay of primary and secondary anchor positions in the major histocompatibility complex binding groove. *Proc Natl Acad Sci USA* 92, 2479-2483 (1995)

23. Domenech N, R. A. Henderson & O. J. Finn: Identification of an HLA-A11-restricted epitope from the tandem repeat of the epithelial tumor antigen MUC1. *J Immunol* 155, 4766-74 (1995)

24. Brossart P, K. S. Heinrich, G. Stuhler, L. Behnke, V. L. Reichardt, S. Stevanovic, A. Muhm, H. G. Rammensee, L. Kranz & W. Brugger: Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. *Blood* 93, 4309-4317 (1999)

25. Mutsunori S, D. Pendleton & J. A. Berzovsky: Broad recognition of cytotoxic T cell epitopes from the HIV-1 envelope protein with multiple Class I histocompatibility molecules. *J Immunol* 148, 1657-1667 (1995)

26. Thomson S, R. Khanna, J. Gardner, S. R. Burrows, B. Coupar, D. J. Moss & A. Suhrbier A: Minimal epitopes expressed in a recombinant polyepitope protein are processed and presented to CD8$^+$ cytotoxic T cells: Implications for vaccine design. *Proc Natl Acad Sci USA* 92, 5845-5849 (1995)

27. Gong J, V. Apostolopoulos, D. Chen, H. Chen, S. Koido, S. J. Gendler, I. F. C. McKenzie & D. Kufe: Selection and characterization of MUC1-specific CD8$^+$ T cells from MUC1 transgenic mice immunized with dendritic-carcinoma fusion cells. *Immunology* 101, 316-324 (2000)

28. Mukherjee P, A. R. Ginardi, C. S. Madsen, C. J. Sterner, M. C. Adriance, M. J Tevethia & S. J. Gendler: Mice with spontaneous pancreatic cancer naturally develop MUC1 specific CTL that eradicate tumors when adoptively transferred. J Immunol 165, 3451-3460 (2000)

29. Pietersz G. A, W. Li, C. Osinski, V. Apostolopoulos & I. F. C. McKenzie: Definition of MHC-restricted CTL epitopes from non-variable number of tandem repeats sequence of MUC1. *Vaccine* 18, 2059-2071 (2000)

30. Garcia K. C, M. Degano, L. Pease, M. Huang, P. A. Peterson, L. Teyton & I. A. Wilson: Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. *Science* 279, 1166-1172 (1998)

31. Agrawal B, M. A. Reddish, B. Christian, A. VanHeele, L. Tang, R. R. Koganty & B. M. Longenecker: The anti-MUC1 monoclonal antibody BCP8 can be used to isolate and identify putative MHC class I associated amino acid sequences. *Cancer Res* 58, 5151-5156 (1998)

32. Collins E. J, D. N. Garboczi & D. C. Wiley: Three-dimensional structure of a peptide extending from one end of a class I MHC binding site. *Nature* 371, 626-629 (1994)

33. Hörig H, A. Young, N. Papadopoulos, T. P. DiLorenzo & S. G. Nathenson: Binding of longer peptides to the H-2K^b heterodimer is restricted to peptides extended at their C terminus: Refinement of the inherent MHC class I peptide binding criteria. *J Immunol* 163, 4434-4441 (1999)

34. Olsen A. C, L. Pedersen, A. S. Hansen, M. H. Nissen, M. Olsen, P. R. Hansen, A. Holm & S. Buus: A quantitative assay to measure the interaction between immunogenic peptides and MHC class I molecules. *Eur J Immunol* 24, 385-392 (1994)

35. Stryhn A, L. O. Pedersen, A. Holm & S. Buus: Longer peptides can be accommodated in the MHC class I binding

site by a protrusion mechanism. *Eur J Immunol* 30, 3089-3099 (2000)

36. Apostolopoulos V, M. Yu, A. L. Corper, G.A. Pietersz, W. Li, L. Teyton, I. F. C. McKenzie & I. A. Wilson: Crystal structures of non-canonical low and high affinity peptide complexes with MHC class I: a novel use of alternative anchors. *J. Exp. Med.* submitted (2001)

37. Reddehase M. J, J. Rothbard & U. Koszinowski, U: A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes. *Nature* 337, 651-653 (1989)

38. Eisen H, Y. Sykulev, & T. Tsomides: Antigen-specific T-cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins. *Adv Protein Chem* 49, 1-56 (1996)

39. Gillanders W. E, H. L. Hanson, R. J. Rubocki, T. H. Hansen & J. M. Connolly: Class I-restricted cytotoxic T cell recognition of split peptide ligands. *Int Immunol* 9, 81-89 (1997)

40. Mandelboim O, G. Berke, M. Fridkin, M. Feldman, M. Eisenstein & L. Eisenbach: CTL induction by a tumor-associated antigen octapeptide derived from a murine lung carcinoma. *Nature* 369, 67-71 (1994)

41. Haurum J. S, I. B. Hoier, G. Arsequell, A. Neisig, G. Valencia, J. Zeuthen, J. Neefjes & T. Elliott: Presentation of cytosolic glycosylated peptides by human class I major histocompatibility complex molecules in vivo. *J Exp Med* 190, 145-150 (1999)

42. Haurum J. S, G. Arsequell, A. C. Lellouch, S. Y. Wong, R. A. Dwek, A. J. McMichael & T. Elliot: Recognition of carbohydrate by major histocampatibility complex class I-restricted, glycopeptide-specific cytotoxic T-lymphocytes. *J Exp Med* 180, 739-744 (1994)

43. Kastrup I. B, S. Stevanovic, G. Arsequell, G. Valencia, J. Zeuthen, H. G. Rammensee, T. Elliot & J. S. Haurum: Lectin purified human class I MHC-derived peptides: evidence for presentation of glycopeptides in vivo. *Tissue Antigens* 56, 129-135 (2000)

44. Abdel-Motal U.M, L. Berg, A. Rosen, M. Bengtsson, C. J. Thorpe, J. Kihlberg, J. Dahmen, G. Magnusson, K. A. Karlsson & M. Jondal: Immunization with glycosylated K^b-binding peptides generates carbohydrate-specific, unrestricted cytotoxic T-cells. *Eur J Immunol* 26, 544-551 (1996)

45. Speir J.A, U. M. Abdel-Motal, M. Jondal and I. A. Wilson: Crystal structure of an MHC class I presented glycopeptide that generates carbohydrate-specific CTL. *Immunity* 10, 51-61 (1999)

46. Glithero A, J. Tormo, J. S. Haurum, G. Arsequell, G. Valencia, J. Edwards, S. Springer, A. Townsend, Y. L. Pao, M. Wormald, R. A. Dwek, E. Y. Jones & T. Elliott: Crystal structures of two H-$2D^b$/glycopeptide complexes suggest a molecular basis for CTL cross-reactivity. *Immunity* 10:63-74 (1999)

47. Jensen T, P. Hansen, L. Galli-Stampino, S. Mouritsen, K. Frishce, E. Meinjohanns, M. Meldal A& O. Werdelin: Carbohydrate and peptide specificity of MHC class II-restricted T cell hybridomas raised against O-glycoslyated self peptides. *J Immunol* 158, 3769-3778 (1997).

48. Deck M. B, P. Sjolin, E. R. Unanue & J. Kihlberg: MHC-restricted, glycopeptide-specific T cells show specificity for both carbohydrate and peptide residues. J Immunol 162, 4740-4744 (1999)

49. Muller S, S. Goletz, N. Packer, A. Gooley, A. M. Lawson & F-G. Hanisch: Localization of O-glycosylation sites on glycopeptide fragments from lactation-associated MUC1. *J Biol Chem* 272, 24780-24798 (1997)

50. Hanisch H-G, S. Muller, H. Hassan, H. Clausen, N. Zacharas, A. A. Gooley, H. Paulsen, K. Alving & J. Peter-Katalinic: Dynamic epigenetic regulation of initial O-Glycosylation by UDP-N-Acetylgalactosamine: peptide N-Acetylgalactosaminyltransferases. *J Biol Chem* 274, 9946-9954 (1999)

51. Muller S, K. Alving, J. Peter-Katalinic, N. Zacharas, A. Gooley & F-G. Hanisch: High density O-glycosylation on tandem repeat peptide from secretory MUC1 of T47D breast cancer cells. *J Biol Chem* 274, 18165-18172 (1999)

52. Theobold M, J. Biggs, J. Hernandez, J. Lustgarden, C. Labadie & L. A. Sherman: Tolerance to p53 by A2.1 restricted CTL. *J Exp Med* 185, 833-841 (1997)

53. Vierboom M. P, H. W. Nijman, R. Offringa, E. I. van der Voort, T. van Hall, L. van der Broek, G. J. Fleuren, P. Kenemans, M. W. Kast & C. J. Melief: Tumor eradiction by wild type p53-specific CTL. *J Exp Med* 186, 695-704 (1997)

54. Cibioti R, J. M. Kanellopoulos, J. P. Cabaniols, O. H. Pannenko, K. Kosmatopoulos, E. Sercarz & P. Kourilsky: Tolerance to a self protein involves its immunodominant but not subdominant determinants. *Proc Natl Acad Sci USA* 89, 416-420 (1992)

55. Sette A, A. Vitielo, B. Reherman, P. Fowler, R. Nayersina, M. W. Kast, C. J. Melief, C. Oserdorf, L. Yuan, J. Ruppert, J. Sidney, M. F. Guercio, S. Southwood, R. T. Kubo, R. W. Chestnut, H.M. Grey & F. V. Chisari: The relationship between class I binding affinity and immunogenicity of CTL epitopes. *J Immunol* 153, 5586-5592 (1994)

56. van der Burg S. H, M. J. W. Visseren, R. M. P. Brandt, W. M. Kast & C. J. Melief: Immunogenicity of peptides bound to MHC class I molecules depends on the MHC peptide complex stability. *J Immunol* 156, 3308-3314 (1996)

57. Tirosh B, K. el-Shami, N. Vaisman, L. Carmon, E. Bar-Haim, E. Vadai, M. Feldman, M. Fridkin & L. Eisenbach:

Immunogenicity of H-2K^b-low affinity, high affinity and covalently-bound peptides in anti-tumor vaccination. *Immunol Lett* 70, 21-28 (1999)

58. Tourdot S, A. Scardino, E. Saloustrou, D. A. Gross, S. Pascolo, P. Cordoopatis, F. A. Lemonnier & K. Kosmatopoulos: A general strategy to enhance immunogenicity of low-affinity HLA-A2-associated peptides: implications in the identification of cryptic tumor epitopes. *Eur J Immunol* 30, 3411-3421 (2000)

59. Apostolopoulos V, S. A. Lofthouse, V. Popovski, G. Chevanayagam, M. S. Sandrin & I. F. C. McKenzie: Peptide mimics of a tumor antigen induce functional cytotoxic T cells. *Nature Biotech* 16, 276-280 (1998)

60. Vaughan H.A, K. R. Oldenburg, M. A. Gallop, J. D. Atkin, I. F. C. McKenzie & M. S. Sandrin: Recognition of an octapeptide sequence by multiple Galalpha(1,3)Gal-binding proteins. *Xenotransplantation* 3, 18-23 (1996)

61. Sandrin M.S, H. A. Vaughan, P. X. Xing & I. F. C. McKenzie: Natural human anti Galalpha(1,3)gal antibodies react with human mucin peptides. *Glycoconj J* 14, 97-105 (1997)

62. Madden D. R: The three-dimensional structure of peptide-MHC complexes. *Annu Rev Immunol* 13, 587-622 (1995)

63. Falk K, O. Rotzschke, S. Stevanovic, G. Jung & H. G. Rammensee: Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351, 290-296 (1991)

64. Jackson M. R, E. S. Song, Y. Yang & P. A. Peterson: Empty and peptide-containing conformers of class I major histocompatibility complex molecules expressed in *Drosophila melanogaster* cells. *Proc Natl Acad Sci USA* 89, 12117-12121 (1992)

65. Molano A, H. Erdjument-Bromage, D. Fremont, I. Messaoudi, P. Tempst & J. Nikolic-Zugic: Peptide selection by an MHC H-2K^b class I molecule devoid of the central anchor (C) pocket. *J Immunol* 160, 2815-2823 (1998)

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