We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

4,400
Open access books available

117,000
International authors and editors

130M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Peptide Presentation Is the Key to Immunotherapeutical Success

Wiebke C. Abels, Alexander A. Celik, Gwendolin S. Simper, Rainer Blasczyk and Christina Bade-Döding

Abstract

Positive and negative selection in the thymus relies on T-cell receptor recognition of peptides presented by HLA molecules and determines the repertoire of T cells. Immune competent T-lymphocytes target cells display nonself or pathogenic peptides in complex with their cognate HLA molecule. A peptide passes several selection processes before being presented in the peptide binding groove of an HLA molecule; here the sequence of the HLA molecule’s heavy chain determines the mode of peptide recruitment. During inflammatory processes, the presentable peptide repertoire is obviously altered compared to the healthy state, while the peptide loading pathway undergoes modifications as well. The presented peptides dictate the fate of the HLA expressing cell through their (1) sequence, (2) topology, (3) origin (self/nonself). Therefore, the knowledge about peptide competition and presentation in the context of alloreactivity, infection or pathogenic invasion is of enormous significance. Since in adoptive cellular therapies transferred cells should exclusively target peptide-HLA complexes they are primed for, one of the most crucial questions remains at what stage of viral infection viral peptides are presented preferentially over self-peptides. The systematic analyzation of peptide profiles under healthy or pathogenic conditions is the key to immunological success in terms of personalized therapeutics.

Keywords: HLA, peptides, peptide prediction, adoptive T-cell therapies, peptide-vaccination
1. Introduction

The immune system of all species has to be able to discriminate self and foreign (nonself) antigens to combat infections without eliciting autoimmune diseases. The presentation of self and nonself occurs through displaying cellular proteins on the cell surface by proteins of the major histocompatibility complex (MHC) gene cluster. In humans, the MHC locus is termed human leukocyte antigen (HLA) and comprises several gene loci with numerous different alleles for most of the genes [1]. One part of the genes is subsumed as HLA class I (HLA-I) with the gene products being expressed on virtually every nucleated cell in the human body. HLA-I molecules present peptides of intracellular proteins on the cell surface. Cytotoxic T cells (CD8+ T cells) as part of the adaptive immune system can recognize these peptide HLA-I (pHLA-I) complexes by the T-cell receptor (TCR) and scan simultaneously the HLA molecule and the peptide [2, 3] to discriminate between healthy and unhealthy cells, for example, virally infected cells. At the same time, natural killer (NK) cells that are part of the innate immune system scan the cell surface of HLA as well. These cells become activated when HLA-I is missing on the cell surface, for example, on virally infected or tumor cells [4].

Peptide loading on HLA-I is a complex mechanism and determines in addition to the HLA allele which peptides will be presented. The central part of this process is the peptide loading complex that is localized in the endoplasmic reticulum (ER). The HLA-I molecule, consisting of a heavy chain and a microglobulin, is stabilized by several chaperons since the structure is unstable when no peptide is bound. The transporter associated with antigen processing (TAP) imports protein fragments that are degraded in the cytosol by the proteasome into the ER. Depending on the sequence, these peptides are trimmed in different ways in the ER [5, 6]. The bridge between TAP and HLA-I is the chaperon tapasin (TPN) that facilitates peptide binding in the peptide binding groove [7].

Because the HLA gene cluster ranks among the most polymorphic region in the human genome [1] and most of these polymorphisms are located in the peptide binding region (PBR) [8, 9], these polymorphisms result in an abundance of structurally different pHLA entities. In this chapter, we focus on the interplay between HLA alleles, bound peptides and the interaction with immune receptors. It is highlighted that even minor differences in the HLA sequence can impact on the bound ligand or the pHLA structure. Every single peptide changes the overall structure of the HLA molecule. Structural alterations that differ from self-pHLA structures will be recognized by the immune system. Therefore, the last parts of the chapter demonstrate the advantage of established immunopeptidomes for immunotherapies.

2. Peptide selection and presentation

The viability of the immune system is governed by interactions between effector cell receptors and their cognate antigenic ligands. Immune effector cells survey HLA-I molecules on the surface of antigen-presenting cells by indirectly scanning the proteomic content of every single cell. The fundamental role of CD8+ T cells, the elimination of pathogens, is elicited through HLA-I molecules complexed to a peptide of foreign (e.g., viral) origin.
Positive and negative selection of T cells in the thymus is a critical step for the development of a mature functional immune system. Immune cells that have not developed immune tolerance against specific pHLA-I complexes during thymus selection will recognize these antigens as foreign. The allele-specific and patient-specific peptides that are presented on the cell surface shape the individual immune response. Even a single alteration in the peptide sequence can be recognized by immune effectors. Single alterations in the sequence of the HLA heavy chain might not affect which peptide sequences can be bound but could lead to a modified overall pHLA-I structure or might affect the strength of peptide binding resulting in pHLA-I complexes with different half-life times. Besides the influence of amino acid (AA) exchanges within the heavy chain, peptides might undergo competition in patients who carry alleles with the same peptide-binding motif. For those reasons, the presentation of a given peptide is dependent on the HLA type and the health status of the patient. Half-life times of pHLA

Figure 1. Viral interference with HLA class I peptide presentation. Depicted are targets for viral interference with peptide presentation on HLA-I molecules. HLA-I maturation and surface presentation of peptides are blocked through different mechanisms early postinfection. Most viruses directly target peptide loading through TAP and peptide optimization by tapasin (HCMV [104, 105], HSV [106], HPV [107], ADV [108], EBV [109]). Additionally, cell surface expression is impeded by retention of HLA-I in the ER (ADV [110], HIV [111], HCMV [112]) or rapid degradation of surface molecules (HHV-8 [113], HIV [114]). Other possibilities include dislocation of the HLA-I heavy chain before any peptide loading can occur (HCMV [115, 116]) and inhibition of proteasomal processing of viral proteins by the host cell (EBV, HCMV [117, 118]).
complexes influence the phenotype and/or functionality of T cells. That has to be taken into account when choosing pHLA-I targets for T-cell therapeutics. Based on these facts the importance of knowledge about allelic peptide specificity becomes obvious. Peptides have to pass several intracellular filters and processing steps before being presented to immune effectors cells. The bottleneck is the peptide loading complex (PLC). Not every available peptide in a cell would be necessarily bound to an HLA molecule and displayed at the cell surface since peptides undergo peptide competition during recruitment through the PLC. The PLC consists of several proteins; each has a specialized function for peptide selectivity and specificity. Proteins of this complex are dedicated targets for viral interference and thus viral immune evasion (Figure 1).

The HLA heavy chain has to adopt a peptide-receptive form and complex with certain proteins of the PLC. It could be demonstrated that certain allelic variants interact differently with proteins of the PLC and thus are more prone to present peptides of malignant origin. Those HLA subtypes can differ from alleles that are strictly dependent on the association with the PLC for peptide loading only by a single amino acid within the heavy chain, altering the structural interface that interacts with the PLC [10–12]. Especially the interaction of TPN, a protein that mediates the binding of high-affinity peptides into the HLA-I PBR, with the HLA-I molecule, is of exquisite importance to produce stable pHLA-I complexes that persist on the cell surface. However, few allelic HLA variants are able to present peptides without the assistance of TPN. That enables those alleles to continuously present viral peptides at an infectious stage where viral interference with TPN occurs; however, the presentation of self-peptides that did not take part in negative T-cell selection would be facilitated and might lead to uncontrollable autoimmune reactions. HLA-I variants that select and load peptides without the assistance of TPN are likely to present a broad range of low-affinity viral-derived peptides during an infection. However, the presentation of viral peptides during an active viral infection is a rare event since still self-peptides are present intracellularly and would compete with viral peptides to fit into the PBR. The viral peptides that would reach the cell surface complexed to an HLA-I molecule could hardly be predicted by peptide prediction tools.

3. Peptide specificity

Due to the fact that peptides undergo different selection steps before being presented by an individual HLA allele, it has to become clear that the individual HLA profile is the major and most distinguished obstacle. Every HLA allele differs from another by the composition of AAs in and/or outside the peptide-binding groove, resulting in allele-specific profiles of the bound peptides [13–15]. Therefore, the knowledge of an individual peptide binding profile can be used as precedence for the measurement of permissivity between HLA subtypes. The sequence, length and immunogenicity of a given peptide determine the half-life time of the whole pHLA complex and furthermore the specificity and reactivity of their cognate immune receptor.
Several studies demonstrated the impact of the sequence and feature of HLA-bound peptides on receptor recognition. This observation holds true for T-cell receptors of the adaptive immune system as well as for NK-cell receptors of the innate immune system. We recently could highlight the potential of the nonclassical HLA-I molecule HLA-E to select and present peptides of extraordinary length and their effect on differential NK-cell recognition. For the nonpolymorphic HLA-E molecule only two functional variants exist distinguished by a single AA difference. That would imply that HLA-E is, in regard to its peptide profile, invariant. However, by sequencing their bound ligands, we found both alleles presenting a different set of peptides [16]. Since HLA-E is an intermediate molecule for the adaptive and innate immune system, supporting the non-PLC-dependent presentation of peptides during HLA downregulation episodes, the invariability of this molecule would certainly make biological sense. However, the finding that HLA-E subtypes differ in their immunity was somehow unexpected. Reconstitution of empty HLA-E molecules with the designated peptides on the surface of artificial APCs resulted in a peptide-specific immune recognition [17].

Two types of NK-cell receptors interact with HLA-I molecules, killer cell immunoglobulin-like receptors (KIRs) and C-type lectin-like receptors. The former ones are highly polymorphic and polygenic in the population and recognize HLA-A, -B and -C alleles [18], whereas the latter ones bind among others to HLA-E [19, 20]. CD8+ T cells recognize endogenous HLA alleles and assess their immune status by virtue of the presented peptide. The display of different peptides thus allows for precise monitoring of the immune status of the cell through the adaptive immune system. However, this also means that these cells have to be primed on the recognition of specific peptides that are usually derived from endogenously expressed proteins [21]. The presented peptide repertoire can be altered by aberrant protein expression as well as the presence of foreign proteins (e.g., viral proteins) in the cell. To counter recognition by CD8+ T cells, many viruses have developed immune evasion strategies that specifically target HLA peptide loading and presentation. For instance, the HCMV protein US6 interferes with peptide translocation at the ER thus depriving the available peptide pool or even directly procures that the HLA heavy chain is degraded through US2 or US11 [22]. However, in the event of such disrupted HLA-I presentation, NK cells become activated. Although NK cells do not recognize the specific HLA allele, the absence of HLA expression on the cell surface triggers NK-cell activation. Nevertheless, NK cells can still recognize certain peptides in the context of the nonclassical HLA molecule HLA-E that presents a very narrow set of peptides derived from the signal sequence of other HLA class I molecules. These peptides are 9 AA in length and are anchored preferably by Met at peptide position p2 and Leu at pΩ, whereas positions p4, p5 and p6 are accessible to the solvent [23, 24]. On NK cells, HLA-E in combination with these peptides is recognized by inactivating the NKG2A/CD94 heterodimeric receptor complex [25]. In the absence of HLA class I molecules or during cell stress, HLA-E was shown to present noncanonical peptides of different length [16, 17], for example, the Hsp60-derived peptide QMRPVSRVL that causes loss of recognition by NKG2A/CD94 [26] or the HIV Gag-derived peptide AISPRTLNA that causes HLA-E upregulation [27]. In the case of HCMV, a peptide from the UL40 protein that closely resembles the sequence of leader
peptides from certain HLA-C allotypes is provided to stabilize HLA-E expression in infected cells. However, in individuals negative for these HLA-C allotypes, the UL40-peptide constitutes the presentation of nonself on HLA-E and can thus elicit a CD8\(^+\) T-cell response [19, 28]. Additionally, HLA-E in complex with other pathogen-derived peptides was shown to stimulate CD8\(^+\) T-cell responses. For instance, the Epstein–Barr virus-derived peptide SQAPLPCVL was shown to be recognized by the αβTCR of a CD8\(^+\)-CD94/NKG2C\(^+\) T-cell clone [29, 30] described HLA-E-restricted *Salmonella enterica serovar Typhi*-specific CD3\(^+\)CD8\(^+\)CD4\(^-\)CD56\(^-\) T cells. These diverse interactions demonstrate the subtle interaction of innate and adaptive immunity through the presented peptide on HLA-E.

### 4. Peptide binding prediction, bioinformatic tools

To identify peptides that would be suitable for application in cellular therapeutic strategies, certain properties have to be analyzed: (1) the peptide-binding motif of the HLA allele of choice and (2) the HLA allele-specific features of the bound peptides such as length and topology. There are several bioinformatic tools that enable scientists and clinicians to predict peptides that would be presented by a certain HLA allele, yet, these tools do not consider allele-specific features and immune dominance of peptides. The kinetics of antigen expression and the competition of peptides to be preferentially bound and presented are also not considered by these bioinformatics prediction tools. Most data available in these tools are based on experimental peptide data (Tables 1 and 2). However, it remains unclear if those predicted peptides would ever be naturally presented. Peptides predicted from for example a viral protein would not necessarily be processed, selected and/or presented by the respective patient awaiting T-cell therapy. Therefore, the pathogen- or peptide-specific T cells that would be transplanted might not be able to find their mutual pHLA molecule. An example of the first successful adoptive transfer of virus-specific T cells described the transfer of HCMV-specific T cells and their reconstitution of antiviral immunity in an immune-deficient bone marrow transplant recipient [31]. The technique of adoptive T-cell transfer could be further improved leading to the selection of specific T-cells based on IFN-γ secretion or pHLA multimer staining and selection following antigen stimulation [32–34]. Both techniques bear the imperative to know which viral peptides are presented on the particular HLA subtype of, for example, HCMV-infected cells. So far, few HLA-restricted peptides have been studied. The majority of peptides are derived from the well-characterized phosphoprotein (pp)65 or the immediately early (IE)1 protein, however, not for every patient responses against these two proteins are immunodominant [35, 36]. Best studied are the pp65-derived peptides NLVPMVATV and TPRVTGGGAM, restricted to HLA-A*02:01 and HLA-B*07:02, respectively. Those peptides are described to induce extremely strong T-cell responses [37–42]. Yet, these peptides have been computationally predicted [43] but not been isolated from HLA molecules. Thus, it remains unproven if they would ever be naturally presented. That might be an explanation for the failure of long-term T-cell transfers [33, 44, 45].
<table>
<thead>
<tr>
<th>Name</th>
<th>Application</th>
<th>Methods</th>
<th>Ref.</th>
<th>Number of HLA class I alleles</th>
<th>Number of HLA class II alleles</th>
<th>Peptide length</th>
<th>Other species</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIMAS</td>
<td>Predicts half-time of dissociation of peptides from protein sequences</td>
<td>Coefficient tables</td>
<td>[58]</td>
<td>41 inc. supertypes</td>
<td>0</td>
<td>8–10</td>
<td>No</td>
<td><a href="https://www-bimas.cit.nih.gov/molbio/hla_bind/">https://www-bimas.cit.nih.gov/molbio/hla_bind/</a></td>
</tr>
<tr>
<td>hla_a2_smm</td>
<td>Predicts binding affinity of peptides, high affinity HLA-A2 binding peptides from protein sequence and mutated peptides with higher affinity</td>
<td>SMM pair coefficients</td>
<td>[60]</td>
<td>1</td>
<td>0</td>
<td>9–10</td>
<td>No</td>
<td><a href="https://zlab.bu.edu/SMM/">https://zlab.bu.edu/SMM/</a></td>
</tr>
<tr>
<td>IEDB T Cell Epitope Prediction Tools</td>
<td>Predicts T cell epitopes from proteins (MHC binding, processing and immunogenicity)</td>
<td>Several tools can be chosen</td>
<td>[61–64]</td>
<td>77</td>
<td>n/s</td>
<td>Class I:8–14</td>
<td>Chimpanzee, cow, gorilla, macaque, mouse, pig, rat for MHC class I; mouse for MCH class II</td>
<td><a href="http://tools.iedb.org/main/tcell/">http://tools.iedb.org/main/tcell/</a></td>
</tr>
<tr>
<td>Mappp</td>
<td>Predicts antigenic peptides to be processed and presented by MHC class I from peptide or protein sequence</td>
<td>Uses BIMAS or SYFPEITHI for binding prediction</td>
<td>[65]</td>
<td>35 inc. supertypes</td>
<td>0</td>
<td>8–10</td>
<td>Mouse, cattle</td>
<td><a href="http://www.mpiib-berlin.mpg.de/MAPP/index.html">http://www.mpiib-berlin.mpg.de/MAPP/index.html</a></td>
</tr>
<tr>
<td>MHC2MIL</td>
<td>Predicts binding affinity of MHC-II peptides from protein sequence</td>
<td>MIL</td>
<td>[66]</td>
<td>0</td>
<td>26</td>
<td>9–25</td>
<td>No</td>
<td><a href="http://datamining-iip.fudan.edu.cn/service/MHC2MIL/index.html">http://datamining-iip.fudan.edu.cn/service/MHC2MIL/index.html</a></td>
</tr>
<tr>
<td>MHC2PRED</td>
<td>Prediction of MHC class II binders</td>
<td>SVM</td>
<td>[67]</td>
<td>0</td>
<td>38 inc. supertypes</td>
<td>9</td>
<td>Mouse</td>
<td><a href="http://crdd.osdd.net/raghava/mhc2pred/index.html">http://crdd.osdd.net/raghava/mhc2pred/index.html</a></td>
</tr>
<tr>
<td>Name</td>
<td>Application</td>
<td>Methods</td>
<td>Ref.</td>
<td>Number of HLA class I alleles</td>
<td>Number of HLA class II alleles</td>
<td>Peptide length</td>
<td>Other species</td>
<td>URL</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>--------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>----------------</td>
<td>---------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>MHCBN</td>
<td>Database with information about allele specific MHC binding peptides, MHC nonbinding, TAP binding, TAP nonbinding peptides and T-cell epitopes</td>
<td>Database</td>
<td>[68, 69]</td>
<td>n/s</td>
<td>n/s</td>
<td>n/s</td>
<td>All</td>
<td><a href="http://crdd.osdd.net/raghava/mhcbn/index.html">http://crdd.osdd.net/raghava/mhcbn/index.html</a></td>
</tr>
<tr>
<td>MHCMIIR</td>
<td>Predicts binding affinity and levels of MHC-II peptides from peptide or protein sequence</td>
<td>MIR                                      [70]</td>
<td>0</td>
<td>13</td>
<td></td>
<td></td>
<td>Mouse</td>
<td><a href="http://ailab.ist.psu.edu/mhcmir/predict.html">http://ailab.ist.psu.edu/mhcmir/predict.html</a></td>
</tr>
<tr>
<td>MHCPRED</td>
<td>Predicts binding affinity of peptides to MHC class I and II molecules and to TAP from protein sequence and calculates binding affinity for heteroclitic peptides</td>
<td>Additive method, partial least square regression</td>
<td>[71–73]</td>
<td>11</td>
<td>3</td>
<td>9</td>
<td>Mouse</td>
<td><a href="http://www.ddg-pharmfac.net/mhcpred/MHCPred/">http://www.ddg-pharmfac.net/mhcpred/MHCPred/</a> <a href="http://www.ddg-pharmfac.net/mhcpred/MHCPred/pepLib.html">http://www.ddg-pharmfac.net/mhcpred/MHCPred/pepLib.html</a></td>
</tr>
<tr>
<td>MMBPred</td>
<td>Predicts mutated high affinity and promiscuous MHC class-I binding peptides from protein sequence, epitope enhancement, 1–3 AAs mutation of nonamer peptides</td>
<td>QM                                       [74]</td>
<td>40 inc. supertypes</td>
<td>0</td>
<td>9</td>
<td></td>
<td>Rhesus macaque, mouse</td>
<td><a href="http://crdd.osdd.net/raghava/mmbpred/">http://crdd.osdd.net/raghava/mmbpred/</a></td>
</tr>
<tr>
<td>MULTIPRED</td>
<td>Predicts binding of peptides to HLA class I and class II DR supertypes and individual genotypes</td>
<td>Uses NetMHCPan and NetMHCIIpan</td>
<td>[75]</td>
<td>13 supertypes</td>
<td>13 supertypes</td>
<td>8–11 for HLA class I and genotype 9 for HLA class II</td>
<td>No</td>
<td><a href="http://cvc.dfci.harvard.edu/multipred2/index.php">http://cvc.dfci.harvard.edu/multipred2/index.php</a></td>
</tr>
<tr>
<td>Name</td>
<td>Application</td>
<td>Methods</td>
<td>Ref.</td>
<td>Number of HLA class I alleles</td>
<td>Number of HLA class II alleles</td>
<td>Peptide length</td>
<td>Other species</td>
<td>URL</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------</td>
<td>------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>NetCTL</td>
<td>Predicts CTL epitopes in protein sequences</td>
<td>ANN</td>
<td>[76]</td>
<td>12 supertypes</td>
<td>0</td>
<td>9</td>
<td>No</td>
<td><a href="http://www.cbs.dtu.dk/services/NetCTL/">http://www.cbs.dtu.dk/services/NetCTL/</a></td>
</tr>
<tr>
<td></td>
<td>(Cleavage, TAP transport, HLA class I binding)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NetMHC</td>
<td>Predicts binding of peptide to MHC class I molecules from peptide or protein sequence</td>
<td>ANN</td>
<td>[19, 77]</td>
<td>81 (or 12 supertypes)</td>
<td>0</td>
<td>8–14</td>
<td>Chimpanzee, rhesus macaque, mouse, cuttle, pig</td>
<td><a href="http://www.cbs.dtu.dk/services/NetMHC/">http://www.cbs.dtu.dk/services/NetMHC/</a></td>
</tr>
<tr>
<td>NetMHCcons</td>
<td>Predicts binding of peptides to any known MHC class I molecule from peptide or protein sequence</td>
<td>Consensus (NetMHC, NetMHCpan and PickPocket)</td>
<td>[78]</td>
<td>User specified</td>
<td>0</td>
<td>8–15</td>
<td>Chimpanzee, gorilla, rhesus macaque, mouse, cuttle, pig</td>
<td><a href="http://www.cbs.dtu.dk/services/NetMHCcons/">http://www.cbs.dtu.dk/services/NetMHCcons/</a></td>
</tr>
<tr>
<td>NetMHCII</td>
<td>Predicts binding of peptides to HLA-DR, HLA-DQ, HLA-DP from peptide or protein sequence</td>
<td>ANN</td>
<td>[79, 80]</td>
<td>0</td>
<td>26</td>
<td>variable</td>
<td>Mouse</td>
<td><a href="http://www.cbs.dtu.dk/services/NetMHCII/">http://www.cbs.dtu.dk/services/NetMHCII/</a></td>
</tr>
<tr>
<td>NetMHCIIpan</td>
<td>Predicts binding of peptides to HLA-DR, HLA-DQ, HLA-DP from peptide or protein sequence</td>
<td>ANN</td>
<td>[81, 82]</td>
<td>User specified</td>
<td>0</td>
<td></td>
<td>Mouse</td>
<td><a href="http://www.cbs.dtu.dk/services/NetMHCIIpan/">http://www.cbs.dtu.dk/services/NetMHCIIpan/</a></td>
</tr>
<tr>
<td>NetMHCpan</td>
<td>Predicts binding of peptides to any known MHC class I molecule from peptide or protein sequence</td>
<td>ANN</td>
<td>[19, 83, 84]</td>
<td>User specified</td>
<td>0</td>
<td>8–14</td>
<td>Chimpanzee, gorilla, rhesus macaque, mouse, cuttle, pig</td>
<td><a href="http://www.cbs.dtu.dk/services/NetMHCpan/">http://www.cbs.dtu.dk/services/NetMHCpan/</a></td>
</tr>
<tr>
<td>nHLAPred: ANNPred</td>
<td>Predicts MHC Class I binding regions in proteins</td>
<td>ANN</td>
<td>[85]</td>
<td>26 inc. supertypes</td>
<td>0</td>
<td></td>
<td>Mouse</td>
<td><a href="http://crdd.osdd.net/raghava/nhlapred/neural.html">http://crdd.osdd.net/raghava/nhlapred/neural.html</a></td>
</tr>
<tr>
<td>nHLAPred: ComPred</td>
<td>Predicts MHC Class I binding regions in proteins</td>
<td>ANN/QM</td>
<td>[85]</td>
<td>59 inc. supertypes</td>
<td>0</td>
<td></td>
<td>Rhesus macaque, mouse</td>
<td><a href="http://crdd.osdd.net/raghava/nhlapred/comp.html">http://crdd.osdd.net/raghava/nhlapred/comp.html</a></td>
</tr>
</tbody>
</table>

**Peptide Presentation: Key to Immunotherapeutical Success**

http://dx.doi.org/10.5772/intechopen.76871
<table>
<thead>
<tr>
<th>Name</th>
<th>Application</th>
<th>Methods</th>
<th>Ref.</th>
<th>Number of HLA class I alleles</th>
<th>Number of HLA class II alleles</th>
<th>Peptide length</th>
<th>Other species</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDPEP</td>
<td>Predicts binding of peptides to HLA class I from peptide or protein sequence</td>
<td>Published coefficient tables</td>
<td>[86]</td>
<td>6</td>
<td>0</td>
<td>8–10 (dependent on the allele)</td>
<td>Mouse</td>
<td><a href="http://margalit.huji.ac.il/Teppred/mhc-bind/index.html">http://margalit.huji.ac.il/Teppred/mhc-bind/index.html</a></td>
</tr>
<tr>
<td>ProPred</td>
<td>Predicts MHC Class II binding regions in an antigen sequence</td>
<td>QM</td>
<td>[87]</td>
<td>51</td>
<td>0</td>
<td>No</td>
<td><a href="http://crdd.osdd.net/raghava/propred/">http://crdd.osdd.net/raghava/propred/</a></td>
<td></td>
</tr>
<tr>
<td>ProPred I</td>
<td>Predicts MHC Class I binding regions in an antigen sequence</td>
<td>QM</td>
<td>[88]</td>
<td>39 inc. supertypes</td>
<td>0</td>
<td>Mouse, cattle</td>
<td><a href="http://crdd.osdd.net/raghava/propred1/index.html">http://crdd.osdd.net/raghava/propred1/index.html</a></td>
<td></td>
</tr>
<tr>
<td>Rankpep</td>
<td>Predicts binding of peptides to MHC class I and class II molecules from peptide or protein sequence</td>
<td>PSSM</td>
<td>[89–91]</td>
<td>n/s</td>
<td>n/s</td>
<td>Dependent on the allele</td>
<td><a href="http://imed.med.ucm.es/Tools/rankpep.html">http://imed.med.ucm.es/Tools/rankpep.html</a></td>
<td></td>
</tr>
<tr>
<td>svmhc</td>
<td>Predicts binding of peptides to MHC class I molecules from peptide or protein sequence</td>
<td>SVM, uses MHCPEP or SYFPEITHI</td>
<td>[92, 93]</td>
<td>31</td>
<td>0</td>
<td>8–10 (dependent on the allele)</td>
<td>No</td>
<td><a href="http://svmhc.bioinfo.se/svmhc/">http://svmhc.bioinfo.se/svmhc/</a></td>
</tr>
<tr>
<td>SYFPEITHI</td>
<td>Database of MHC ligands and peptide motifs and epitope prediction</td>
<td>Matrix/motif-based, published motifs</td>
<td>[94]</td>
<td>33</td>
<td>6</td>
<td>8–11 for HLA class I, 15 for HLA class II</td>
<td>No</td>
<td><a href="http://www.syfpeithi.de/">http://www.syfpeithi.de/</a></td>
</tr>
<tr>
<td>TEPITOPEpan</td>
<td>Predicts tissue-specific binding of peptides to MHC class II molecules from peptide or protein sequence</td>
<td>PSSM</td>
<td>[95]</td>
<td>0</td>
<td>50</td>
<td>9–25</td>
<td>No</td>
<td><a href="http://datamining-tip.fudan.edu.cn/service/TEPITOPEpan/index.html">http://datamining-tip.fudan.edu.cn/service/TEPITOPEpan/index.html</a></td>
</tr>
</tbody>
</table>

Abbr. HMM = hidden Markov model, SVM = support vector machine, PSSM = position-specific scoring matrix, QM = quantitative matrices, ANN = artificial neuronal networks, SMM = stabilized matrix method, MIL = multiple instance learning, MIR = multiple instance regression, n/s = not specified.

Table 1. Listing of peptide prediction tools available on the web [Accessed November 2017].
<table>
<thead>
<tr>
<th>Name</th>
<th>Underlying database/data source</th>
<th>URL for matrices/training data</th>
</tr>
</thead>
</table>
| BIMAS       | Coefficient tables deduced from the published literature by Dr. Kenneth Parker, Children's Hospital Boston | https://www-bimas.cit.nih.gov/cgi-bin/molbio/hla_coefficient_viewing_page  
| hla_a2_smm  | BIMAS [58], SYFPEITHI [94], data described in Peters, Tong [60]                                | https://zlab.bu.edu/SMM/                                                                          |
| IEDB T Cell Epitope Prediction Tools | IEDB [61], Sette lab, Buss lab, uses diverse predictions methods (see webpage) | http://tools.iedb.org/mhci/download/  
http://tools.iedb.org/mhcii/download/ |
| Mappp       | BIMAS [58], SYFPEITHI [94], coefficient tables deduced from the literature by Kenneth Parker, Children’s Hospital Boston | —                                                  |
| MHC2MIL     | Data by Wang, Sidney [98]                                                                     | —                                                  |
| MHC2PRED    | JenPep [19], MHCBN [68]                                                                       | —                                                  |
| MHCBN       | MHCBN [68]                                                                                     | —                                                  |
| MHC2MIR     | IEDB [61]                                                                                      | —                                                  |
| MHC2PRED    | JenPep [19]                                                                                    | —                                                  |
| MMBPRed     | MHCBN [68]                                                                                     | —                                                  |
| MULTIPRED   | See NetMHCpan and NetMHCIIpan                                                                   | —                                                  |
| NetCTL      | See NetMHC                                                                                     | —                                                  |
| NetMHC      | Trained for 81 HLA alleles including HLA-A, -B, -C and –E, n/s                                 | —                                                  |
| NetMHCcons  | IEDB [61]                                                                                      | —                                                  |
| NetMHCpan   | IEDB [61], IMGT/HLA database [1]                                                                | —                                                  |
| nHLAmpred   | MHCBN [68]                                                                                     | —                                                  |
| nHLAmpred: ComPred | MHCBN [68], BIMAS [58]                                                                           | http://crdd.osdd.net/raghava/nhlapred/matrix.html                                                 |
| PREDPEP     | Pairwise potential table by Miyazawa and Jernigan [99]                                         | —                                                  |
5. Analysis of naturally presented peptides

The analysis of the individual patient and cell-type-specific immunopeptidome can be realized through sequencing the HLA-bound peptides. It is imperative for all ongoing peptide studies and cellular therapies to find peptides that are (1) naturally presented by the distinct allele, (2) immunogenic for (at best) a public T-cell repertoire and (3) preferentially presented when different peptides are available. A study from Yaciuk et al. showed for example that the peptides isolated from HIV-infected T cells differ from predicted peptides and exhibit different T-cell reactions, factors that have to be considered in designing immunotherapies [46]. That information is only available after immunopeptidome analyses.

In the past, different methods have been applied to answer these questions comprehensively. There are two reliable methods to determine peptide sequences from selected HLA alleles. First, membrane-bound HLA molecules from recombinant single-antigen-presenting cells [47,48] or from donor cells [49,50] can be captured by affinity chromatographic methods and the bound peptides isolated and sequenced by mass spectrometry. Second, the most realizable method is the soluble HLA technology [16,51]. Vectors encoding for soluble forms of HLA molecules (Exon 1–4) are transfected or lentivirally transduced into the cell line of choice. An optional recombinant tag (e.g., V5 tag) engineered at the C-terminus of the protein enables specific purification of the recombinant HLA molecule of choice without the challenge of contamination by cellular-self-HLA molecules. Both methods have been compared by Scull et al. [52] and indicated as an equivalent for the determination of allele-specific peptides. Furthermore, Badrinath et al. [10] could demonstrate that sHLA molecules associate during peptide acquisition with the loading complex as well. These results prove evidence that the use of sHLA technology for understanding allele-specific peptide-binding motifs, the prerequisite for updating peptide prediction databases, is the most time- and cost-efficient implementation.

For the development of tailor-made T-cell-based immunotherapeutic strategies, the identification of tumor-specific HLA ligands is imperative. The production of recombinant sHLA-expressing cells derived from various tissues of malignant origins would guide towards understanding immune dominance through peptide competition. One of the most innovative applications is the peptide fishing from tumor tissue. Immunological tolerance is mediated through T cells that

<table>
<thead>
<tr>
<th>Name</th>
<th>Underlying database/data source</th>
<th>URL for matrices/training data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rankpep</td>
<td>MHCPEP [102], SYFPEITHI [94],</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GenBank [103]</td>
<td></td>
</tr>
<tr>
<td>SYFPEITHI</td>
<td>Published literature</td>
<td></td>
</tr>
<tr>
<td>TEPITOPEpan</td>
<td>n/s</td>
<td><a href="http://datamining-iip.fudan.edu.cn/service/TEPITOPEpan/TEPITOPEpan.html">http://datamining-iip.fudan.edu.cn/service/TEPITOPEpan/TEPITOPEpan.html</a></td>
</tr>
</tbody>
</table>

Abbr. n/s = not specified.

Table 2. Listing of the underlying databases/data sources for peptide binding prediction.
are primed in the thymus by self-peptides. Therefore, the comprehensive knowledge of the HLA immunopeptidome from diseased cells is fundamental for the development of efficient immunotherapeutic strategies. The presentation of peptides depends on the health state of a patient. During infections, the expression of HLA molecules and thus peptide presentation, including presentation of self-peptides, is diminished through an immune escape mechanism of the invasive pathogen.

6. Peptide vaccination

The treatment of cancer represents a great challenge due to the fact that the vast majority of HLA-restricted peptides differs from tissue to tissue and is dependent on the tumor entity. For that reason, it becomes obvious how fundamentally important the knowledge of the tumor-specific peptidome is. For personalized cancer immunotherapies, the knowledge of naturally presented peptides [53] represents the exclusive possibility for therapeutical success. The analysis of the mutanome, the proteomic content of a diseased cell, includes the discovery of neo-antigens or post-translational-modified peptides and the avoidance of targeting self-antigens from healthy tissue. The results of such individual mutanomes might alter during the course of tumor progression [16]. In peptide vaccination trials, the use of multiple peptides in combination [54, 55] represents a useful method for targeting all MHC-presenting cells with the peptide of choice. Yet, since the cell type where the peptide(s) bind to cannot be traced, the rates of antitumor immune responses might differ from patient to patient and certain tumor cells where, for example, low MHC expression rates might remain undetected from the immune system. To achieve a comprehensive and precise analysis of presented tumor antigens, the method of antigen discovery and appropriate T-cell assay for knowledge of immunogenicity of the dedicated antigen for vaccination is the key factor [56, 57].

7. Conclusion

Peptide selection and presentation is an exquisite biological and immunological event. Every single peptide is a mirror of the health state of a distinct cell and determines the outcome of immune recognition and responses. For all cellular therapies, the knowledge of the HLA-subtype specific proteome is crucial for the utilization of ligand prediction tools, which have to be implemented where no experimental data are available, yet.

Author details

Wiebke C. Abels, Alexander A. Celik, Gwendolin S. Simper, Rainer Blasczyk and Christina Bade-Döding*

*Address all correspondence to: bade-doeding.christina@mh-hannover.de

Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany
References


[7] Zarling AL et al. Tapasin is a facilitator, not an editor, of class I MHC peptide binding. The Journal of Immunology. 2003;171(10):5287-5295

[8] Carreno BM et al. The peptide binding specificity of HLA class I molecules is largely allele-specific and non-overlapping. Molecular Immunology. 1992;29(9):1131-1140


[34] Schmitt A et al. Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation. Transfusion. 2011;51(3):591-599


[38] Gibson L et al. Human cytomegalovirus proteins pp65 and immediate early protein 1 are common targets for CD8+ T cell responses in children with congenital or postnatal human cytomegalovirus infection. Journal of Immunology. 2004;172(4):2256-2264


[52] Scull KE et al. Secreted HLA recapitulates the immunopeptidome and allows in-depth coverage of HLA a*02:01 ligands. Molecular Immunology. 2012; 51(2):136-142


[56] Purcell AW, Croft NP, Tscharke DC. Immunology by numbers: Quantitation of antigen presentation completes the quantitative milieu of systems immunology! Current Opinion in Immunology. 2016; 40:88-95


[58] Parker KC, Bednarek MA, Coligan JE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. Journal of Immunology. 1994; 152(1):163-175


[61] Vita R et al. The immune epitope database (IEDB) 3.0. Nucleic Acids Research. 2015;43(Database issue):D405-D412


[64] Moutaftsi M et al. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. Nature Biotechnology. 2006;24(7):817-819


[76] Larsen MV et al. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007;8:424


[81] Karosiene E et al. NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. Immunogenetics. 2013;65(10):711-724


[84] Jurtz V et al. NetMHCpan-4.0: Improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. Journal of Immunology. 2017;199(9):3360-3368


[86] Schueler-Furman O et al. Structure-based prediction of binding peptides to MHC class I molecules: Application to a broad range of MHC alleles. Protein Science. 2000;9(9):1838-1846


[89] Reche PA et al. Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. Immunogenetics. 2004;56(6):405-419


Bennett EM et al. Cutting edge: Adenovirus E19 has two mechanisms for affecting class I MHC expression. Journal of Immunology. 1999;162(9):5049-5052

Horst D et al. Specific targeting of the EBV lytic phase protein BNLF2a to the transporter associated with antigen processing results in impairment of HLA class I-restricted antigen presentation. Journal of Immunology. 2009;182(4):2313-2324

Beier DC et al. Association of human class I MHC alleles with the adenovirus E3/19K protein. Journal of Immunology. 1994;152(8):3862-3872


Coscoy L, Ganem D. Kaposi’s sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(14):8051-8056


