

REVIEW ARTICLE

The immunoinformatics of cancer immunotherapy

D. S. DeLuca & R. Blasczyk

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

Key words

antigen presentation; immunoinformatics; immunotherapy

Correspondence

Dr Rainer Blasczyk
 Institute for Transfusion Medicine
 Hannover Medical School
 Carl-Neuberg-Street 1
 30625 Hannover
 Germany
 Tel: +49 511 532 6700
 Fax: +49 511 532 2079
 e-mail: blasczyk.rainer@mh-hannover.de

Received 16 July 2007; accepted 16 July 2007

doi: 10.1111/j.1399-0039.2007.00914.x

Abstract

We review here the developments in the field of immunoinformatics and their present and potential applications to the immunotherapeutic treatment of cancer. Antigen presentation plays a central role in the immune response, and as a result in immunotherapeutic methods such as adoptive T-cell transfer and antitumor vaccination. We therefore extensively review the current technologies of antigen presentation prediction, including the next generation predictors, which combine proteasomal processing, transporter associated with antigen processing and major histocompatibility complex (MHC)-binding prediction. Minor histocompatibility antigens are also relevant targets for immunotherapy, and we review the current systems available, SNEP and SiPep. Here, antigen presentation plays a key role, but additional types of data are also incorporated, such as single nucleotide polymorphism data and tissue/cell-type expression data. Current systems are not capable of handling the concept of immunodominance, which is critical to immunotherapy, but efforts have been made to model general aspects of the immune system. Although tough challenges lie ahead, when measuring the field of immunoinformatics on its contributions thus far, one can expect fruitful developments in the future.

Introduction

The cells of the immune system hold great potential to be harnessed for their therapeutic effects against cancer. Potential therapies include adoptive transfer of *ex vivo*-expanded antigen-specific T cells (1–3), as well as *in vivo* vaccination (4). In the case of adoptive transfer, a highly specific reactivity can be achieved using peptide-pulsed or transfected cells, as well as using artificial antigen-presenting cells (5–8). In addition to successful immunotherapeutic applications in mouse tumor models (9), clinical plausibility in humans is continually becoming established (10–14). The identification of the optimal antigens to use either in *ex vivo* T-cell stimulation or as anticancer vaccines is a crucial step in the development of immunotherapies (15, 16). Here, there is major potential for support from the field of computational biology.

Immunoinformatics is emerging as a field with growing significance and application in the clinical setting (17). For example, intense effort has been made to computationally represent the antigen presentation process (18–20). For immunotherapy, antigen targets can be of a tumor-specific nature (21). In this case, experiments are necessary to identify tumor-specific target proteins, at which point antigen presentation prediction can be very useful for

identifying immunoreactive domains. Further experimentation by major histocompatibility complex (MHC)-binding assays and cytotoxic T lymphocyte (CTL) assays is then required to select the functionally relevant antigens from the list of theoretical candidates (22).

In the context of hematopoietic stem cell transplantation, minor histocompatibility antigens (mHags) arise from the polymorphic genetic profile of a particular patient with respect to that of a stem cell donor (23). The graft *vs* leukemia (GvL) effect, which occurs when donor lymphocytes eliminate the residual malignant cells of the patient, can be considered the most established form of immunotherapy (24). Exploiting the GvL effect, while minimizing graft *vs* host disease (GvHD), is the major challenge of the hematopoietic stem cell transplantation field. Here, immunoinformatics contributes to the effort to identify GvL-inducing mHags by mining polymorphism data, expression profiles as well as antigen presentation predictions (25, 26).

Antigen presentation

Antigen presentation is the process leading to the presentation of potential T-cell epitopes on the cell surface by MHC. In the

case of presentation by MHC class I, this process involves protein degradation, peptide transport into the endoplasmic reticulum via transporter associated with antigen processing (TAP), and MHC binding (27). The solved crystal structures of these components are depicted in Figure 1. Algorithms have been developed to predict each of these fundamental steps in this process, as well as combination algorithms, which integrate the individual types of prediction. An overview of such algorithms is shown in Table 1.

Protein cleavage

The proteasome is the central focus of the efforts of immunoinformatics to describe protein degradation. There are two sources of data, which are used to train cleavage prediction systems. On the one hand, there are peptide sequences generated by the *in vivo* degradation of specific proteins (28–30). These data are extremely insightful into the manner in which the proteasome operates. In particular, it is clear from these digestion assays that, while there are

favorable cleavage sites, the same protein can be cleaved at different sites. The result is a pool of peptides, which in many cases overlap in the original protein sequence. The limiting aspect of this high-quality data is simply that few proteins have been analyzed in this way and that a larger training set is desirable. This has caused other immunoinformaticians to turn to a more plentiful source of data: naturally presented peptides eluted from MHC proteins. It is assumed that such natural peptides must have been cleaved by the proteasome and can therefore be used in training predictors in combination with the source protein sequences. The inclusion of peptides, which have been eluted from MHC proteins having differing binding motifs, should weaken an MHC-binding bias inherent in this data. However, as we will discuss later, these kinds of systems may still be predicting MHC binding to some extent. The unavoidable consequence of this kind of data, however, is the fact that cleavage sites in proteins will be missed, when the resulting peptide does not bind MHC, and is not included in the eluted peptide dataset as a result.

In addition to the proteasome, aminopeptidases are responsible for shortening the peptides, which are ultimately presented by MHC (31, 32). These enzymes act on the N-terminus of the peptide. Because there is currently no system for predicting aminopeptidase digestion, a certain gray area surrounds the determination of the N-terminus of predicted peptides. Because of this fact, it is arguable that predicting digested protein fragments should concentrate mainly on the C-terminus, with the assumption that for a given C-terminal cleavage site, peptidases will produce an array of peptides with differing N-terminal sites. This is supported by the observation of naturally presented peptides that result from the same C-terminal cleavage site but have differing lengths (33). There is another caveat concerning peptide length that should be mentioned here. The requirement by some prediction systems that a peptide be C-terminally cleaved but not contain internal, 'peptide-destroying' cleavage sites may be too strict. The *in vivo* digestion assays show the existence of peptides stemming from overlapping sequences in the original protein. This strongly suggests that an internal cleavage site does not disqualify a sequence region as a potential peptide. This is further supported by the latest attempts at including the internal cleavage site as a disqualifier, which did not improve proteasomal cleavage prediction performance (20).

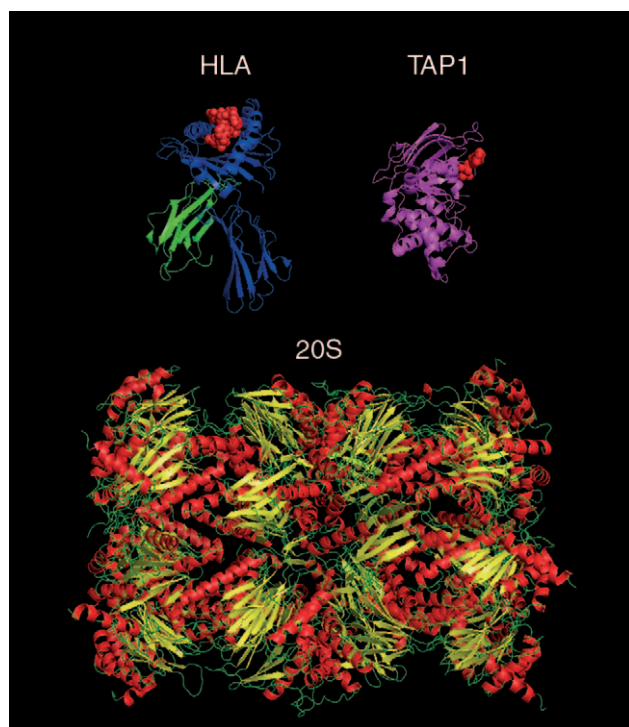


Figure 1 The major players in antigen presentation – human leukocyte antigen (HLA), transporter associated with antigen processing (TAP) and the proteasome. The crystal structures of three proteins central to the antigen presentation process are depicted in their relative sizes to one another. The HLA protein is in complex with β_2 microglobulin in green and a decamer peptide in red. TAP1 is shown in isolation because the details of TAP complex formation are still unknown. The ADP-binding site is shown in red. The barrel-like 20S subunit of the yeast proteasome is colored according to secondary structure. The following PDB files were used: 1HHH (71), 1RYP (72), and 1JJ7 (73).

Transporter associated with antigen processing

After proteasomal cleavage, peptide sequences are transported into the endoplasmic reticulum by the TAP protein. This process is assisted by various chaperones, such as tapasin, calreticulin and the disulfide isomerase ERp57 (34).

The peptide-binding motif of human TAP has been deciphered by combinatorial libraries. This and other

Table 1 Overview of antigen presentation prediction systems^a

Resource	Proteasomal processing	TAP binding	HLA binding	Availability
Multistep epitope prediction				
NetCTL 1.2 (20)	NetChop 3.0 (65)	SMM (39)	NetMHC 3.0 (45)	http://www.cbs.dtu.dk/services/NetCTL/
IEDB (66)	SMM (18)	SMM (39)	ANN, ARB, and SMM	http://www.immuneepitope.org/
EpiJen v1.0 (19)	Quantitative matrix (67)	Tap additive model (68)	MHCpred (69)	http://www.jenner.ac.uk/EpiJen/
Minor histocompatibility antigen prediction				
SiPep (25)	None	None	BIMAS (41), SYF (43), nHLAPred (70)	http://www.sipep.org/
SNEP (26)	None	None	SYF (43)	http://elchtools.de/SNEP/

ANN, artificial neural networks; ARB, average relative binding; HLA, human leukocyte antigen; IEDB, immune epitope database and analysis resource; SMM, stabilized matrix method; SYF, SYFPEITHI; TAP, transporter associated with antigen processing.

^a Numbers in parentheses are literature citations.

studies showed significant amino acid preferences, particularly at the C-terminus and the three N-terminal positions (35, 36). Peptides of up to 16 amino acids are preferred, with lengths of 8–12 binding most efficiently (37). For the purpose of predicting the rate at which TAP transports a particular peptide into the ER, TAP-peptide-binding affinity can be used as an estimator of transport rate, because these two values have been shown to correspond (38). In the pioneering work of Peters et al., weight matrices were fitted to TAP-peptide affinity data (39). Phenylalanine, tyrosine, and arginine were shown to be particularly favored at the C-terminus. We will discuss the implications of this effort in the section on combined predictions below.

Putting it together with MHC-binding prediction

The final step in antigen presentation is a classical subject of immunoinformatics: MHC binding. This is the most restrictive step involved in antigen presentation. It is estimated that only 1 out of 200 peptides will bind a given MHC class I allele with sufficient strength to elicit a CTL response (40). Many approaches have been taken to predict MHC–peptide binding (41–48), and it is useful at this point to discuss the prediction of all steps together, which are needed for comprehensive prediction of the entire presentation pathway.

The means of evaluating multistep antigen presentation prediction is to use a dataset of naturally processed MHC-bound peptides in combination with the original peptide sequences (the SYFPEITHI database being the accepted gold standard for curated naturally processed peptides). In the work of Tenzer et al., the stabilized matrix method (SMM) for TAP transport was combined with a novel SMM-based cleavage prediction and MHC-binding prediction algorithm (18). The novel cleavage prediction method, dubbed ProteaSMM, showed improvements over older methods such as PAPROC and NetChop 2.0. Furthermore, a significant improvement was demonstrated when

combining prediction steps. In particular, the combined prediction showed marked improvement over proteasomal processing prediction alone. However, a ‘breakthrough’ improvement over MHC-binding prediction alone was not observed. This is also true for NetCTL, the artificial neural network-based attempt at multistep antigen presentation prediction, in which only a minimal improvement over MHC-binding prediction alone was shown (20). It is difficult to make conclusions here about how this reflects the biology of these processes, or whether this is a result of training or testing artifacts. On the one hand, MHC-binding predictors could have hidden proteasomal cleavage and TAP transports element to them if these motifs are found directly in the presented peptides. On the other hand, MHC binding is considered the bottleneck in the presentation pathway, and one would therefore expect that the respective prediction step would weigh the most heavily.

Vaccination

The appeal of antitumor vaccination is the fact that it takes advantage of *in vivo* processes and has the potential to harness the full power of the immune system, in contrast to the more artificial *ex vivo* expansion of T cells. The use of synthetic peptide vaccines enables one to achieve a high level of specificity, with relative ease of production (49). It has been shown that the peptides predicted to bind MHC can elicit a tumor-killing CTL response (50). An effective methodology for determining new tumor-specific peptide epitopes involves the application of antigen prediction algorithms to a tumor-associated protein, then experimental confirmation of the MHC-binding affinity, and finally stimulation of CTLs with peptide-loaded dendritic cells (51).

Class II MHC-bound epitopes play an important role in the antitumor response by the activation of CD4+ T cells and help maintain effective CTL response (52, 53). Historically, predicting peptide binding of class II MHC has been much more challenging than that of class I (54–56).

The reason for this lies in the fact that class II proteins bind to peptides of variable length and that the core anchor residues cannot be readily identified. Application of artificial neural networks has been a successful endeavor, at least for predicting the high-affinity side of the peptide binder spectrum (54). A review of class II predictions has shown that such neural network-based predictions are effective when sufficient peptide-binding data are available but that motif-based versions are favored for small peptide datasets (56). Clearly, prediction for class II alleles needs to be improved and expanded to include more allelic variants, considering the central role that it plays in the immune response. This holds true not only for antitumor vaccination but presumably for the immune response to mHags as well (23).

mHags prediction

mHags are peptides, which are presented by MHC on the cell surface of an individual, and cause an alloreactive immune response as a result of their absence in the individual from whom the attacking lymphocytes originate. A database of known minors is hosted by the Leiden University Medical Center and can be accessed online at <http://www.lumc.nl/5033/dbminor/>. In the context of a hematopoietic stem cell transplantation, such antigens can cause GvHD (57) as well as the therapeutic GvL effect (24). Finding target antigens that minimize the former and maximize the latter is the goal of immunoinformatic efforts, which combine antigen presentation with additional biological data including polymorphism and gene expression data (25, 26).

Polymorphism data are necessary to identify gene variants, which could result in the differential expression of peptides. The majority of known 'minors' result from a single nucleotide polymorphism (SNP) in a coding region of a gene, resulting in an amino acid substitution. However, insertions, deletions, frameshift mutations, mutations resulting in stop codons, mutations eliminating stop codons, splice site and promoter mutations and whole gene deletions (58) are all conceivable. Most of these kinds of mutations are computable with conventional means using databases such as the dbMHC. What will remain difficult in the immediate future is the identification of peptide variations that result from mutations outside of the gene encoding the peptide (e.g. mutations that disable activator proteins). The SiPep web service, which aims to predict mHags, used the dbMHC as well as data from the HapMap project to compute variant peptides (25). In this system, coding nonsynonymous mutations are considered. Because most known minors do result from this type of polymorphism, it is not unreasonable to concentrate solely on them. Future applications, however, would do well to consider the other types of relevant mutations. Another mHag prediction system is SNEP (25), which also focuses

on coding nonsynonymous SNPs. In this system, the CONFLICT and VARIANT annotations in SWISS-PROT provide the polymorphism data.

For leukemia treatment, if mHags are to be used to expand T cell for adoptive transfer, they should induce GvL without GvHD. This would be the case when the minor is either restricted to the malignant cells or restricted to the cells of the hematopoietic origin. This latter case is acceptable because the patient's blood system will be replaced by the graft. Indeed, hematopoietic expression is the criteria recommended by the authors of SiPep. In this system, the types of tissue in which the peptide candidates are expressed are obtained from Stanford University's SOURCE database (<http://hrweb.stanford.edu/source/>). SNEP does not address the issue of cell- or tissue-specific gene expression. Additionally, while SNEP is limited to the MHC-binding step of antigen presentation, SiPep includes proteasomal processing. Another advantage of SiPep is the inclusion of SNP frequency data. Our own unpublished investigation of SNP frequencies from dbSNP entries resulted in the following: of an 100 patients typed for nonvalidated SNPs, no SNP could be confirmed; for SNPs that were validated with frequencies from the HapMap project, the majority were confirmed. This strongly underscores the importance of such frequency data. The SNEP system however relies on SWISS-PROT, and not on dbSNP, so the polymorphism data it used could be of a higher quality than nonvalidated dbSNP data, in particular for the VARIANT entries that are validated.

Immunodominance – the next frontier

Despite the great advances in the prediction algorithms mentioned above, the understanding of immunodominance is still a distant, elusive goal. The ability to predict TAP binding is an important milestone because peptide presentation has been shown to vary greatly dependent on TAP affinities (59). There are of course additional factors that influence the peptide landscape found on the cell surface, many of which are probably still unknown. For example, before proteasomal processing, the expression levels and turnover rates of proteins could influence the final concentrations in which they are ultimately presented on the cell surface. The utilization of existing protein turnover prediction programs should be considered (60). Furthermore, it has been shown that the cleavage specificities vary between the constitutive proteasome and the immunoproteasome (28). Clearly, the induction of immunoproteasome expression by cytokines could influence immunodominance. *In vitro* assays for proteasomal digestion and human leukocyte antigen binding have shown poor correlation to the respective *in silico* predictions from the late 1990s (61). This underscores

a need for further assays to evaluate the latest generation of prediction algorithms. To make things more complicated, there is evidence that the dominant peptides presented in a given MHC protein change depending on which additional MHC proteins an individual carries (62).

A path that could take us closer to understanding immunodominance is *in silico* modeling of the immune system. Biological processes, including the immune system, can be modeled using differential equations (63) or agent-based models (64). Differential equations can be used to mathematically describe the concentrations, number or levels of biological entities with respect to each other and with respect to time. While such models are very elegant, they are quite difficult to implement correctly and are very inflexible. For these reasons, there is a tendency toward agent-based systems of modeling the immune system. Here, individual entities are represented in the computer, given coordinates in space, and behaviors are defined, which describe how the entities interface with the immediate surrounding environment. Each time step is then calculated iteratively, and the progress of the system can be observed. Currently, such immune system simulations have found more applications outside of the field of immunology than inside (64). This is the innovative field of artificial immune systems, in which immune system-inspired computer algorithms are applied to 'real-world' engineering applications. This field is based on a dialogue between immunologists and computer scientists, and although the computer scientists are benefiting more than the immunologists from this field at the moment, there is good hope that further advances in immune system simulations will be useful in generating scientific knowledge.

Concluding remarks

The immunoinformatic community has made an important contribution to the effort to develop T-cell-based cancer therapies by providing comprehensive antigen presentation systems. Breakthroughs in the biological model of antigen presentation will have to be met with further experimental data-driven bioinformatics. The hurdle of reliable class II peptide binding prediction is a high priority, considering its significance in the immune system. The quality of mHag prediction systems has the potential to improve as more and more of the entries in the underlying databases become validated. Whether immune system modeling can take the quantum leap to predict immunodominance is unknown. Perhaps, the vision of agent-based representations of peptide sets, competing for access to the antigen presentation machinery, will become a reality. This, together with the extension of the model to include T cell receptor (TCR) interaction and signaling downstream of the TCR, could provide useful insights into this complicated biological process.

References

1. Yee C. Adoptive T cell therapy: addressing challenges in cancer immunotherapy. *J Transl Med* 2005; **3**: 17.
2. Knutson KL, Wagner W, Disis ML. Adoptive T cell therapy of solid cancers. *Cancer Immunol Immunother* 2006; **55**: 96–103.
3. Rosenberg SA. Progress in human tumour immunology and immunotherapy. *Nature* 2001; **411**: 380–4.
4. Ribas A, Butterfield LH, Glaspy JA, Economou JS. Current developments in cancer vaccines and cellular immunotherapy. *J Clin Oncol* 2003; **21**: 2415–32.
5. Maus MV, Thomas AK, Leonard DG et al. Ex vivo expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. *Nat Biotechnol* 2002; **20**: 143–8.
6. Oelke M, Maus MV, Didiano D, June CH, Mackensen A, Schneck JP. Ex vivo induction and expansion of antigen-specific cytotoxic T cells by HLA-Ig-coated artificial antigen-presenting cells. *Nat Med* 2003; **9**: 619–24.
7. Yee C, Gilbert MJ, Riddell SR et al. Isolation of tyrosinase-specific CD8+ and CD4+ T cell clones from the peripheral blood of melanoma patients following in vitro stimulation with recombinant vaccinia virus. *J Immunol* 1996; **157**: 4079–86.
8. Yee C, Thompson JA, Byrd D et al. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. *Proc Natl Acad Sci U S A* 2002; **99**: 16168–73.
9. Melief CJ, Toes RE, Medema JP, van der Burg SH, Ossendorp F, Offringa R. Strategies for immunotherapy of cancer. *Adv Immunol* 2000; **75**: 235–82.
10. Hsu FJ, Benike C, Fagnoni F et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat Med* 1996; **2**: 52–8.
11. Nestle FO, Aljagic S, Gilliet M et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998; **4**: 328–32.
12. Marchand M, van Baren N, Weynants P et al. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. *Int J Cancer* 1999; **80**: 219–30.
13. Thurner B, Haendle I, Roder C et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med* 1999; **190**: 1669–78.
14. Kolb HJ, Holler E. Adoptive immunotherapy with donor lymphocyte transfusions. *Curr Opin Oncol* 1997; **9**: 139–45.
15. Offringa R, van der Burg SH, Ossendorp F, Toes RE, Melief CJ. Design and evaluation of antigen-specific vaccination strategies against cancer. *Curr Opin Immunol* 2000; **12**: 576–82.
16. Rosenberg SA. A new era for cancer immunotherapy based on the genes that encode cancer antigens. *Immunity* 1999; **10**: 281–7.
17. Brusic V, Petrovsky N. Immunoinformatics – the new kid in town. *Novartis Found Symp* 2003; **254**: 3–13; discussion 13–22, 98–101, 250–2.

18. Tenzer S, Peters B, Bulik S et al. Modeling the MHC class I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. *Cell Mol Life Sci* 2005; **62**: 1025–37.
19. Doytchinova IA, Guan P, Flower DR. EpiJen: a server for multistep T cell epitope prediction. *BMC Bioinformatics* 2006; **7**: 131.
20. Larsen MV, Lundegaard C, Lamberth K et al. An integrative approach to CTL epitope prediction: a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. *Eur J Immunol* 2005; **35**: 2295–303.
21. Van den Eynde BJ, van der Bruggen P. T cell defined tumor antigens. *Curr Opin Immunol* 1997; **9**: 684–93.
22. Del Val M, Schlicht HJ, Ruppert T, Reddehase MJ, Koszinowski UH. Efficient processing of an antigenic sequence for presentation by MHC class I molecules depends on its neighboring residues in the protein. *Cell* 1991; **66**: 1145–53.
23. Hambach L, Goulmy E. Immunotherapy of cancer through targeting of minor histocompatibility antigens. *Curr Opin Immunol* 2005; **17**: 202–10.
24. Barrett AJ, van Rhee F. Graft-versus-leukaemia. *Baillieres Clin Haematol* 1997; **10**: 337–55.
25. Halling-Brown M, Quartey-Papafio R, Travers PJ, Moss DS. SiPep: a system for the prediction of tissue-specific minor histocompatibility antigens. *Int J Immunogenet* 2006; **33**: 289–95.
26. Schuler MM, Donnes P, Nastke MD, Kohlbacher O, Rammensee HG, Stevanovic S. SNEP: sNP-derived epitope prediction program for minor H antigens. *Immunogenetics* 2005; **57**: 816–20.
27. Pamer E, Cresswell P. Mechanisms of MHC class I –restricted antigen processing. *Annu Rev Immunol* 1998; **16**: 323–58.
28. Emmerich NP, Nussbaum AK, Stevanovic S et al. The human 26 S and 20 S proteasomes generate overlapping but different sets of peptide fragments from a model protein substrate. *J Biol Chem* 2000; **275**: 21140–8.
29. Toes RE, Nussbaum AK, Degermann S et al. Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. *J Exp Med* 2001; **194**: 1–12.
30. Tenzer S, Stoltze L, Schonfisch B et al. Quantitative analysis of prion-protein degradation by constitutive and immuno-20S proteasomes indicates differences correlated with disease susceptibility. *J Immunol* 2004; **172**: 1083–91.
31. Craiu A, Akopian T, Goldberg A, Rock KL. Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide. *Proc Natl Acad Sci U S A* 1997; **94**: 10850–5.
32. Serwold T, Gaw S, Shastri N. ER aminopeptidases generate a unique pool of peptides for MHC class I molecules. *Nat Immunol* 2001; **2**: 644–51.
33. Barnea E, Beer I, Patoka R et al. Analysis of endogenous peptides bound by soluble MHC class I molecules: a novel approach for identifying tumor-specific antigens. *Eur J Immunol* 2002; **32**: 213–22.
34. Abele R, Tampe R. The ABCs of immunology: structure and function of TAP, the transporter associated with antigen processing. *Physiology (Bethesda)* 2004; **19**: 216–24.
35. van Endert PM, Riganelli D, Greco G et al. The peptide-binding motif for the human transporter associated with antigen processing. *J Exp Med* 1995; **182**: 1883–95.
36. Uebel S, Kraas W, Kienle S, Wiesmuller KH, Jung G, Tampe R. Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries. *Proc Natl Acad Sci U S A* 1997; **94**: 8976–81.
37. Scholz C, Tampe R. The intracellular antigen transport machinery TAP in adaptive immunity and virus escape mechanisms. *J Bioenerg Biomembr* 2005; **37**: 509–15.
38. Gubler B, Daniel S, Armandola EA, Hammer J, Caillat-Zucman S, van Endert PM. Substrate selection by transporters associated with antigen processing occurs during peptide binding to TAP. *Mol Immunol* 1998; **35**: 427–33.
39. Peters B, Bulik S, Tampe R, Van Endert PM, Holzthutter HG. Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. *J Immunol* 2003; **171**: 1741–9.
40. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annu Rev Immunol* 1999; **17**: 51–88.
41. Parker KC, Bednarek MA, Coligan JE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. *J Immunol* 1994; **152**: 163–75.
42. Rognan. Molecular dynamics simulation of MHC-peptide complexes as a tool for predicting potential T cell epitopes. *Biochemistry* 1994; **33**: 11476–86.
43. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 1999; **50**: 213–9.
44. Rognan D, Lauemoller SL, Holm A, Buus S, Tschinke V. Predicting binding affinities of protein ligands from three-dimensional models: application to peptide binding to class I major histocompatibility proteins. *J Med Chem* 1999; **42**: 4650–8.
45. Buus S, Lauemoller SL, Wornig P et al. Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach. *Tissue Antigens* 2003; **62**: 378–84.
46. Nielsen M, Lundegaard C, Wornig P et al. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. *Protein Sci* 2003; **12**: 1007–17.
47. Reche PA, Glutting JP, Zhang H, Reinherz EL. Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. *Immunogenetics* 2004; **56**: 405–19.
48. DeLuca DS, Khattab B, Blasczyk R. A modular concept of HLA for comprehensive peptide binding prediction. *Immunogenetics* 2007; **59**: 25–35.
49. Disis ML, Cheever MA. HER-2/neu oncogenic protein: issues in vaccine development. *Crit Rev Immunol* 1998; **18**: 37–45.
50. Lu J, Celis E. Use of two predictive algorithms of the world wide web for the identification of tumor-reactive T-cell epitopes. *Cancer Res* 2000; **60**: 5223–7.

51. Rongcun Y, Salazar-Onfray F, Charo J et al. Identification of new HER2/neu-derived peptide epitopes that can elicit specific CTL against autologous and allogeneic carcinomas and melanomas. *J Immunol* 1999; **163**: 1037–44.
52. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. *J Exp Med* 1998; **188**: 2357–68.
53. Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J Exp Med* 1998; **188**: 2199–204.
54. Brusic V, Rudy G, Honeyman G, Hammer J, Harrison L. Prediction of MHC class II-binding peptides using an evolutionary algorithm and artificial neural network. *Bioinformatics* 1998; **14**: 121–30.
55. Marshall KW, Wilson KJ, Liang J, Woods A, Zaller D, Rothbard JB. Prediction of peptide affinity to HLA DRB1*0401. *J Immunol* 1995; **154**: 5927–33.
56. Yu K, Petrovsky N, Schonbach C, Koh JY, Brusic V. Methods for prediction of peptide binding to MHC molecules: a comparative study. *Mol Med* 2002; **8**: 137–48.
57. Goulmy E, Schipper R, Pool J et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med* 1996; **334**: 281–5.
58. Murata M, Warren EH, Riddell SR. A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J Exp Med* 2003; **197**: 1279–89.
59. Fruci D, Lauvau G, Saveanu L et al. Quantifying recruitment of cytosolic peptides for HLA class I presentation: impact of TAP transport. *J Immunol* 2003; **170**: 2977–84.
60. Bachmair A, Finley D, Varshavsky A. In vivo half-life of a protein is a function of its amino-terminal residue. *Science* 1986; **234**: 179–86.
61. Kessler JH, Beekman NJ, Bres-Vloemans SA et al. Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J Exp Med* 2001; **193**: 73–88.
62. Paston SJ, Dodi IA, Madrigal JA. Progress made towards the development of a CMV peptide vaccine. *Hum Immunol* 2004; **65**: 544–9.
63. Chan CC, Stark J, George AJ. Analysis of cytokine dynamics in corneal allograft rejection. *Proc Biol Sci* 1999; **266**: 2217–23.
64. Forrest S, Beauchemin C. Computer immunology. *Immunol Rev* 2007; **216**: 176–97.
65. Nielsen M, Lundegaard C, Lund O, Kesmir C. The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage. *Immunogenetics* 2005; **57**: 33–41.
66. Peters B, Sidney J, Bourne P et al. The immune epitope database and analysis resource: from vision to blueprint. *PLoS Biol* 2005; **3**: e91.
67. Doytchinova IA, Flower DR. Class I T-cell epitope prediction: improvements using a combination of proteasome cleavage, TAP affinity, and MHC binding. *Mol Immunol* 2006; **43**: 2037–44.
68. Doytchinova I, Hemsley S, Flower DR. Transporter associated with antigen processing preselection of peptides binding to the MHC: a bioinformatic evaluation. *J Immunol* 2004; **173**: 6813–9.
69. Guan P, Hattotuwigama CK, Doytchinova IA, Flower DR. MHCpred 2.0: an updated quantitative T-cell epitope prediction server. *Appl Bioinformatics* 2006; **5**: 55–61.
70. Bhasin M, Raghava GP. A hybrid approach for predicting promiscuous MHC class I restricted T cell epitopes. *J Biosci* 2007; **32**: 31–42.
71. Madden DR, Garboczi DN, Wiley DC. The antigenic identity of peptide-MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2. *Cell* 1993; **75**: 693–708.
72. Groll M, Ditzel L, Lowe J et al. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* 1997; **386**: 463–71.
73. Gaudet R, Wiley DC. Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing. *EMBO J* 2001; **20**: 4964–72.