ArrayPitope: Automated Analysis of Amino Acid Substitutions for Peptide Microarray-Based Antibody Epitope Mapping

Identification of epitopes targeted by antibodies (B cell epitopes) is of critical importance for the development of many diagnostic and therapeutic tools. For clinical usage, such epitopes must be extensively characterized in order to validate specificity and to document potential cross-reactivity. B cell epitopes are typically classified as either linear epitopes, i.e. short consecutive segments from the protein sequence or conformational epitopes adapted through native protein folding. Recent advances in high-density peptide microarrays enable high-throughput, high-resolution identification and characterization of linear B cell epitopes. Using exhaustive amino acid substitution analysis of peptides originating from target antigens, these microarrays can be used to address the specificity of polyclonal antibodies raised against such antigens containing hundreds of epitopes. However, the interpretation of the data provided in such large-scale screenings is far from trivial and in most cases it requires advanced computational and statistical skills. Here, we present an online application for automated identification of linear B cell epitopes, allowing the non-expert user to analyse peptide microarray data. The application takes as input quantitative peptide data of fully or partially substituted overlapping peptides from a given antigen sequence and identifies epitope residues (residues that are significantly affected by substitutions) and visualize the selectivity towards each residue by sequence logo plots. Demonstrating utility, the application was used to identify and address the antibody specificity of 18 linear epitope regions in Human Serum Albumin (HSA), using peptide microarray data consisting of fully substituted peptides spanning the entire sequence of HSA and incubated with polyclonal rabbit anti-HSA (and mouse anti-rabbit-Cy3). The application is made available at: www.cbs.dtu.dk/services/ArrayPitope.
Characterization of binding specificities of bovine leucocyte class I molecules: impacts for rational epitope discovery.

The binding of peptides to classical major histocompatibility complex (MHC) class I proteins is the single most selective step in antigen presentation. However, the peptide-binding specificity of cattle MHC (bovine leucocyte antigen, BoLA) class I (BoLA-I) molecules remains poorly characterized. Here, we demonstrate how a combination of high-throughput assays using positional scanning combinatorial peptide libraries, peptide dissociation, and peptide-binding affinity binding measurements can be combined with bioinformatics to effectively characterize the functionality of BoLA-I molecules. Using this strategy, we characterized eight BoLA-I molecules, and found the peptide specificity to resemble that of human MHC-I molecules with primary anchors most often at P2 and P9, and occasional auxiliary P1/P3/P5/P6 anchors. We analyzed nine reported CTL epitopes from *Theileria parva*, and in eight cases, stable and high affinity binding was confirmed. A set of peptides were tested for binding affinity to the eight BoLA proteins and used to refine the predictors of peptide-MHC binding NetMHC and NetMHCpan. The inclusion of BoLA-specific peptide-binding data led to a significant improvement in prediction accuracy for reported *T. parva* CTL epitopes. For reported CTL epitopes with weak or no predicted binding, these refined prediction methods suggested presence of nested minimal epitopes with high-predicted binding affinity. The enhanced affinity of the alternative peptides was in all cases confirmed experimentally. This study demonstrates how biochemical high-throughput assays combined with immunoinformatics can be used to characterize the peptide-binding motifs of BoLA-I molecules, boosting performance of MHC peptide-binding prediction methods, and empowering rational epitope discovery in cattle.

General information
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Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, Immunological Bioinformatics, International Livestock Research Institute, University of Copenhagen, United States Department of Agriculture, University of Vermont
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Uncovering the Peptide-Binding Specificities of HLA-C: A General Strategy To Determine the Specificity of Any MHC Class I Molecule

MHC class I molecules (HLA-I in humans) present peptides derived from endogenous proteins to CTLs. Whereas the peptide-binding specificities of HLA-A and -B molecules have been studied extensively, little is known about HLA-C specificities. Combining a positional scanning combinatorial peptide library approach with a peptide-HLA-I dissociation assay, in this study we present a general strategy to determine the peptide-binding specificity of any MHC class I molecule. We applied this novel strategy to 17 of the most common HLA-C molecules, and for 16 of these we successfully generated matrices representing their peptide-binding motifs. The motifs prominently shared a conserved C-terminal primary anchor with hydrophobic amino acid residues, as well as one or more diverse primary and auxiliary anchors at P1, P2, P3, and/or P7. Matrices were used to generate a large panel of HLA-C-specific peptide-binding data and update our pan-specific NetMHCpan predictor, whose predictive performance was considerably improved with respect to peptide binding to HLA-C. The updated predictor was used to assess the specificities of HLA-C molecules, which were found to cover a more limited sequence space than HLA-A and -B molecules. Assessing the functional significance of these new tools, HLA-C*07:01 transgenic mice were immunized with stable HLA-C*07:01 binders; six of six tested stable peptide binders were immunogenic. Finally, we generated HLA-C tetramers and labeled human CD8(+) T cells and NK cells. These new resources should support future research on the biology of HLA-C molecules. The data are deposited at the Immune Epitope Database, and the updated NetMHCpan predictor is available at the Center for Biological Sequence Analysis and the Immune Epitope Database.
Comparison of Vaccine-Induced Effector CD8 T Cell Responses Directed against Self- and Non-Self-Tumor Antigens: Implications for Cancer Immunotherapy

It is generally accepted that CD8 T cells play a major role in tumor control, yet vaccination aimed at eliciting potent CD8 T cell responses are rarely efficient in clinical trials. To try and understand why this is so, we have generated potent adenoviral vectors encoding the endogenous tumor Ags (TA) tyrosinase-related protein-2 (TRP-2) and glycoprotein 100 (GP100) tethered to the invariant chain (Ii). Using these vectors, we sought to characterize the self-TA-specific CD8 T cell response and compare it to that induced against non-self-Ags expressed from a similar vector platform. Prophylactic vaccination with adenoviral vectors expressing either TRP-2 (Ad-Ii-TRP-2) or GP100 (Ad-Ii-GP100) had little or no effect on the growth of s.c. B16 melanomas, and only Ad-Ii-TRP-2 was able to induce a marginal reduction of B16 lung metastasis. In contrast, vaccination with a similar vector construct expressing a foreign (viral) TA induced efficient tumor control. Analyzing the self-TA-specific CD8 T cells, we observed that these could be activated to produce IFN-gamma and TNF-alpha. In addition, surface expression of phenotypic markers and inhibitory receptors, as well as in vivo cytotoxicity and degranulation capacity matched that of non-self-Ag-specific CD8 T cells. However, the CD8 T cells specific for self-TAs had a lower functional avidity, and this impacted on their in vivo performance. On the basis of these results and a low expression of the targeted TA epitopes on the tumor cells, we suggest that low avidity of the self-TA-specific CD8 T cells may represent a major obstacle for efficient immunotherapy of cancer.
Discovering naturally processed antigenic determinants that confer protective T cell immunity

CD8(+) T cells (TCD8) confer protective immunity against many infectious diseases, suggesting that microbial TCD8 determinants are promising vaccine targets. Nevertheless, current T cell antigen identification approaches do not discern which epitopes drive protective immunity during active infection information that is critical for the rational design of TCD8-targeted vaccines. We employed a proteomics-based approach for large-scale discovery of naturally processed determinants derived from a complex pathogen, vaccinia virus (VACV), that are presented by the most frequent representatives of four major HLA class I supertypes. Immunologic characterization revealed that many previously unidentified VACV determinants were recognized by smallpox-vaccinated human peripheral blood cells in a variegated manner. Many such determinants were recognized by HLA class I-transgenic mouse immune TCD8 too and elicited protective TCD8 immunity against lethal intranasal VACV infection. Notably, efficient processing and stable presentation of immune determinants as well as the availability of naive TCD8 precursors were sufficient to drive a multifunctional, protective TCD8 response. Our approach uses fundamental insights into T cell epitope processing and presentation to define targets of protective TCD8 immunity within human pathogens that have complex proteomes, suggesting that this approach has general applicability in vaccine sciences.

General Information
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Peptide Binding to HLA Class I Molecules: Homogenous, High-Throughput Screening, and Affinity Assays

The Human MHC Project aims at large-scale description of peptide-HLA binding to a wide range of HLA molecules covering all populations of the world and the accompanying generation of bioinformatics tools capable of predicting binding of any given peptide to any given HLA molecule. Here, the authors present a homogenous, proximity-based assay for detection of peptide binding to HLA class I molecules. It uses a conformation-dependent anti-HLA class I antibody, W6/32, as one tag and a biotinylated recombinant HLA class I molecule as the other tag, and a proximity-based signal is generated through the luminescent oxygen channeling immunoassay technology (abbreviated LOCI and commercialized as AlphaScreen (TM)). Compared with an enzyme-linked immunosorbent assay-based peptide-HLA class I binding assay, the LOCI assay yields virtually identical affinity measurements, although having a broader dynamic range, better signal-to-background ratios, and a higher capacity. They also describe an efficient approach to screen peptides for binding to HLA molecules. For the occasional user, this will serve as a robust, simple peptide-HLA binding assay. For the more dedicated user, it can easily be performed in a high-throughput screening mode using standard liquid handling robotics and 384-well plates. We have successfully applied this assay to more than 60 different HLA molecules, leading to more than 2 million measurements. (Journal of Biomolecular Screening 2009: 173-180)
We present a new release of the immune epitope database analysis resource (IEDB-AR, http://tools.immuneepitope.org), a repository of web-based tools for the prediction and analysis of immune epitopes. New functionalities have been added to most of the previously implemented tools, and a total of eight new tools were added, including two B-cell epitope prediction tools, four T-cell epitope prediction tools and two analysis tools.
A novel system for continuous protein refolding and on-line capture by expanded bed adsorption

A novel two-step protein refolding strategy has been developed, where continuous renaturation-by-dilution is followed by direct capture on an expanded bed adsorption (EBA) column. The performance of the overall process was tested on a N-terminally tagged version of human beta(2)-microglobulin (HAT-h beta(2)m) both at analytical, small, and preparative scale. In a single scalable operation, extracted and denatured inclusion body proteins from Escherichia coli were continuously diluted into refolding buffer, using a short pipe reactor, allowing for a defined retention and refolding time, and then fed directly to an EBA column, where the protein was captured, washed, and finally eluted as soluble folded protein. Not only was the eluted protein in a correctly folded state, the purity of the HAT-h beta(2)m was increased from 34% to 94%, and the product was concentrated sevenfold. The yield of the overall process was 45%, and the product loss was primarily a consequence of the refolding reaction rather than the EBA step. Full biological activity of HAT-h beta(2)m was demonstrated after removal of the HAT-tag. In contrast to batch refolding, a continuous refolding strategy allows the conditions to be controlled and maintained throughout the process, irrespective of the batch size; i.e., it is readily scalable. Furthermore, the procedure is fast and tolerant toward aggregate formation, a common complication of in vitro protein refolding. In conclusion, this system represents a novel approach to small and preparative scale protein refolding, which should be applicable to many other proteins.

General information
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Main Research Area: Technical/natural sciences
Loudness of complex sounds as a function of the standard stimulus and the number of components

The purpose of this study was twofold: to determine if the measured loudness level of a signal depends on the standard stimulus used and to measure loudness as a function of the number of components in a wide-band signal. The stimuli were a pure tone, tone complexes with frequency separations of 231 and 1592 Hz, and noise bands with widths of 220 and 1592 Hz. The center frequency was 1 kHz and the loudness level was approximately 65 phons. Loudness matches between all combinations of stimuli showed that the measured loudness of the sounds did not depend on the standard stimulus used and the measured loudness level of a wide-band sound increased as a function of the number of components. Individual observers were consistent in their loudness estimations; the greatest source of variability was among subjects. Additional measurements indicated that the rate at which loudness increased beyond the critical band appeared to be greater for noise bands than for two-tone complexes.
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Web of Science (2003): Indexed yes
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In Vitro Protein Refolding: Development of a Method for Continuous on-line refolding

Department of Systems Biology
Period: 01/08/2001 → 05/12/2005
Number of participants: 7
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