Immunopeptidome Analysis of Mouse MHC Class I

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KEY FINDING

Combinatorial approaches are optimal for pull-down of H-2K^b and H-2D^b complexes for antigen identification.

ABSTRACT

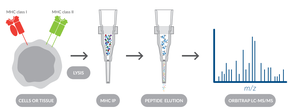
MHC class I is required for antigen presentation to CD8 T cells. These cell surface proteins are heterodimers consisting of a variable alpha chain associated with β2 microglobulin and are expressed on nucleated cells at varying levels. The antigen specificity of the interaction between MHC, peptide, and T cell receptor can be exploited in the development of personalized cancer vaccines and other immunotherapies against cancer. MHC sequences are species-specific and there are many different haplotypes of MHC class I. For this reason, the antibodies used for immunoprecipitation of MHC complexes are a critical component of the workflow for identification of antigens presented by MHC.

Subclasses of mouse MHC class I are divided into classical (H-2D, H-2K, and H-2L) and non-classical (H-2Q, H-2M, and H-2T), and haplotypes of each are known for homozygous inbred laboratory strains of mice. One common strain is C57BL/6, which expresses H-2D^b and H-2K^b classical MHC molecules. Antibody clone M1/42, which reacts with H-2 expressed on nucleated cells from mice of a, b, d, j, k, s, and u haplotypes, is commonly referred to as a pan-MHC class I antibody. Antibody clones Y3 and HB27 recognize H-2K^b and H-2D^b haplotypes, respectively.

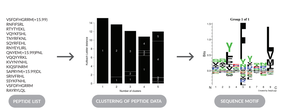
In this study, we aimed to compare different antibodies to mouse MHC class I complexes for their efficiency of associated peptide recovery. Input was murine EL-4 cells, which are T lymphoblast cells derived from a C57BL/6 background. We performed immunoprecipitation (IP) with different antibodies either singly or sequentially to enrich MHC class I, followed by gentle elution of associated peptides, and finally peptide analysis by mass spectrometry. Each IP workflow yielded a different (but overlapping) cohort of peptides, suggesting optimal methodologies for analyses of immunopeptidomes from common laboratory mouse strains.

WORKFLOW FOR IMMUNOPEPTIDOME PROFILING

PEPTIDE ISOLATION AND LC-MS/MS ANALYSIS



DATA OUTPUT

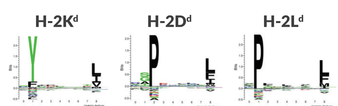


- Cells/tissue are lysed, and the resulting preparations are subjected to immunoaffinity capture. Following multiple washes, peptides are eluted from the MHC cleft with acid.
- Purified peptides are analyzed by LC-MS/MS using nano-scale chromatography combined with an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer using collision-induced dissociation (CID) or electron-transfer/higher-energy collision dissociation (EThcD) fragmentation.
- PEAKS is used for peptide identification and quantitation. Processed data can be further interrogated using bioinformatics tools. For example, NetMHC or GibbsCluster allow the elucidation of binding strengths and motifs.

PROBLEM

THE "PAN" MOUSE MHC CLASS I ANTIBODY M1/42 DOES NOT PRECIPITATE THE H-2D^b HAPLOTYPE

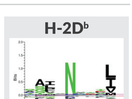
Balb/c Haplotype
H-2K^d
H-2D^d
H-2L^d



Experimentally Identified Motifs

C57BL/6 Haplotype
H-2K^b
H-2D^b

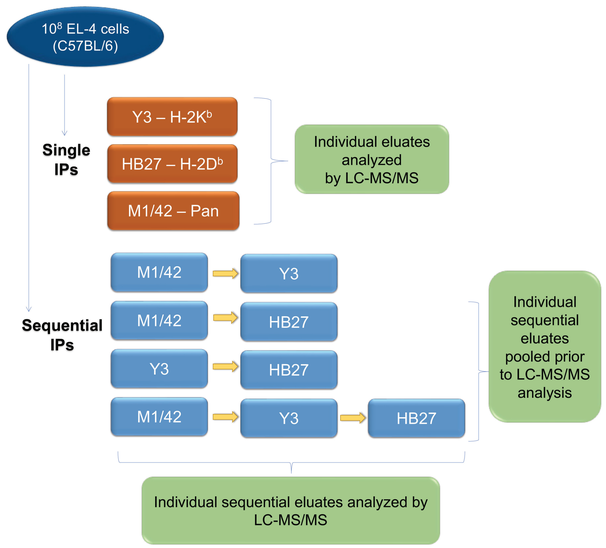
Experimentally Identified Motifs



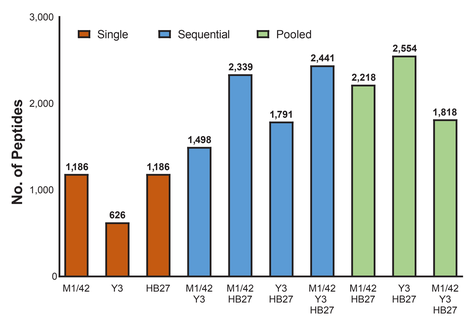
NOT FOUND

M1/42 IP results in MHC class I-associated peptides from all haplotypes expressed in the Balb/c strain but does not yield peptides associated with the H-2D^b haplotype expressed in the C57BL/6 strain.

EXPERIMENTAL APPROACH

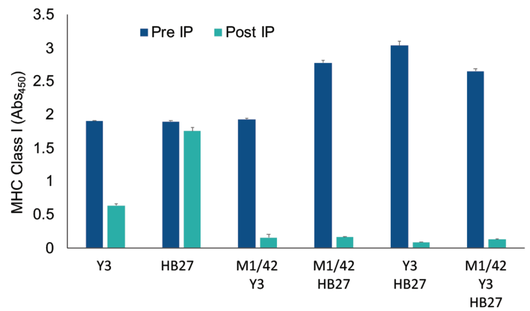


RESULTS



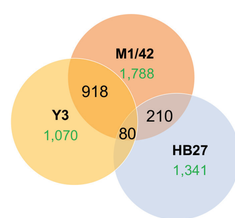
TOTAL PEPTIDE NUMBERS

Peptide counts from individual initial samples are shown according to the analysis method. Sequential indicates peptides from each sample were analyzed individually and peptide lists were combined. Pooled indicates eluates were pooled prior to mass spec.



QUALITY CONTROL

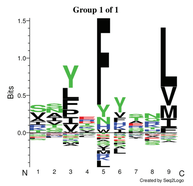
ELISA was used to compare levels of MHC class I molecules in cell lysates before and after IP with single and sequential approaches. Clone HB-51, which recognizes H-2K^b and H-2D^b, was used as the capture antibody, and clone M1/42 was used as the detection antibody. Post IP levels were lower when using clones that recognize H-2K^b, Y3, and M1/42, suggesting effective precipitation.



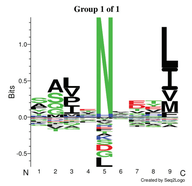
PEPTIDE OVERLAP

Venn diagram showing the unique and common peptides from each individual IP. Numbers in green are the total number of peptides. Numbers in black are the number of overlapping peptides.

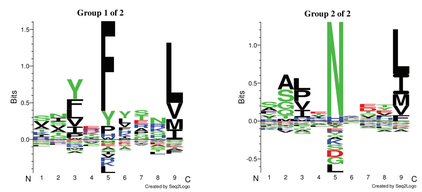
Y3 Alone



HB27 After Y3



Y3 and HB27 Eluates Pooled



MOTIF ANALYSIS

An unbiased motif analysis was performed with GibbsCluster to identify motifs in sequential or pooled samples. MHC class I peptide sequences clustered into two motifs, which resemble consensus. Similar results are obtained with sequential and pooled IP approaches.

SUMMARY

- Enrichment of peptides from different mouse MHC haplotypes can be tailored using clones Y3, HB27, and M1/42 in single or sequential IP approaches.
- Similar results are obtained with sequential and pooled MS/MS analysis – if haplotype-specific peptide sequences are not necessary, eluates can be pooled without compromising data.
- Strain differences in MHC class I haplotype expression require careful selection of antibodies for IP.

RESOURCES

1. GibbsCluster: https://services.healthtech.dtu.dk/service.php?GibbsCluster-2.0
2. MotifViewer: https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1

Learn more about our Immunopeptidome Profiling services and download this poster by scanning the code