

Identification and motif determination of naturally processed peptides from the murine Type I diabetes associated MHC class I allele H-2K^d

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Type 1 diabetes (T1D) in humans and in the non-obese diabetic (NOD) mouse is a result of a T cell-mediated autoimmune process directed against pancreatic islet β -cells. Most of the diabetogenic CD8⁺ T cells in NOD mice are restricted to the class I MHC allele, H-2K^d. The majority of literature on H-2K^d bound peptides has been elucidated by peptide/H-2K^d complex's ability to stimulate T cells *in vitro*. Past work to identify the sequence of self-peptides presented on the cell surface by other alleles has offered insight into motif determination, defined allele differences, deciphered the determinant capture hypothesis, and offered potential to provide putative auto-immune peptides. We provide here a spectrum of naturally processed peptides presented by H-2K^d.

The sources of H-2K^d-bound peptides were two different cell lines: Nit-1, an insulinoma, and NOD.C3, an APC line generated from NOD splenocytes. Both cell lines were grown in large numbers (5-10 $\times 10^9$ cells), and H-2K^d-peptide complexes were affinity purified using a SF1.1.1 monoclonal antibody conjugated to Sepharose beads. Peptides were acid-eluted and separated by off-line strong-cation exchange chromatography. Ten eluted fractions were then analyzed by on-line capillary HPLC nanoESI-MS and MS/MS using an LCQ-Deca quadrupole ion-trap mass spectrometer. Product ion spectra were submitted to database searching using SEQUEST. For binding analyses, synthetic peptides corresponding to the naturally processed ones were tested for cell-surface binding to H-2K^d by using the T-2K^d cell line, which has an intrinsic defect in peptide loading onto the class I molecules and therefore very few stable H-2K^d class I molecules on the cell surface. Surface H-2K^d is stabilized and up-regulated by the binding of a high affinity peptide. Synthetic peptides corresponding to identified naturally processed sequences (or mutated sequences) were mixed with T-2K^d cells at varying concentrations. The cells were then stained with a biotinylated anti-H-2K^d (SF1.1.1) monoclonal primary antibody and binding of peptide was determined with Phyco-erythrin (PE)-labeled streptavidin and reported by mean fluorescence intensity as measured by FACS.

We identified a total of 93 peptides: 58 peptides are from Nit-1 whereas 45 peptides are from NOD.C3. There are 10 peptides that were common to both cell lines. Almost all peptides are derived from proteins present in the intracellular compartments, which is characteristic of class I MHC-bound epitopes. Approximately 80% of the peptides have a motif defined by a Tyr or a Phe at P2 and a small hydrophobic residue, usually Ile/Leu or Val, at P9. Mutational binding analysis confirmed this motif, as changing the P2 Tyr or Phe to an Ala abolished binding (Figure 1).

The majority of peptides are 8-10 amino acids in length; however, we saw several longer peptides (~20%) up to 18 amino acids (Figure 2). Interestingly, the longer peptides (>10mers) also bind to H-2K^d, and this was affected by both the anchor residues and/or the peptide length (Figure 1). This would indicate that the longer peptides may form a loop structure at the center of the peptide, or the peptide could protrude from either the carboxy- or amino terminal. Both of the models would allow the binding dependent amino acids to maintain interaction with the class I molecule. These results suggest that H-2K^d could bind longer peptides *in vivo* and play an undetermined role in the onset of Type I diabetes.

Future work will focus on: (1) defining the presence or absence of *in vivo* CD8+ T-cells which are restricted to longer peptides (>10 amino acids) presented by H-2K^d in the NOD mouse, (2) correlating X-ray crystal data of H-2K^d with peptides which are 8-10 amino acids in length and those which are longer, and (3) exploring the mechanism by which longer peptides interact with H-2K^d by using H/D exchange and mass spectrometry.

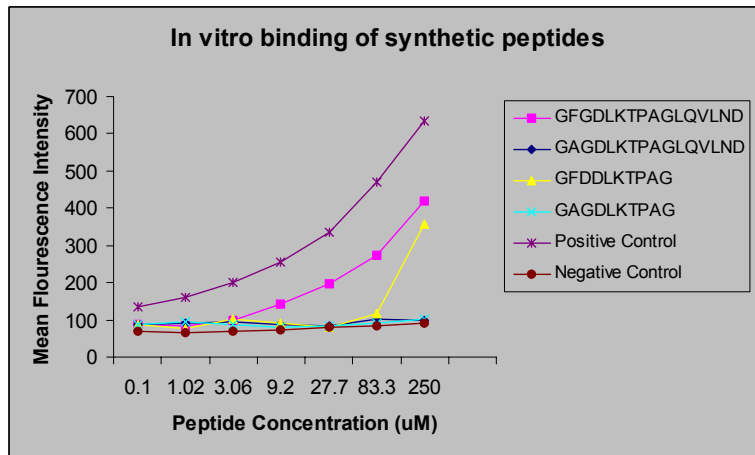


Figure 1. *In vitro* binding of select synthetic peptides. The **identified peptide** binds with the greatest affinity. The **truncated variant** binds, however with weaker affinity indicating that the extension of the carboxy terminus contributes to the interaction with the MHC molecule. Peptides mutated at P2 lose the ability to bind.

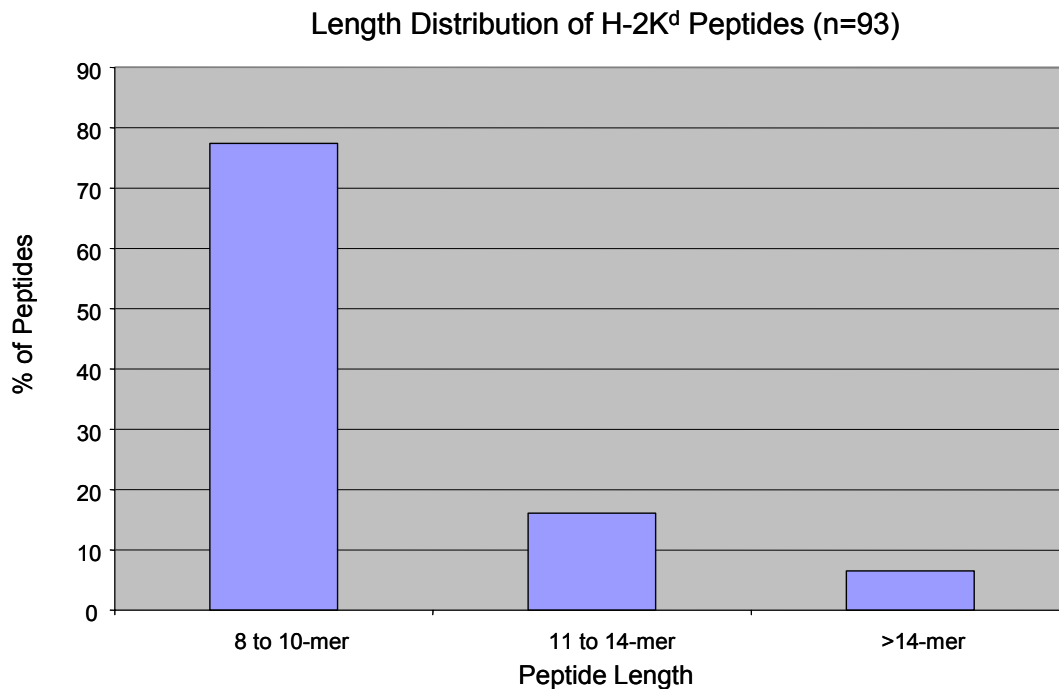


Figure 1. Length Distribution of peptides from H-2K^d identified by LC-MS/MS