AUTOMATED PROCEDURES FOR THE COMPARATIVE ANALYSIS OF HLA CLASS I-BOUND PEPTIDE REPERTOIRES

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HLA class I molecules present to the immune system a wide spectrum of the protein content of cells as a pool of peptides arising mainly from proteasome-mediated protein degradation pathways. The sequences of these peptides have allotype-specific patterns, designated as the binding motif. Some class I allotypes are associated with protection against infection or predisposition to disease. Such is the case of HLA-B27, which is strongly associated with ankylosing spondylitis as well as with slow progression of AIDS. It is assumed that the pathogenetic role of HLA-B27 is peptide-mediated, either through presentation of specific peptides leading to a break of immunological tolerance, or through more general effects on other biological properties of the molecules, such as folding and stability.

The relationships between the peptidomes of closely related class I allotypes can be properly analyzed by systematic comparison of the HLA-bound peptide pools extracted form immunopurified HLA/peptide complexes, by means of chromatographic fractionation and mass spectrometry. A software tool was developed, based on the high probability that species with the same m/z values and retention times in closely matched chromatograms correspond to identical peptides. The algorithm provided a fast and reliable estimation of the overlap between peptide repertoires of related allotypes. By implementing additional functions, the program was also used to interpret data from stable isotope labeling studies aimed at determining the proteasome dependence of MHC class I-bound peptide pools

Sequencing of HLA class I ligands by mass spectrometry poses specific difficulties related to the fact that the search engines generally used in the interpretation of MS/MS spectra and protein identification are optimized for tryptic peptides. Thus we are developing a specific tool for *de novo* interpretation of MS/MS spectra of peptides, independently of their origin, whose basis and power will be discussed.