Proteomics: Quantification of Human Neutrophil Peptides by Mass Spectrometry

This research focuses on the development of mass spectrometry-based approaches for detecting and quantifying biomarkers of human diseases. Human neutrophil peptides (HNPs) are cysteine-rich antimicrobial peptides. The very similar structure of HNPs -1, -2, and -3 renders them exceptionally difficult to study individually in biological samples (Fig. 1). For the first time, we describe a method of individually identifying the HNPs -1, -2, and -3 using stable isotope labeling and matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

I.V. Turko (Div. 831), L. Thompson (Baylor College of Medicine), and F. Murad (U.of Texas)

This study was performed on mucus from tracheal aspirates (TA) from infants with respiratory syncytial virus infection at acute illness and at convalescence. *In vitro*, convalescent, and acute illness TAs were labeled with D_0- and D_3-acrylamides, respectively, and mixed 1:1. TA proteins are separated by one-dimensional gel electrophoresis and then identified by mass spectrometry-based peptide mass fingerprinting. The ratio of signal intensities for the isotopically normal (D_0-labeled) and heavy (D_3-labeled) forms of the N-terminal peptide reveals the relative increase in each peptide with the illness (Fig. 2).

HNPs are important components of innate immunity against viruses, fungi and bacteria. Specifically, *in vitro* studies have demonstrated differences in killing efficiencies among the HNPs against a variety of pathogens. Our study presents a novel method for identifying the individual HNPs -1, -2, and -3 by mass spectrometry and demonstrates how the method can quantify the increases in HNPs -1-3 found in biological samples during illness. If future work proves the hypothesis of increased HNP levels with immune activation to be true, it may be found with repeated exposure to other pathogens as well. Thus, individual quantification of the HNPs-1-3 may point to the peptide affording the greatest protection.

Publication

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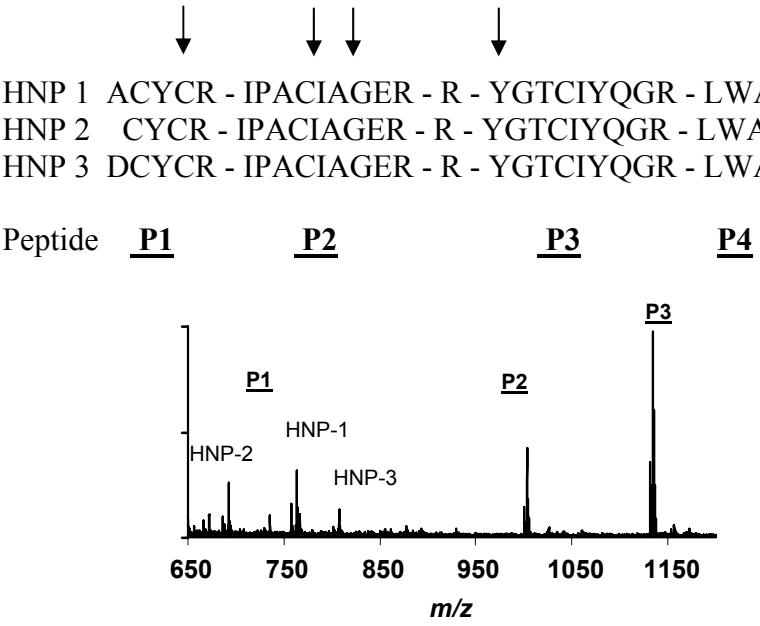

Figure 1: The arrows show the sites of cleavage by trypsin, yielding 4 peptides.

Only P1, the N-terminal peptide, shows variation in structure. A representative MALDI-TOF spectrum shows peaks for P1 of HNPs-1, -2, and 3 as well as P2 and P3 of total HNP-1-3.

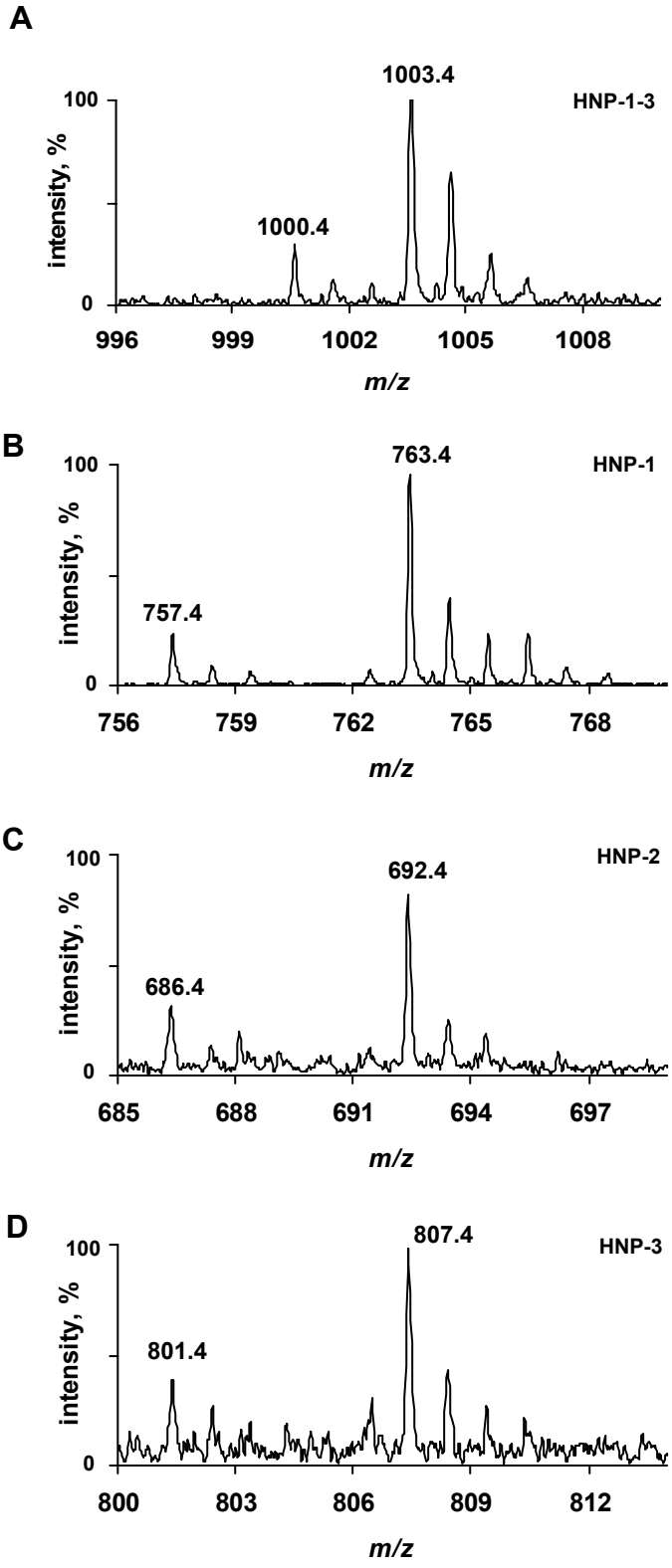

Fig 2. Representative MALDI-TOF spectra used for quantification.

A, P2 of HNP-1-3. B, P1 of HNP-1. C, P1 of HNP-2. D, P1 of HNP-3. D_0-acrylamide labeled peaks at $[M+H]^+$ m/z 1000.4, 757.4, 686.4, and 801.4 represent convalescence. D_3-acrylamide labeled peaks at $[M+H]^+$ m/z 1003.4, 763.4, 692.4, and 807.4 represent acute illness.