DESI Cyclic IMS enables the detection and spatial distribution of proteins in tuberculosis granulomas

Mass Spectrometry

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Tuberculosis, caused by Mycobacterium tuberculosis (Mtb), is the leading cause of death by a single infectious agent and remains a serious global health threat. New therapeutic approaches are vitally needed to minimize the lengthy drug treatment regimens, the emergence of multi-drug resistance and the lung damage caused by the aberrant immune response. The TB granuloma is a dynamic three-dimensional structure that contains a highly complex mix of innate and adaptive immune cells that have diverse phenotypes. Imaging of proteins in TB granulomas has the potential to inform on host-responses that lead to granuloma formation to enable the identification of novel signaling pathways and new therapeutic interventions. DESI cIMS was utilized to determine the spatial distribution of proteins in TB granulomas. Rabbits were infected with Mtb strain HN878 for 16-20 weeks. Upon euthanasia, lungs were resected and cellular, necrotic and cavity containing lesions dissected and snap frozen. For sterilization, TB lesions were gamma irradiated whilst frozen and then transferred to a -80C freezer until use. Lesions were sectioned at 10 µm and transferred to positively charged glass slides for histology and DESI Cyclic IMS mass spectrometry imaging. A Waters SELECT SERIES cIMS equipped with a DESI-XS source was used to collect positive mode data at 75 □m (pixel size). Initial experiments were performed using one pass along the Ion Mobility cell. Several pass (up to 5) experiments were then used to increase the mobility resolution for specific classes of proteins.

Intact protein imaging by mass spectrometry is now possible due to the application of DESI enabling multiply charged ions to be detected directly from tissue sections. The sensitivity of protein imaging is being further enhanced with the application of a novel cyclic ion mobility instrument. Preliminary DESI Cyclic IMS MSI data analysis from TB infected lung granulomas detected a number of doubly charged ions, with one pass along the Ion Mobility cell. Two regions of these species were found between 750 and 800 m/z and between 1100 and 1200 m/z. Ion mobility clearly separated these ions from their corresponding singly charged isobaric ions without any requirement for prior sample preparation. The spatial distribution of the protein ions were predominantly detected in the normal alveolar space and in regions of immune cell infiltration. Several proteins were also detected with higher signal intensity in distinct populations of immune cells surrounding larger caseous granulomas. On-going studies will extend the mass range of analysis and focus on multiple pass experiments in the cyclic ion mobility with the aim to further enhance the number of proteins detected and to improve the ion mobility resolution. Additional experiments are being conduct to annotate the proteins detected.