

Pilot Project Abstracts

Development of advanced proteomic methods to monitor baseline and postimmunization mucosal immunological profiles of the human gastrointestinal tract via stool samples

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The major goal of this project is to develop transformational technology advances in proteomics so that “deep and wide” proteomic measurements can be made directly from human fecal material so that baseline measurements can be obtained to determine the immunological protein components of the gastrointestinal (GI) tract. This will be leveraged in a longitudinal study of individuals following the administration of the licensed Ty21a attenuated oral typhoid fever vaccine to evaluate how protective immunity alters the proteomic signatures in healthy adult and elderly subjects. The human GI tract represents one of the most direct interfaces between human immunity and microorganisms, both pathogens and commensal flora, as well as innocuous dietary antigens. The GI tract is, thus, a key area for the study of human mucosal immunology, particularly in exploring the immune response against enteric pathogens. The application of mass spectrometry-based proteomics has had a significant impact on our understanding of healthy and diseased states, but this effort has primarily focused on specific cell types and serum/plasma studies. While fecal material has long been known to be a good proxy for the underlying physiology of the human GI tract, there has been virtually no effort in defining a “normal healthy” GI tract via the analyses of the proteome of fecal material. This approach has primarily been hindered by the difficulty in successful extraction of proteins from fecal material in a format amenable to proteomic analyses and limited application of emerging advanced mass spectrometry techniques that can handle the complexity and dynamic range problems posed by fecal components.

- **Specific Aim 1:** Development of sample preparation methods for unbiased and effective

extraction of the entire fecal proteome as a proxy for activity in the gastrointestinal tract, with sample preparation optimization focused on maximizing measurement of immunological components.

- **Specific Aim 2:** Development of advanced liquid chromatography-mass spectrometry methods for shotgun proteome analyses and deep coverage of the “healthy normal” fecal proteome from several human subjects, as well as an elderly population, in order to define core fecal immunological components.

- **Specific Aim 3:** Longitudinal shotgun proteome studies of fecal material for identification and analysis of mucosal immunological changes in response to vaccination with the licensed Ty21a attenuated oral typhoid fever vaccine administered in “non-protective” (1 dose) or “protective” (4 doses) regimens.

The successful completion of these specific aims will develop a new analytical tool that can provide deep insight into the mucosal immunological response and is an ideal complement to studies already underway in the UMB-CCHI center.