Nivedita Lahiri, PhD
(Post-Doctoral Fellow, Brigham and Women's Hospital, Harvard Medical School, Boston MA)

Immunotherapeutic approach to infectious diseases and cancer using biomarkers

BACKGROUND

Infectious diseases like Tuberculosis and Leishmaniasis flourish are caused by pathogens that establish themselves by deactivation of the immune responses of the host. The deactivation of the immune responses, mainly cell mediated immunity is mediated by virulent cell wall glycolipids or proteins present in the pathogens. These virulent molecules are involved in the alteration of cytokine and chemokine profile, suppression of free radicals like superoxide anion and nitric oxide generation and normal signal transduction in the infected macrophage. However, the latest medications are not being able to effectively control the diseases due to the emergence of different drug resistant forms of the pathogens (MDR- multi, XDR- extensive, and TDR- totally-).
Along with the suppression of the host immune system, alteration of cell signaling and the emergence of the various drug resistant forms of diseases, the infectious diseases have now become a major global concern. With increasing rates and increasing breadth of drug resistance, including newly identified totally drug resistant strains, generating markers of the drug resistant state and mechanistic understanding fitness impairments represent key priorities in anti-microbial research. Cell signaling is also altered along with metabolic dysfunctions, producing unnatural molecules during the process of carcinogenesis. Unfortunately, modern medical research has not yet been able to control these aspects of drug resistance, immune and signaling dysfunction in these life-threatening diseases.

RESEARCH ACCOMPLISHMENTS

During my research career spanning 10 years (PhD and post-doc) on tuberculosis, I have tried to address some of the above mentioned problems. Mycobacteria contain a virulent cell-wall glycolipid Lipoarabinomannan (LAM), which induces differential signaling alterations and cytokine release in murine macrophages. It is known that Ara-LAM (Arabinosylated-LAM), isolated from non-pathogenic mycobacteria, induces the release of pro-inflammatory cytokines causing inflammatory reactions in a cell leading to apoptosis. I demonstrated for the first time that Ara-LAM administration also evokes the release of an unusual form of IL-10, having pro-inflammatory nature like TNF-α or IL-12. This property of Ara-LAM could be exploited for inducing Th1 cytokines, as well as restoration of impaired cell mediated immune response in *in vivo* murine TB model, via the induction of TLR-2. My research demonstrates the effectiveness of Ara-LAM as a novel cost-effective alternative to available anti-TB drugs through immunomodulation and favorable regulation of the signal transduction mechanisms.

Addressing the problem of drug resistance, I have worked intensely for 3 years on rifampin resistant *Mycobacterium tuberculosis* and tried to explain the effect of drug resistance on the cell wall lipid changes of the resistant pathogen. By generating panel mutants that mimic the specific RpoB changes seen *in vivo*, I undertook an organism-wide study to identify the extent of cellular change and identify the particular molecules changed as after alteration of the rifamping binding site (RNA polymerase B).
With the help of a recently validated mass spectrometry system (in my post-doctoral laboratory at Harvard Medical School), than can scan among more than 10,000 molecular species of lipids in one organism, the MycoMass and MycoMap databases were created to identify unnamed lipids of known mass. I demonstrated for the first time that rifampin resistance is not a neutral event, but instead influences physiology of the organism. Several aspects of these data indicate that changes in mycobactin, carboxymycobactin, phthiocerol dimycocerosate and sulfoglycolipid are likely characteristic rifampin binding site mutations. I identified these four classes of lipids that were changed in all or all but one mutant with similar findings in strain backgrounds derived from North American or Euro-Beijing reference strains.

FUTURE GOALS

Being involved in research in both Immunology and lipid biochemistry fields; my research is oriented in diverse directions, in different infectious diseases, as well as cancer. I am also open to pursue immunotherapeutic as well as study of the structure of different infectious pathogens *like Leishmania donovani* and their surface lipids as virulent factors. Besides, another project I was working on which showed a lot of promise was detecting TB specific biomarkers, which are generally small molecules/metabolites/lipids in plasma/urine/sputum of TB patients in comparison to that of healthy controls and latent TB controls. My focus is on 3 areas of research:

1. **Immunotherapeutic approach to different diseases using pathogenic cell wall molecules: Induction of apoptosis:** Some cell wall lipids/proteins are pro-inflammatory in nature, and which hold the promise of inducing apoptosis of infected cells. I already have a patent on this and would like to further my work, go into the deeper mechanisms how the protective mechanism might be activated *in vitro* first; effect on Toll like receptors, protein kinases and phosphatases and transcription factors. This research plan, tracing the key modulators of different cell signaling molecules could be a great tool for identifying not only the key targets in infectious diseases, but also certain forms of cancer that involve disarrays of cell signaling. With these plans in mind, I would extend my research on cancers like multiple myelomas, hematomas, colon cancer, etc.

   Related publications

- Majumder N, Bhattacharjee S, Dey R, Bhattacharyya (Majumdar) S, Pal NK, Majumdar S, Arabinosylated Lipoarabinomannan Modulates The Impaired Cell Mediated Immune Response In *Mycobacterium tuberculosis* H37Rv Infected C57BL/6 Mice. **Microbes Infect** 10 (4):349-357
- Bhattacharjee S, Majumder N, Bhattacharya P, Bhattacharyya (Majumdar) S, Majumdar S. 2007. Immunomodulatory role of Arabinosylated Lipoarabinomannan on *L. donovani* infected murine macrophages: Generation of protective immune response. **Ind J Biochem Biophys** 44:366-372
- Majumder Lahiri N, Das S, Bhattacharjee S, Gupta G, Bhattacharyya (Majumdar) S, Majumdar S. TLR-2 Signaling as a target for Ara-LAM mediated protection in Man-LAM induced pathogenesis. (**J. Biomed. Sci,** 2013 doi: 10.3823/1081)

PATENT

- Majumder N, Majumder S. A process for preparing pure arabinosylated lipoarabinomannan effective against tuberculosis and leishmaniasis. 2010. Patent application No.772/KOL/2005, **Govt. of India. Patent no. 237901**

2. **The underlying mechanisms for the cell wall lipid changes (changes in siderophores) in rifampin resistant M. tb cell wall**: Changes at the genetic level (whether IdeR dependent or independent);
    - the effect of rifampin resistant M tb on diacylated sulfoglycolipid mediated T cell response of macrophages.
    - Animal studies: study with mice and with human lymphocytes.

    The reproducible nature of the changed molecules among replicates, mutation sites and strain backgrounds supports some early insights and future studies into candidate mechanisms for fitness costs based on the known functions of the four classes of lipids identified here. Mycobactin and carboxymycobactin function are coordinately a system for uptake of iron from extracellular stores into the cytosol. Sulfoglycolipids and PDIM are both abundant polyketides with known or proposed roles in promoting virulence *in vivo*. Both complex lipids localize to the outer cell wall through complex, multi-step export pathways that involve MmpL transporters. Both their abundance and surface localization have suggested possible roles in direct interface with host membranes or alterations in cell wall permeability, pointing toward future studies of infected cells and drug permeability of rifampin resistant organisms.

    Related publications

    - Lahiri N., Shah Rupal R. Layre E, Young D.C, Cheng T.Y, Murray Megan B., Fortune Sarah M. and Moody D. Branch, Unbiased Lipidomics of Rifampin Monoresistant Mycobacterium Tuberculosis Strains Identifies Downregulated Siderophore Production. Submitted, 2013.

3. **Identification of specific biomarkers from patient samples, which is unique for certain diseases including cancer**.
    My goal is to devise a rapid diagnostic test for tuberculosis, leishmaniasis and other diseases which can be figured out easily by mass spectrometry of biomarkers (lipids/proteins/metabolites) extracted from body fluids. An accurate diagnostic test could be devised using antibodies to common pathogenic biomarkers. The next step after detection is to slow down the progression of the disease. This should act as a first-aid until detailed medical procedures follow. This first-aid would either (a) necrotize or lead to apoptosis of the cell/cell-groups that are already affected by the pathogen or, (b) stimulate or activate the generation of free radicals immediately, to destroy the pathogens in a target specific manner. For example, in a person diagnosed with breast cancer, such a first-aid would target and kill only the HER-2+ hypoxic cells irrespective of their location, *in situ* or metastasized.

SIGNIFICANCE OF MY RESEARCH

My research plan primarily focuses on targeting the altered signaling molecules, or unnatural molecules (biomarkers) for fast diagnosis of the pathogenic condition. If the immune system could be stimulated in a specific and a controlled manner against the development of disease (by drug resistant organisms) or carcinogenesis (altered signaling), the problem of drug resistance could be mitigated. Immunotherapeutic treatments could be more reliable than the existing medications, especially for metastasizing cancers.

POTENT FUTURE COLLABORATORS

- Srinivas V Koduru PhD **Founder, CEO and Managing Editor at Vedic Research International, Vedic Research Inc., USA; Research assistant professor at Yale University School of Medicine,** *experienced scientist working on cell signaling of multiple myeloma and hematomas.*
- Pallavi Tawde, PhD, **Marketing Applications Scientist, Biotherapeutics at Molecular Devices,** *working to develop diagnostic methods to detect TB at a mass scale.*
- Sanjib Chowdhury, PhD, **Assistant Professor at University of Nebraska Medical Center** *Cancer biologist working on the identification and characterization of potential molecular targets associated with cancer metastasis.*