BIOGRAPHICAL SKETCH

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NAME: Singhal, AMIT

eRA COMMONS USER NAME (credential, e.g., agency login): AMIT_SINGHAL

POSITION TITLE: Principal Investigator

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Kurukshetra University, India	BSc	06/1997	Biochemistry
National Dairy Research Inst., Karnal, India	MSc	06/2000	Animal Biotechnology
All India Inst. of Medical Sciences, Delhi, India	PhD	03/2007	Infectious Disease
Institute Pasteur, Brussels, Belgium	Postdoc	05/2007-06/2008	Immunology of TB
Novartis Institute of Tropical Disease, Singapore	Postdoc	07/2008-06/2010	Pharmacology and Immunology of TB

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

My laboratory studies host-pathogen interaction taking *M. tuberculosis* as a model microorganism. We apply various omics approaches to dissect the modulation in host intracellular signaling upon infection and to understand the mechanisms utilized by bacteria for evading immune defense system. The information gained from these experiments is being currently exploited for designing new biomarkers and host-directed therapy for TB. We have recently identified that metformin, an anti-diabetic drug can be repurposed as adjunctive anti-TB therapy and that host sirtuin 1 can be developed as a novel target for TB. My interest in infectious disease and in particular TB grew during my early work as a PhD fellow at All India institute of Medical Sciences, India. My work on TB began with basic clinical investigations in the peripheral cells from TB patients, which are on going. In parallel now I utilize many *in vitro* and animal models for TB i.e. mouse and Rat, the model which I developed while working at Institute Pasteur and Novartis. In addition my interest also lies on developing new point-of-care diagnostic tests, which can be used at resource-limited settings. My goal is to carry fundamental and modern translational immunological research in human populations and to establish clinically relevant diagnostic and therapeutic interventions in infectious diseases.

B. Positions and Honors

Positions and Employment

Date	Position Title	Organization
2010 – 2012	Research Scientist	Singapore Immunology Network (SIgN), A*STAR
2012 – 2017	Senior Research Scientist	Singapore Immunology Network (SIgN), A*STAR
2014 – 2017	Project Leader	Singapore Immunology Network (SIgN), A*STAR
2016 – present	Adjunct Asst. Professor	LKC Medicine, NTU
2017 – present	Principal Investigator	Singapore Immunology Network (SIgN), A*STAR

Other Experience and Professional Memberships

2000 - 2001 Member, Association of Microbiologist of India
2001 - 2007 Member, Indian Immunological Society

2011 - present Member, Singapore Society of Immunologist
2010 - present Leading BSL3 lab at SIgN

Honors

1997 - 1999 Junior Research Fellowship, Indian Council of Agricultural Research (ICAR), India, 2nd rank at all India level
2001 - 2003 Junior Research Fellowship, University Grants Commission (UGC), India through national eligibility test (NET)
2003 - 2006 Senior Research Fellowship, UGC, India
2016 Singapore (A*STAR) -India (DST) Cooperation award

C. Contribution to Science

1. Understanding host-pathogen interaction and TB treatment - Host directed therapy

My top research priority is to understand the basic biology behind *Mycobacterium tuberculosis* (Mtb) infection and to utilize this information for designing new interventions against TB. In this respect I have recently identified that drugs targeting immuno-metabolic axis of the host cell can be developed as an adjunct therapies to enhance the efficacy of anti-TB drugs. In the past we have also taken a chemical biology approach to improve the efficacy of Ethionamide a first line anti-TB drug.

- Cheng CY, Martinez N, Marzuki MB, Lu X, Foreman TW, Paleja B, Lee B, Balachander A, Chen J, Tsenova L, Kurepina N, Teng KWW, West K, Mehra S, Zolezzi F, Poidinger M, Kreiswirth B, Kaushal D, Korfeld H, Newell E, Singhal A. Host SIRT1 regulates mycobacterial immunopathogenesis and represents a therapeutic target against tuberculosis. ***Science Immunology***. 24th March 2017. Doi:10.1126/sciimmunol.aaj1789. PMC: 5505666
- Singhal A, Jie L, Kumar P, Hong GS, Khee-Shing ML, Tsenova L, Kurepina N, Chen J, Zolezzi F, Kreiswirth B, Poidinger M, Chee C, Kaplan G, Wang YT, De Libero G. Metformin as adjunct anti-tuberculosis therapy. 2014. ***Science Translational Medicine.*** 19th Nov doi:10.1126/scitranslmed. 3009885 **(Corresponding author).** PMID: 25411472
- Willand N, Dirié B, Carette X, Bifani P, Singhal A, Desroses M, Leroux F, Willery E, Mathys V, Déprez-Poulain R, Delcroix G, Frénois F, Aumercier M, Locht C, Villeret V, Déprez B, Baulard AR. 2009. Synthetic EthR inhibitors boost antituberculous activity of ethionamide. ***Nat Med.*** 15(5):537-44. PMID: 19412174
- Singhal A, Jaiswal A, Arora VK, Prasad HK. 2007. Modulation of interferon gamma receptor 1 by *Mycobacterium tuberculosis*: A potential immune escape mechanism. ***Infect Immun*** 75(5):2500-10. PMID: 17339358
- Chauhan S, Kumar A, Singhal A, Tyagi JS, Krishna Prasad H. 2009. CmtR, a cadmium-sensing ArsR-SmtB repressor, cooperatively interacts with multiple operator sites to autorepress its transcription in *Mycobacterium tuberculosis*. ***FEBS J.*** 2009. 276(13):3428-39. PMID: 19456862

2. TB models

To control TB spread and progression understanding of the quiescent form of the disease in humans is very important. However it is still lacking due to the unavailability of small animal model representing whole spectrum of the disease. I explored the use of Wistar rats (an outbred rat) to study the protective immunity and control of Mtb infection and found that these rats control Mtb infection and also potentially establish the latent TB. These properties together with the ease of manipulation, relatively low cost and well-established use of rats in toxicology and pharmacokinetic analyses make the rat a good animal model for TB drug discovery.

- Singhal A, Mathys V, Kiass M, Creusy C, Delaire B, Aliouat el M, Dartois V, Kaplan G, Bifani P. 2011. BCG induces protection against *Mycobacterium tuberculosis* infection in the Wistar rat model ***PLoS One***. 6(12):e28082. **(Co-corresponding author)** PMID: 22162757
- Singhal A, Aliouat el M, Hervé M, Mathys V, Kiass M, Creusy C, Delaire B, Tsenova L, Fleurisse L, Bertout J, Camacho L, Foo D, Tay HC, Siew JY, Boukhouchi W, Romano M, Mathema B, Dartois V, Kaplan G, Bifani P. 2011. Experimental tuberculosis in the Wistar rat: a model for protective immunity and control of infection. ***PLoS One***. 12;6(4):e18632. PMID: 21533270
- Heng Y, Seah PG, Siew JY, Tay HC, Singhal A, Mathys V, Kiass M, Bifani P, Dartois V, Hervé M. 2011. Mycobacterium tuberculosis infection induces hypoxic lung lesions in the rat. ***Tuberculosis*** Jul;91(4):339-41. PMID: 21636324

- Foo DG, Tay HC, Siew JY, Singhal A, Camacho L, Bifani P, Dartois V, Hervé M. 2011. ***Antimicrob Agents Chemother***. T cell monitoring of chemotherapy in experimental rat tuberculosis. Aug;55(8):3677-83. PMID: 21628535

3. TB diagnostics

   Another main area of my research is to develop new point-of-care diagnostic assays / devices for TB and other disease with better performance than smear microscopy, which is important to improve the diagnostic yield and to reduce the proportion of TB cases. While working in India I have developed nested-PCR assays to detect Mtb (in different clinical specimens) and to differentiate it from M. bovis. Presently I am trying to utilize the advances in nanotechnology and microfluidics to design new approaches for TB diagnosis in resource-constrained settings. In this respect I have recently developed a simple and inexpensive microchip TB immunoassay (MTBI) for rapid (~15 min) detection of anti-mycobacterial lipid IgG's in the plasma (Patent Application No: 10201503435Q). Currently we are validating this assay in additional clinical samples.

   - Mani V, Paleja B, Larbi K, Kumar P, Tay JA, Siew JY, Inci F, Wang S, Chee C, Wang YT, Demirci U, De Libero G, Singhal A***.*** Microchip-based ultrafast serodiagnostic assay for tuberculosis. ***Scientific Report***. Oct 2016. doi: 10.1038/srep35845. PMID:27775039
   - Inci F, Filippini C, Baday M, Ozen MO, Calamak S, Durmus NG, Wang S, Hanhauser E, Hobbs KS, Juillard F, Kuang PP, Vetter ML, Carocci M, Yamamoto HS, Takagi Y, Yildiz UH, Akin D, Wesemann DR, Singhal A, Yang PL, Nibert ML, Fichorova RN, Lau DT, Henrich TJ, Kaye KM, Schachter SC, Kuritzkes DR, Steinmetz LM, Gambhir SS, Davis RW, Demirci U.A Multiplexed, Quantitative Nanoplasmonic Electrical-field Enhanced Resonating Device for Point-of-Care Diagnostics. ***Proc Natl Acad Sci U S A***. 2015 Aug 11;112(32):E4354-63. doi: 10.1073/pnas. PMID: 26195743
   - Mani V, Wang S, Inci F, De Libero G, Singhal A, Demirci U. Emerging Technologies for Monitoring Drug-Resistant Tuberculosis at the Point-of-Care. ***Adv Drug Deliv Rev.*** 2014. June 2. doi:10.1016/j.addr.2014. 05.015 (**Co-corresponding author)**. PMID: 24882226
   - Wang S, Inci F, De Libero G, Singhal A, Demirci U. ***Biotechnol Adv***. Point-of-care assays for tuberculosis: role of nanotechnology / microfluidics. 2013 Jul;31(4):438-49. **(Co-corresponding author)**
   - Shah N, Singhal A, Jain A, Kumar P, Upal SS, Shrivatsva MVP, Prasad HK. 2006. Occurrence of overlooked zoonotic tuberculosis: Detection of *M. bovis* infection in human CSF. ***J Clin Microbiol***. 44(4): 1352-8. **(Joint first author)** PMID: 16597862

4. Epidemiology of TB

   Alleviating the burden of tuberculosis (TB) requires an understanding of the genetic basis that determines the emergence of drug resistant mutants. These studies throw light on the alternative mechanism(s) or pathway(s) associated with the drug resistance. I have conducted studies on the resistant genotypes of second line TB drugs (PAS) and new drug candidates (PA-824), which may contribute to future efforts in monitoring clinical strain susceptibility associated with them.

   - Haver HL, Chua A, Ghode P, Lakshminarayana SB, Singhal A, Mathema B, Wintjens R, Bifani P. Mutations in the F420 biosynthetic pathway and a nitroreductase enzyme are the primary resistance determinants in spontaneous in vitro selected PA-824 mutants of Mycobacterium **tuberculosis**. ***Antimicrob Agents Chemother***. 2015 Sep;59(9):5316-23. doi: 10.1128/AAC.00308-15. PMID: 26100695
   - Mathys V, Wintjens R, Lefevre P, Bertout J, Singhal A, Kiass M, Kurepina N, Wang XM, Mathema B, Baulard A, Kreiswirth BN, Bifani P. 2009. Molecular genetics of para-aminosalicylic acid resistance in clinical isolates and spontaneous mutants of Mycobacterium tuberculosis. ***Antimicrob Agents Chemother***. 53(5):2100-9. PMID: 19237648

5. T cell Immunity

   Non-classical T cells that recognize nonpeptidic antigens represent an important effectors of the immune response. They are present in large numbers in circulating blood and tissues and are as abundant as T cells recognizing peptide antigens. They recognize complexes of nonpeptidic antigens such as lipid and glycolipid molecules, vitamin B2 precursors, and phosphorylated metabolites of the mevalonate pathway. Non-classical T cells exert multiple functions including immunoregulation, tumor control, and protection against infections. I have carried out studies in characterizing these T cells.

- Lepore M, Kalinicenko A, Colone A, Paleja B, Singhal A, Tschumi A, Lee B, Poidinger M, Zolezzi F, Quagliata L, Sander P, Newell E, Bertoletti A, Terracciano L, De Libero G, Mori L. Parallel T-cell cloning and deep sequencing of human MAIT cells show stable oligoclonal TCRβ repertoire. ***Nat Commun***. 2014. May 15;5:3866. PMID: 24832684
- De Libero G, Singhal A, Lepore M, Mori L. Nonclassical T cells and their antigens in tuberculosis. ***Cold Spiring Harb Perspect Med.*** 2014. Jul 24 doi: 10.1101/cshperspect.a01873. PMID: 25059739
- **Singhal A**, Mori L, De Libero G. T cell recognition of non-peptidic antigens in infectious diseases. *IJMR*. 2013 Nov; 138(5):620-31. PMID: 24434317
- Facciotti F, Ramanjaneyulu GS, Lepore M, Sansano S, Cavallari M, Kistowska M, Forss-Petter S, Ni G, Colone A, Singhal A, Berger J, Xia C, Mori L, De Libero G. Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. ***Nat Immunol***. 2012 Mar 18;13(5):474-80. PMID: 22426352

D. Patents: US20150209308, 10201503435Q, 10201600326T

E. Research Support

ACTIVE

RO1 (PI: Hardy Kornfeld) 05/2016-04/2019
NIH
This project investigates the interaction of diabetes and tuberculosis.
Role: Co-Investigator

A*STAR-INDIA DST Program of Cooperation Award (PI: Singhal) 01/2016-12/2018
A*STAR-DST
This project investigates the host-pathogen interaction during *Mycobacterium tuberculosis* infection with the aim of finding new drug targets in the pathogen.

A*STAR JCO-Carrier Developmental Program (PI: Singhal) 12/2015-11/2018
A*STAR
This project aims to find new host-directed therapeutic candidates for TB.

NMRC CBRG grant (PI: Kevin Pethe) 08/2015-07/2018
National Medical Research Council, Singapore
This project characterizes the antibacterial and anti-inflammatory properties of Q203, a promising clinical candidate for the treatment of drug-resistant strains. Role: Co-Investigator

SIgN Core grant (PI: Singhal) *(no expiry, discussed every 3 year)* 05/2014-04/2020
SIgN, A*STAR
*A **s**ystematic **m**olecular **a**pproach to investigate the immunology of Mycobacterium tube**r**culosis infection and explore novel **T**uberculosis treatments (SMART).* This project investigates the mechanisms by which Mtb evades immune defense system and develops novel diagnostic and therapeutic strategies to combat infection.

COMPLETED

BMRC-SERC Diagnostic grant (PI: Gennaro De Libero) 04/2012-09/2015
A*STAR
Biomarkers in tuberculosis: lipid-specific immune response. This study investigates the lipid specific immune response in TB patients. Based on this development of novel diagnostic assays have been planned.
Role: Co-Investigator

ASTAR-CIMIT grant 1187 (PI: Singhal) 11/2012-07/2014
A*STAR
Microfluidics for diagnosis of tuberculosis. This project aims at to develop a point-of-care test for TB diagnosis. In collaboration with Utkan Demirci (Stanford University) we have developed a highly specific microfluidic based CHIP assay that can detect mycobacterial anti-lipid antibodies in patient serum within 15 minutes. The sensitivity of the assay is 75%.