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EDUCATION

- **1999-2006**: Ph.D. in Molecular Parasitology, School of Life Sciences. Jawaharlal Nehru University, New Delhi, India; Final Grade Point Average: 8.6/10, Grade: A^+ (Ph.D. course work).

- **1997-1999**: Master in Life Sciences, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India; Final Grade Point Average: 8.42/10, Grade: A.

- **1994-1997**: Bachelor in Biochemistry (Hons.), Delhi University, New Delhi, India; First division with 71.88%.

RESEARCH AND PROFESSIONAL EXPERIENCE

07/08/2019 ***Selected and offered the post of Assistant Professor in Biochemistry at Lovely Professional University, Punjab, India.***

11/2015-11/2018 ***Science and Engineering Research Board (SERB)-Young Scientist, Hosting lab of Dr. Anurag Agrawal, Director- Institute of Genomics and Integrative Biology (IGIB), Mall Road, Delhi, India.***
Job: Project Investigator of a self-conceptualized *project on a novel role of microRNA function in innate immunity to tuberculosis,* ***project management*** *and management of* ***users and resources in Biosafety level3*** *of Mall Road Campus.*

11/2013-11/2015 ***Activites during this period include- applied for Science and Engineering Research Board (SERB)-Young Scientist-Start-Up Grant, worked as a Guest Researcher in Dr. Yogendra Singh lab at IGIB-Mall Road, authored a book chapter, worked as a reviewer for Bio-protocol and attended conference.***

11/2009-11/2013 ***Postdoctoral Fellow, New York University School of Medicine (NYUSoM), USA***
Department of Medicine- Infectious Diseases, Dr. Jennifer A. Philips lab (Associate Professor-Washington University).

Study: Knowledge of host-*Mycobacterium tuberculosis* (Mtb) interactions in tuberculosis (TB), of which **very few are known**, can help in better understanding of the molecular mechanisms of impaired innate immune responses and can be exploited for drug development.

- Demonstrated a **novel** host-pathogen (Hrs-EsxH) interaction that impairs host Endosomal Sorting Complex Required for Transport (ESCRT) leading to phagosome maturation arrest during Mtb infection in macrophages. ***First author publication in PLOS Pathogens, 2013*.**
- Demonstrated **alteration of Hrs localization** in EsxH dependent manner during Mtb infection (***Published in mBio, 2018*).**
- **Established** retrovirally transduced GFP1-10 RAW 264.7 cell line and Mtb overexpressing GFP11-tagged EsxH strain to detect **secretion** by type VII secretion system during infection by **GFP complementation**.
- Examined **HIV replication in macrophages co-infected with mycobacteria** using high-content imaging.

04/2008-09/2009 ***Research Scientist/ Research Associate, Immunology Group.***
International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India.

Study: Host microRNA (miRNA) regulation of innate immune responses in TB.

- Demonstrated **microRNA-99b to be specifically induced in MYD88** dependent manner in murine dendritic cells. (***Published in Journal of Biological Chemistry, 2013***).
  - **Initiation** of the project: designed miRNA expression profiling experiment, identified locked nucleic acid (LNA) based tools for miRNA study, validation of hits from microarray and miRNA target prediction.

10/2006-04/2008 ***Postdoctoral Scholar, Karolinska Institute. Sweden.***
Department of Biosciences and Nutrition, Dr. Inderpreet Sur (senior postdoc) in Prof. Rune Toftgård lab.

Study: Understanding context dependent functions of Krüppel like factor 5 (KLF5), a transcription factor, in positive and negative regulation of proliferation of non-transformed and cancer cells, respectively.

- **Designed** the study on identification of KLF5 interactors- **conducted** yeast two hybrid library screen for transient binary interactors and **standardized** co-immunoprecipitation for identifying stable interactors by mass spectrometry.
- Promoter-reporter assays, expression analysis of K5tTA X TRE-KLF5 bi-transgenic embryos.

1999-2006 ***Ph.D., School of Life Sciences, Jawaharlal Nehru University (JNU), India***
Molecular Biology and Biochemistry (Parasitology), Prof. Alok Bhattacharya lab.

Thesis title "***Study of Cell Surface Molecules of Entamoeba histolytica"*** .
Study: The protozoan parasite, *Entamoeba histolytica* (*E. histolytica*), causes enteric disease amebiasis. Genetic knockouts to understand function of hypothetical genes has not been possible in this organism due to varied ploidy levels. I initiated work on three of it's unknown proteins in the lab:

- **First functional study** of B1 transmembrane kinases (B1 TMKs) of the parasite demonstrating their role in **serum-dependent proliferation** of the parasite using cells expressing ***tetracycline-inducible dominant negative*** B1 TMK. ***First author paper in Infection and Immunity, 2006***.
- **First functional study** of lysine-rich protein (KRP) ***in vitro*** **cytotoxicity** by either over or antisense-induced underexpression of *krp* in the parasite. ***First author paper in Am J Biochem and Biotec, 2007***.

08/2003- 11/2003 ***Visiting Research Scholar at Weizmann Institute of Science, Israel***

Worked on ***identification of peptide backbone of Proteophospholycan,*** specifically present on the surface of the virulent species of *E. histolytica,* by selective organic extraction from cells, acid cleavage of phosphoglycans and purification for *de novo* sequencing by mass spectrometry ***to identify the gene encoding it***.

08/1998- 04/1999 ***Masters project in Bioinformatics, Bioinformatics Centre, Jawaharlal Nehru University, India***
Supervisor: Prof. Alok Bhattacharya.

Thesis: "**Analysis of coding sequences for predicting protein domain structure using the software GeneScan ver2.2**"
GeneScan is an in-house developed algorithm based on variation in three-base periodicity of a coding region.

AWARDS/HONORS

03/2019 Marie Skłodowska-Curie Actions (MSCA) Seal of Excellence by European Commission for my proposal submitted to H2020-MSCA-IF-2018 call with Helmholtz Centre for Infection Research, Germany as the host.

07/2015 SERB-Young Scientist (Start-Up) award by SERB, India (applied in August 2014 and approved on 3/07/2015).
Project Grading *is* ***"Good"*** *as assessed by SERB upon completion of 3-year duration of project.*

01/2015 Invited to attend Young Investigators' Meeting (YIM), Gulmarg, Srinagar, India (IndiaBioscience sponsors India's best life sciences researchers for YIM by selection of applicants).

07/2012 Potts Memorial Foundation Award (New York) for post-doctoral fellowship for the project "Mechanism of EsxH-dependent modulation of host ESCRT machinery".

03/2006 First prize for Oral presentation at Biosparks, Fourth Annual Research Festival, School of Life Sciences, Jawaharlal Nehru University, India.

2000-2005 Ph.D. research scholarship from Council for Scientific and Industrial Research (CSIR), India.

1998-1999 Jawaharlal Nehru Memorial Fund Award for standing first in Masters in Life Sciences, Jawaharlal Nehru University.

MEMBERSHIPS OF PROFESSIONAL BODIES/ PROFESSIONAL ACTIVITIES

04/2016 onwards: **Associate Editor (Immunology) of Bio-protocol**, an indexed (ESCI edition of Web of Science) and a peer-reviewed e-journal based in USA.

Protocols edited & published can be viewed at *https://bio-protocol.org/ProtocolEdited.aspx?id=1004096*, *few are listed below:*
IDe3701: **SMART (Single Molecule Analysis of Resection Tracks) Technique for Assessing DNA end-Resection in Response to DNA Damage**, *from Nucleic Acids Research*.
IDe3494: **Automated Analysis of Cell Surface Ruffling: Ruffle Quantification Macro**, *from Journal of Cell Biology*.
IDe3369: **Quantitation of TLR4 Internalization in Response to LPS in Thioglycollate-Elicited Peritoneal Mouse Macrophages by Flow Cytometry,** *from Nature Immunology*.
IDe3335**: *In vitro* Differentiation of Thymic T_{reg} Cell Progenitors to Mature Thymic T_{reg} Cells,** *from Nature Immunology.*
IDe2857**: Ectopic Gene Expression in Macrophages Using *in vitro* Transcribed mRNA,** *from PNAS.*

09/2014-03/2016: Member of Bio-protocol **Reviewer Board (by invitation). Protocols reviewed can be viewed at *https://bio-protocol.org/ProtocolReviewed.aspx?id=1004096***

01/2010-01/2012: Membership of **New York Academy of Sciences**.

01/2011-01/2012: Membership of **American Society of Microbiology**.

SUPERVISION & MENTORING ACTIVITIES

- Managed **Biosafety level 3 facility** at Mall Road Campus, IGIB, Delhi, India .
- Trained technicians and Ph.D. students on lab techniques and biosafety level 3 procedures for *in vitro* Mtb culture and infection.
- Supervised M.Sc. thesis: (1) Jyoti Srivastava (2002): "Purification of Lipopeptidophosphoglycan of *Entamoeba histolytica*".
  (2) Kokil Agarwal (2003): "Cloning and expression of Kinase domain of a Receptor Kinase of *E. histolytica*".

PUBLICATIONS

- **Published Manuscripts**

1. Mittal E, Skowyra M, Uwase G, Tinaztepe E, **Mehra A**, Köster S, Hanson P, and Philips JA. **(2018)** *Mycobacterium tuberculosis* Type VII secretion system effectors differentially impact the ESCRT endomembrane damage response. **mBio** Nov 27; 9(6): pii: e01765-18. PMID: 30482832. ***Citations***: 20 (Scopus)

2. Portal-Celhay C, Tufariello JM, Srivastava S, Zahra A, Klevorn T, Grace PS, **Mehra A**, Park HS, Ernst JD, Jacobs Jr. WR, and Philips JA. **(2016)** *Mycobacterium tuberculosis EsxH inhibits ESCRT-dependent CD4+ T cell activation.* **Nature Microbiology** Dec5 (2) 16232. PMID: 27918526, ***Citations***: 32 (Scopus).

3. **Mehra A**. **(2014)**. *Phagolysosomal Trafficking Assay*. **Bio Protoc.** 4 (13) pii: e1163**;** DOI: 10.21769/BioProtoc. e1163. PMID: 29675446. ***Views***: ***12407*.**

4. **Mehra A*** and Philips JA*. **(2014)**. *Analysis of Mycobacterial Protein Secretion*. **Bio Protoc.** 4 (12) pii: e1159**;** *Co-corresponding author**.** DOI: 10.21769/BioProtoc. e1159**.** PMID: 29675445. ***Views***: 10792. ***Citations***: 1 (CrossRef)

5. Siegrist MS, Steigedal M, Ahmad R, **Mehra A**, Dragset MS, Schuster BM, Philips JA, Carr SA, Rubin EJ. **(2014)**. *Mycobacterial Esx-3 requires multiple components for iron acquisition*. **mBio.** 5 (3) e01073-14**.** PMID: 24803520. ***Citations***: 50 (Scopus).

6. **Mehra A***, Zahra A*, Thompson V, Sirisaengtaksin N, Wells A, Porto M, Köster S, Penberthy K, Kubota Y, Dricot A, Rogan D, Vidal M, Bean AJ, Hill D, Philips JA. **(2013)**. *Mycobacterium tuberculosis type VII secreted effector EsxH targets host ESCRT to impair trafficking*. **PLOS Pathogens.** 9(10) e1003734. * Equal contribution. PMID: 24204276. ***Citations***: 82 (Scopus).

7. Singh Y, Kaul V, **Mehra A**, Chatterjee S, Tousif S, Dwivedi VP, Suar M, Kaer LV, Bishai WR and Das G. **(2013)**. *Mycobacterium tuberculosis controls microRNA-99b (miR-99b) expression in infected murine dendritic cells to modulate host immunity*. **Journal of Biological Chemistry.** Feb 15; 288(7): 5056-61. PMID: 23233675. ***Citations***: **98** (Scopus).

8. Juranek J, Kothary P, **Mehra A**, Hays A, Brannagan III TH, Schmidt AM. **(2013)**. *Increased expression of the receptor for advanced end-glycation products in human peripheral neuropathies.* **Brain and Behaviour.** Nov 3(6) 701-9. PMID: 24363972. ***Citations***: 15 (Scopus).

9. **Mehra A**, Bhattacharya S and Bhattacharya A. **(2007)**. *Identification and functional characterization of a novel lysine-rich protein from Entamoeba histolytica.* **American J Biochemistry and Biotechnology**. 3(4): 193-198. ***Citations***: 1(Scopus).

10. **Mehra A**, Fredrick J, Petri WA Jr, Bhattacharya S and Bhattacharya A. **(2006)**. *Expression and function of a family of transmembrane kinases from the protozoan parasite Entamoeba histolytica.* **Infect and Immun**. Sep; 74(9): 5341-51. PMID: 16926429. ***Citations***: **21** (Scopus).

11. Arya R, **Mehra A**, Bhattacharya S, Vishwakarma RA and Bhattacharya A. **(2003)**. *Biosynthesis of Entamoeba histolytica Proteophosphoglycan in vitro.* **Mol. Biochem. Parasitol**. Jan, 126 (1): 1-8. PMID: 12554078. ***Citations***: **7** (Scopus).

- **Published Book Chapters**

1. **Mehra A**. **(2015)**. *Microbial CRISPR-Cas System: From Bacterial Immunity to Next-Generation Antimicrobials*. **Microbial Factories** (Springer India 2015, ISBN No.: 978-81-322-2594-2) Volume 2, Chapter 14. 217-234. **DOI: 10.1007/978-81-322-2595-9_14**. ***Downloads***: **1200**, for latest numbers visit this link; https://link.springer.com/chapter/10.1007/978-81-322-2595-9_14

SCIENTIFIC CONFERENCES AND PRESENTATIONS (presenting authors are underlined)

29/Apr 2020 **Attended**: Webinar on "**Redefine immunology with multiomics single cell and spatial characterization" by 10x Genomics**.

20-22/Feb 2017 **Attended: Participated in Indo-US workshop on "Flow Cytometry and Metabolomics in Studying Infectious Diseases in Animals and Humans", South Asian University, Delhi, India.**

02-03/Feb **Oral talk as Project Investigator on progress of SERB funded project: SERB-Young Scientist Group**

2017 **Monitoring Workshop, Bhubaneshwar, Odisha, India.**

14-15/Jan 2017 **Attended International Conference "GI Immunology and Inflammation 2017"** organized by **All India Institute of Medical Sciences** at the Oberoi Hotel, New Delhi.

27-28/Mar 2015 **Poster: Young Investigators' Meeting, Using local systems to study biology, Srinagar, India.**
Mycobacterium tuberculosis Type VII secreted effector, EsxH alters Hrs localization to impair phagosome maturation in macrophages. **Mehra A**, Portal-Celhay C, Zahra A, Tufariello JM, Porto M, Dricot A, Vidal M, Hill D, Jacobs WR Jr, Philips JA.

2-3/Mar 2015 **Poster**: **International Conference on "Antimicrobial Resistance, Novel Drug Discovery and Development: Challenges and Opportunities"** organized by **SRM University** at India Habitat Centre, India.
Mycobacterium tuberculosis Type VII secreted effector, EsxH targets host ESCRT to inhibit intracellular trafficking.
Mehra A, Zahra A, Thompson V, Sirisaengtaksin N, Wells A, Porto M, Penberthy K, Kubota Y, Dricot A, Rogan D, Vidal M, Bean AJ, Hill D, Philips JA

13-18/Mar 2013 **Poster: Keystone X7 symposia, Host Response in Tuberculosis, Whistler, Canada.**
Mycobacterium tuberculosis Type VII secreted effector, EsxH targets host ESCRT to inhibit intracellular trafficking.
Mehra A, Zahra A, Thompson V, Sirisaengtaksin N, Wells A, Porto M, Penberthy K, Kubota Y, Dricot A, Rogan D, Vidal M, Bean AJ, Hill D, Philips JA.

21-24/May 2011 **Poster:** 111th **American Society of Microbiology General meeting, New Orleans, USA.** *ESX-Dependent Modulation of the Host ESCRT Machinery by Mycobacteria.* **Mehra A**, Wells A, Porto M, Dricot A, Penberthy K, Rogan D, Hill D, Vidal M, Philips JA.

15-20/Jan 2011 **Poster: Keystone Symposia, Tuberculosis- Immunology, Cell Biology and Novel Vaccination Strategies, Canada.**
A systematic approach to understand Mycobacterium tuberculosis survival strategy suggests Esx-dependent modulation of the host ESCRT machinery. **Mehra A**, Wells A, Porto M, Dricot A, Penberthy K, Hill D, Vidal M, Philips JA.

9-10/Mar 2006 ***1st Prize in Oral presentation***: **Biosparks 2006**: **4th Annual Research Festival, School of Life Sciences, Jawaharlal Nehru University India.** *A Family of Transmembrane Kinases from the Protozoan Parasite Entamoeba histolytica: Expression and Function.* **Mehra A**, Bhattacharya S, Bhattacharya A.

Sep 2005 **Poster: Molecular Parasitology Meeting, Woodshole, Massachusetts, USA.**
B1 family of Receptor Protein Kinase from the protozoan parasite Entamoeba histolytica is involved in cellular proliferation.
Mehra A, Frederick JR, Petri WA Jr, Bhattacharya S, Bhattacharya A.

11-14/Feb 2002 **Poster: International conference on Amebiasis and the biology of *Entamoeba* (org anized by Prof. Alok and Sudha Bhattacharya), Agra, India.** *In vitro biosynthesis of cell surface glycoconjugate, proteophosphoglycans, of Entamoeba histolytica*. Arya R, Vats D, **Mehra A**, Anand MT, Bhattacharya S, Bhattacharya A.

TECHNICAL EXPERTISE

- **Computational Skills**
  - ***Web-based bioinformatics tools*** for nucleic acid and protein sequence analysis, miRNA target prediction, User of **Graph Pad InStat**.
  - ***Courses done (Jan2020- till date): Data Science in Python*** *(Internshala)****, Statistics and R****(edX)*, ***InZight Visual Inference Tool*** for data visualization, analysis and inference, ***Machine learning: Predictive modeling in Octave*** *(Coursera****), Introduction to Bioconductor in R*** *(edX)*
    - ***Predictive Modeling methods***: Supervised learning algorithms: Linear and Logistic Regression, Decision trees and Un-Supervised learning algorithm: K-means Neural Networks, Support Vector machine, Principal Component Analysis.
    - ***Genomics data analysis*****:** Microarray and Next-Generation Sequencing data analysis in R.
- **Tissue culture techniques**
  - Mammalian cell lines: various cancer and non-transformed cell lines.
  - Primary mammalian cells: murine and human macrophages, murine dendritic cells and keratinocytes from neonate mice skin.
- **Biosafety level (BSL) 3 and BSL2 work for mycobacteria culture and macrophage infections**
- **Animal Handling**
  - ***Laboratory Animal Science course certificate*** from Karolinska Insitute in accordance with Federation of European Laboratory Animal Science Association (FELASA) category C and European legislation
- **Molecular biology, biochemical and immunological techniques**
  - Lentiviral transduction of RAW264.7 macrophages and DNA transfection in mammalian cells for stable and transient gene expression.
  - Molecular techniques: Cloning, RNA isolation, DNA isolation- Plasmid and genomic, Southern and Northern blot analysis, PCR, Real-time PCR, Electrophoresis: Agarose and polyacrylamide gel electrophoresis, co-immunoprecipitation, Western blot analysis.
  - Protein purification: expression and purification proteins in *E. coli,* Ni^{+}-affinity, hybrophobic interaction and gel filtration chromatography.
- **Cell-based assays for bacterial trafficking in macrophages using epifluorescence microscopy and image analysis using Image J**

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RESEARCH ARTICLE
Host-Microbe Biology

Mycobacterium tuberculosis Type VII Secretion System Effectors Differentially Impact the ESCRT Endomembrane Damage Response

Ekansh Mittal,[a,b] Michael L. Skowyra,[c] Grace Uwase,[a,b] Emir Tinaztepe,[d] Alka Mehra,[d] Stefan Köster,[d]* Phyllis I. Hanson,[c] Jennifer A. Philips[a,b,d]

[a]Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

[b]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA

[c]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri, USA

[d]Division of Infectious Diseases, Department of Medicine, New York University School of Medicine, New York, New York, USA

ABSTRACT Intracellular pathogens have varied strategies to breach the endolysosomal barrier so that they can deliver effectors to the host cytosol, access nutrients, replicate in the cytoplasm, and avoid degradation in the lysosome. In the case of *Mycobacterium tuberculosis*, the bacterium perforates the phagosomal membrane shortly after being taken up by macrophages. Phagosomal damage depends upon the mycobacterial ESX-1 type VII secretion system (T7SS). Sterile insults, such as silica crystals or membranolytic peptides, can also disrupt phagosomal and endolysosomal membranes. Recent work revealed that the host endosomal sorting complex required for transport (ESCRT) machinery rapidly responds to sterile endolysosomal damage and promotes membrane repair. We hypothesized that ESCRTs might also respond to pathogen-induced phagosomal damage and that *M. tuberculosis* could impair this host response. Indeed, we found that ESCRT-III proteins were recruited to *M. tuberculosis* phagosomes in an *ESX-1*-dependent manner. We previously demonstrated that the mycobacterial effectors EsxG/TB9.8 and EsxH/TB10.4, both secreted by the ESX-3 T7SS, can inhibit ESCRT-dependent trafficking of receptors to the lysosome. Here, we additionally show that ESCRT-III recruitment to sites of endolysosomal damage is antagonized by EsxG and EsxH, both within the context of *M. tuberculosis* infection and sterile injury. Moreover, EsxG and EsxH themselves respond within minutes to membrane damage in a manner that is independent of calcium and ESCRT-III recruitment. Thus, our study reveals that T7SS effectors and ESCRT participate in a series of measures and countermeasures for control of phagosome integrity.

IMPORTANCE *Mycobacterium tuberculosis* causes tuberculosis, which kills more people than any other infection. *M. tuberculosis* grows in macrophages, cells that specialize in engulfing and degrading microorganisms. Like many intracellular pathogens, in order to cause disease, *M. tuberculosis* damages the membrane-bound compartment (phagosome) in which it is enclosed after macrophage uptake. Recent work showed that when chemicals damage this type of intracellular compartment, cells rapidly detect and repair the damage, using machinery called the endosomal sorting complex required for transport (ESCRT). Therefore, we hypothesized that ESCRT might also respond to pathogen-induced damage. At the same time, our previous work showed that the EsxG-EsxH heterodimer of *M. tuberculosis* can inhibit ESCRT, raising the possibility that *M. tuberculosis* impairs this host response. Here, we show that ESCRT is recruited to damaged *M. tuberculosis* phagosomes and that

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Editor Samuel I. Miller, University of Washington

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ARTICLES

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Mycobacterium tuberculosis EsxH inhibits ESCRT-dependent CD4$^+$ T-cell activation

Cynthia Portal-Celhay[1], JoAnn M. Tufariello[2,3], Smita Srivastava[1], Aleena Zahra[1], Thais Klevorn[1], Patricia S. Grace[1], Alka Mehra[1], Heidi S. Park[1], Joel D. Ernst[1], William R. Jacobs Jr[2,4‡*] and Jennifer A. Philips[1†‡*]

Mycobacterium tuberculosis **(Mtb) establishes a persistent infection, despite inducing antigen-specific T-cell responses. Although T cells arrive at the site of infection, they do not provide sterilizing immunity. The molecular basis of how Mtb impairs T-cell function is not clear. Mtb has been reported to block major histocompatibility complex class II (MHC-II) antigen presentation; however, no bacterial effector or host-cell target mediating this effect has been identified. We recently found that Mtb EsxH, which is secreted by the Esx-3 type VII secretion system, directly inhibits the endosomal sorting complex required for transport (ESCRT) machinery. Here, we showed that ESCRT is required for optimal antigen processing; correspondingly, overexpression and loss-of-function studies demonstrated that EsxH inhibited the ability of macrophages and dendritic cells to activate Mtb antigen-specific CD4$^+$ T cells. Compared with the wild-type strain, the *esxH*-deficient strain induced fivefold more antigen-specific CD4$^+$ T-cell proliferation in the mediastinal lymph nodes of mice. We also found that EsxH undermined the ability of effector CD4$^+$ T cells to recognize infected macrophages and clear Mtb. These results provide a molecular explanation for how Mtb impairs the adaptive immune response.**

Host defences, both innate and adaptive, are subverted by *Mycobacterium tuberculosis* (Mtb). During Mtb infection, there is a delay in priming antigen-specific CD4$^+$ and CD8$^+$ cells by dendritic cells (DCs) in the lymph node[1]. When the effector CD4$^+$ T cells traffic to the site of infection in the lungs, although they promote the antimycobacterial activity of macrophages by secreting cytokines such as interferon (IFN)-γ[2], they fail to generate sterilizing immunity. Currently, we lack a comprehensive and detailed understanding as to why major histocompatibility complex class II (MHC-II) antigen presentation fails during Mtb infection. In naive macrophages, Mtb can act through Toll-like receptor 2 to block IFN-γ-induced MHC-II transcription[3–5], although the contribution this plays *in vivo* is unclear[6]. How Mtb impairs antigen presentation in macrophages already expressing MHC-II is less well understood. One proposed mechanism is that by blocking phagosome maturation Mtb impairs efficient processing of antigen and the MHC-II-associated invariant chain[7,8]. However, there have been contradictory results regarding the impact of phagosome maturation on antigen presentation[8–11]. Contradictory results have also been reported in DCs. Some studies have demonstrated that Mtb inhibits DCs maturation, thereby impairing mobilization of MHC-II molecules to the cell surface[12], whereas others have shown that Mtb upregulates DC expression of MHC-II, co-stimulatory molecules and inflammatory cytokines[13,14]. More recent data have shown that Mtb-infected DCs are inefficient at priming antigen-specific CD4$^+$ T cells[15–17]. Rather, bystander uninfected cells take up Mtb antigen and prime CD4$^+$ T cells[18,19].

Our group recently found that Mtb EsxH inhibits phagosome maturation by targeting the host endosomal sorting complex required for transport (ESCRT)[20]. EsxH forms a heterodimer with EsxG ($EsxGH_{Mt}$), which is secreted by the Esx-3 type VII secretion system. $EsxGH_{Mt}$ is involved in iron and zinc acquisition[21–25], and recent work has demonstrated that $EsxGH_{Mt}$ also plays an additional role in virulence[20,26,27]. $EsxGH_{Mt}$ binds the host protein hepatocyte growth factor-regulated tyrosine kinase substrate (HGS, also known as HRS), a component of the ESCRT machinery[20]. ESCRT plays a well-described role in directing cell-surface receptors into intraluminal vesicles of multivesicular bodies so they can be degraded in the lysosome[28]. We found that ESCRT is also required for phagosome maturation[20,29], and we hypothesized that ESCRT, and by extension $EsxGH_{Mt}$, regulates MHC-II antigen presentation. In this study, we showed that ESCRT promotes T-cell activation during Mtb infection by facilitating antigen processing. We demonstrated that $EsxGH_{Mt}$ impairs the ability of macrophages and DCs to present mycobacterial antigens and activate CD4$^+$ T cells, resulting in impaired T-cell priming and defective effector function. Overall, our data support a model in which EsxH inhibits ESCRT, thereby undermining two key aspects of the adaptive immune response: (1) efficient priming of naive T cells, and (2) recognition of Mtb-infected cells by CD4$^+$ T cells.

Results

ESCRT promotes antigen presentation during Mtb infection. To test the hypothesis that ESCRT contributes to antigen presentation, we depleted HRS and TSG101, components of ESCRT-0 and ESCRT-I, from bone marrow-derived macrophages (BMDMs) (Fig. 1a). Following ESCRT depletion, BMDMs were activated with IFN-γ to induce MHC-II expression and infected the following day with Mtb. The ability of the infected macrophages to activate CD4$^+$ T cells was assessed using T-helper 1 (T_H1) polarized CD4$^+$ effector cells that express a transgenic T-cell antigen receptor (TCR-Tg) specific for peptide 25 (amino acids 240–254) of Mtb Ag85B protein (P25TCR-Tg T_H1 cells)[30], which secrete IFN-γ when co-cultured

[1]Division of Infectious Diseases, Department of Medicine, New York University School of Medicine, New York, New York 10016, USA. [2]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, USA. [3]Center for Microbial Pathogenesis, Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia 30303, USA. [4]Howards Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York 10461, USA. [†]Present address: Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St Louis, Missouri 63110, USA. [‡]These authors contributed equally to this work. *e-mail: philips.j.a@wustl.edu; jacobsw@hhmi.org

Microbial CRISPR–Cas System: From Bacterial Immunity to Next-Generation Antimicrobials

14

Alka Mehra

Abstract

Microbes live in multi-microbial communities called microbiome. Discoveries that can help in the regulation of the composition of the microbiome are likely to impact diverse functions of microbes from health, environment, to biotechnology. Antimicrobials offer such regulatory potential and are slowly but surely evolving for the benefit of human health and biotechnology. Antibiotics are the first discovered antimicrobials which are low molecular weight natural microbial products that inhibit the growth of other microbes. However, emergence of microbial resistance to conventional antibiotics has presented an urgent need for novel antimicrobials. Here, we describe another native microbial machinery, CRISPR ("clustered regularly interspaced short palindromic repeats")–Cas ("CRISPR associated") system, that confers adaptive immunity to microbes by employing CRISPR RNAs to recognize and destroy complementary nucleic acids of invasive foreign genetic elements. Further, sequence-based targeting by CRISPR–Cas system has been leveraged for the development of sequence-specific novel antimicrobials, genome editing, and genome regulation tools.

14.1 Introduction

14.1.1 A Microbial Immune System

Microbes need to survive under a constantly changing environment. They can adapt to the environment by acquiring new traits which confer selective advantage leading to genome evolution. Therefore, there is exchange of genetic material between microbes by horizontal gene transfer (HGT) (Nakamura et al. 2004). HGT can occur by transformation of DNA from the

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Phagolysosomal Trafficking Assay

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Abstract

Phagolysosomal trafficking is an important innate defense pathway that clears microbes by delivering them to lysosomes, the degradative compartment of the cell. *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis, subverts this host defense mechanism by arresting maturation of the phagosome. The ability of Mtb to arrest its delivery to the lysosome can be demonstrated by the prolonged co-localization of bacteria containing phagosomes/vacuole with early phagosomal markers [such as, Ras-related proteins in the brain 5 (Rab5) and Transferrin receptor (TfR)], and a failure to acquire late phagosomal and lysosomal markers (such as Rab7 and LAMP1) (Deretic and Fratti, 1999, Mehra *et al.*, 2013). Here, a protocol is outlined for infection of macrophages with mycobacterial species like pathogenic Mtb, vaccine strain *Mycobacterium bovis*- bacillus Calmatte- Guérin (BCG) and rapidly dividing non-pathogenic *Mycobacterium smegmatis* (Msmeg), followed by indirect-immunofluorescence microscopy to visualize host vacuolar markers. Thereafter, automated quantification of degree of co-localization between mycobacteria and host vacuolar markers like TfR and LAMP1 is done by processing the binary images of bacteria using mathematical tools. This results in quantification of the mean fluorescence intensity (MFI) of these host markers directly around the bacteria/bacterial clusters with increased sensitivity relative to when done manually. By manipulating host or pathogen, this assay can be used to evaluate host or bacterial determinants of intracellular trafficking. The basic method can be applied to studying trafficking of other bacteria or particles like beads, although the kinetics of infection and phagosome maturation will depend upon the phagocytic cargo. The mathematical analysis tools are available in many standard imaging analysis programs. However, any adaption for similar analysis should be confirmed by the individual user with their imaging and analysis platform.

Materials and Reagents

Note: All work with live Mtb must be performed in a Biosafety Level 3 (BSL3) facility according to institutional standards of practice.

1. Macrophages, either primary macrophages, such as C57BL/6 bone marrow-derived macrophages (BMDMs) or a macrophage cell line (such as RAW264.7)

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Analysis of Mycobacterial Protein Secretion

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Abstract

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis. Analysis of proteins secreted by Mtb has been of interest to the field of tuberculosis research since certain secreted proteins interact with the host to promote virulence, while others may be important antigens or serve as biomarkers of infection. Here, we describe a protocol to prepare whole cell extracts (WCE) and short term culture filtrate (CF) from Mtb or the vaccine strain *Mycobacterium bovis*-bacillus Calmatte- Guérin (BCG) (Mehra *et al.*, 2013). These are both slow growing mycobacteria, but the same basic procedure can easily be adapted to analyze secreted proteins from rapidly growing mycobacteria, such as *Mycobacterium smegmatis* (Msmeg), a non-pathogenic species commonly used in the laboratory. The fractions obtained can be analyzed by western blotting to examine proteins of interest or by mass spectrometry if antibodies are not available or to examine the entire secretome. Genetic knockout mutants for the gene of interest serve as a negative control. Additionally, levels of a cytosolic protein such as the chaperone GroEL or the pyruvate dehydrogenase E2 component sucB (Rv2215/dlaT) should be assessed in the CF fraction to rule out the possibility that a positive signal in CF is due to bacterial lysis (see Figure 1). By varying the growth conditions of the strain, this *in vitro* secretion assay can be used to examine conditions that alter the secretome. We are thankful to Magnus Stiegedal for helpful tips on TCA (trichloroacetic acid) precipitation.

Materials and Reagents

Note: All work with live Mtb must be performed in a Biosafety Level 3 (BSL3) facility.

1. Middlebrook 7H9 Broth (Difco, catalog number: 271310)
2. Tween-80 (Sigma-Aldrich, catalog number: P4780)
3. Glycerol (Sigma-Aldrich, catalog number: G5516)
4. Albumin-dextrose-catalase (ADC) (BD, catalog number: 212352)
5. Oleic-albumin-dextrose-catalase (OADC) (BD, catalog number: 212351)
6. Potassium phosphate (monobasic) (KH_2PO_4) (Sigma-Aldrich, catalog number: P9791)
7. L-asparagine monohydrate (Sigma-Aldrich, catalog number: A8381)

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