d. Signed details of the excellence in research work for which the Sun Pharma Research Award is claimed, including references and illustrations. The candidate should duly sign on the details (Max: 2.5 MB)

A smart pathogen like *Mycobacterium tuberculosis* (Mtb), lacks typical toxins but uses multiple spatio-temporally regulated **multiple virulent factors** to subvert protective responses of the host to favor its survival (***J. Immunol. [2018]201:1421***; ***J. Biol. Chem.[2013]288:24956***). Since the discovery of INH in the 1940s, no effective antimycobacterial drugs has been commercially available making the disease a huge public burden especially with the emergence of MDR/XDR strains. To desist convergent adaptive evolution of the pathogen, host-directed therapy appears to be a futuristic and an attractive alternative to drug-based therapeutic strategy to control tuberculosis. As a first step towards this direction, an understanding of the host-pathogen interaction is imperative. Identification of important virulent proteins of Mtb is important for TB care and management program. The arsenal of Mtb is equipped with several such proteins which help the bacterium to avoid and weaken protective host immune responses. **Dr Mukhopadhyay has made immense and excellent contribution in 'Disease Biology' with a focus to understand molecular mechanism of pathogenesis of *Mycobacterium tuberculosis* (Mtb) based on her studies in in vitro cell culture and animal model as well as in clinical settings**. Her work contributed to the knowledge on how the innate and adaptive (Th1/Th2) immune response of host are hijacked by two mycobacterial proteins like ESAT-6 and PPE18 which are potential drug targets. Also she has highlighted pathogenic properties of PPE2, PE11 and PknG proteins of *M. tuberculosis* that also play crucial roles in successful establishment of the bacteria inside host.

Research work carried out in her laboratory has significantly advanced the knowledge in the areas of Tuberculosis pathogenesis and its cross-talk with the host's immune mechanisms which laid solid platforms for translational research opportunities in these areas. Her work established important clues about immunosuppression caused by excess free radicals produced during infection (***Blood[2006]107:1513***) and use of N-acetyl cysteine (NAC) as a IL-12-Th1-based adjuvant to boost anti-TB immune response based on the data obtained from in vitro and clinical settings (***J. Immunol.[2010]184:2918)***.. She for the first time hints about the pleiotropic effect of ESAT-6 in manipulating several protective functions of host through its

direct interaction with beta-2 microglobulin (β2M) of host macrophages (***PLoS Pathog.[2014]10:e1004446***). β2M is an important molecule that binds to MHC class I and regulate MHC class I antigen presentation and CD8 T cell function. Also it is an interacting component of HFE that is involved in intracellular iron uptake as well β2M binds to CD1 which is the only one involved in mycobacterial lipid antigen presentation. Thus her work describes the diverse moonlighting functions relating to pathogenesis of ESAT-6. In her next approaches, she identified two FDA approved drugs (Mirabegron and Olsalazine) that bind to ESAT-6, thus preventing its direct interaction with β2M. This results in improved host responses that are associated directly with β2M resulting in reduced bacterial survival (***J. Immunol.[2019]203:1918; J. Immunol[2020]*** **205:3095**). Thus, her research ushers promising therapeutic effect of Mirabegron and Olsalazine to be used either as standalone therapy or as an adjunct to current DOTs based regime against *M. tuberculosis.*

Also she for the first time showed that Mtb PPE18 protein activates IL-10/Th2 environment (known to favor Mtb infection) by targeting the TLR2 ectodomain and downstream PKCε-IRAK3-MKP-1 signaling cascades (***J. Immunol.[2016]197:1776***). Based on these findings, she identified TLR2-LRR 11~15 domain as a novel target for designing drugs to block excess IL-10/Th2 response. She identified two FDA approved drugs which can block TLR2-PPE18 interaction and thus can be repurposed for blocking excess IL-10 with simultaneous increase of IL-12/Th1 response, this is funded by the TATA Innovation Fellowship by DBT. Govt of India (2017-2018). Also, she for the first time found that Mtb PPE2 protein mimics eukaryotic transcription factor to translocate to nucleus where it sterically inhibits transcription from iNOS promoter (***Sci. Rep[2017]7:39706***). In addition, the protein has SH3 domain that allows it to interact with $p67^{phox}$ resulting in inhibition of reactive oxygen species (***J. Immunol[2019]203:1218***). PPE2 also inhibits mast cells and myeloid haematopoiesis ((***Immunobiology[2020] 226:152051***). This discovery has fetched an USA patent (***USA patent granted[2013***]. Also, for the first time she showed that Mtb PE11 protein with esterase activity plays a crucial role in regulating cell wall architecture and provide defense to the bacilli against antibiotics and cellular stressors. Thus, inhibitors/drugs that reduce esterase activity of PE11 appears to be a promising drugs that can alter *M. tuberculosis* cell wall architecture and can be used as novel anti-TB therapeutics as well as may increase efficiency of currently available anti-TB drugs to treat MDR/XDR (***Sci. Rep[2016] 6:21624***). Again, for the first time, we showed a role of Rab7l1 in phagosome-lysosome fusion and Mtb PknG protein targets the Rab7l1 signaling to block phagosome-lysosome fusion for favoring Mtb survival inside

macrophages (***J. Immunol.[2018]201:1421***). She showed involvement of ESAT-6, PPE18 and PPE2 in pathogenesis of tuberculosis in patients with active TB which indicate that these proteins are crucial virulent factors of Mtb and potential targets for designing of anti-TB therapeutics.

Most Significant Outputs

Identified for the first time novel virulence mechanism of ESAT-6, PPE2, PPE18, PE11 and PknG proteins of *M. tuberculosis* and its implication as potential drug targets

Identification and validation of FDA approved drugs for selective blocking of PPE18-TLR2 LRR 11~15 domain interaction with an aim to inhibit the non-protective anti-inflammatory responses with simultaneous increasing of Th1-type anti-TB protective immune responses.

Immunosuppressive role of free radicals and application of antioxidants as therapeutics to treat people undergoing immunosuppression during stress/infection

Role of Mast cell in regulation of inflammation and designing of novel molecules that target mast cell signalling to control inflammatory disorders like inflammatory bowel disease (IBD), tissue injury and scar-less wound healing.

Translational Potential

a. Application of antioxidants to boost immune system to control tuberculosis

b. Designing of novel drugs against tuberculosis

c. Designing of therapeutics to control IBD, inflammation/tissue injury and wound healing.

Societal Relevance

India accounts about a quarter of global TB burden and more than 4 million people are dying every year. With the emergence of MDR TB, the situation has become alarming. With no new drug developed in the last 40 years, there is an urgent need for developing effective therapeutic drug to control the menace. Understanding the molecular etiology of bacterial virulence and its interaction with the host is necessary to identify suitable drug target. Targeting bacterial virulence factors and/or cell-to-cell signalling pathways is thought to be more rational than traditional antibiotic-based therapies. In this context, she has made seminal contributions to understand the mechanism of pathogenesis of tuberculosis. This basic research laid an excellent foundation for carrying out further translational research to design better and efficacious drugs and therapeutic interventions to control tuberculosis. In addition, she is striving to design novel

biological molecule to treat inflammation/tissue injury and scar-less wound healing that has important impact on drug market.

Media Highlights

1. https://www.thehindu.com/sci-tech/health/novel-mechanism-may-lead-to-better-tb-control/article6764393.ece

2. http://vigyanprasar.gov.in/isw/A-new-boost-to-anti-tb-crusade.html

Dr. Mukhopadhyay has published 62 research papers in high impact factor peer reviewed Journals and one USA patent has been granted. She has filed two Indian patents and one International patent in the area of Sepsis and Inflammation. This Scientific contribution is well recognized by the 'ASH, USA, 2007' and 'AAI, USA, 2011' and by 'The National Academy of Sciences of India', 2010'; 'The Indian Academy of Sciences, Bangalore, 2013'; 'The Indian National Science Academy, New Delhi, 2016' and 'The Telangana Academy of Sciences, Telangana' by electing her as a 'Fellow'. Also, she is serving as member of various Institutional committees as well as committee of National importance like 1) Member of DST-WOS-A, Govt of India, 2) Member of Research Progress Committee of Nirma University, Ahmedabad, 3) Member of the CSIR (Medical Science) and DBT Task Force Committee (Infectious Disease); 4) Member of the Twinning R&D program for NER (Medical Biotechnology of DBT; M.Sc Biotechnology Advisory Committee of University of Hyderabad. Member of the Scientific Advisory Committee of NIBMG, West Bengal. She was also a Special Invitee of the RAP-SAC committee of NCCS, Pune, 2015 and 2016, Committee member of INSA (Health Science), DST INSPIRE, CSIR SKM Fellowship, INSA JRD-TATA, Inspiring Science Award and Chairperson of various Institutional (CDFD) committees. Also she served as reviewer of several grant proposals of Indo-Taiwan, DBT, Govt. of India and DST, Govt. of India, CSIR, Govt of India, UPE-II Research Project proposals of University of Hyderabad, Dr. D.S. Kothari Postdoctoral Fellowship, Pune. She also served as peer reviewer of several National and International journals like Indian Journal of Medical Research, Current Science, PLoS ONE, Journal of Biosciences, Journal of Infectious Diseases, Genes to Cell, DNA and Cell Biology, Journal of Medical Microbiology, Scientific Reports, IUBMB Life etc. and examiner of PhD/M.Sc students of several Universities and Institutes of India and abroad.

Detailed description of the work

Objective 1. Studying how various candidate proteins of *Mycobacterium tuberculosis* (ESAT-6, PPE18, PPE2, PE11 and PknG) interfere with macrophage signaling cascades to modulate host's protective responses against the bacilli and identification of novel therapeutic targets to improve anti-tuberculosis immunity of host.

Development of effective therapeutics and vaccines against tuberculosis (TB) has been one of the priority areas of global biomedical research, including India. Among the infectious diseases, tuberculosis is one of the major killers in India which has nearly one-third of the world's TB patients. Downregulation of the host's protective response by mycobacteria is one of the factors responsible for successful establishment of the pathogen. Virulence of *Mycobacterium tuberculosis* is governed by multiple factors and therefore understanding the mechanism of pathogenesis of tuberculosis is essential for identification of suitable drug target and candidate TB vaccines. Interference with bacterial virulence and/or cell-to-cell signaling pathways is an especially compelling approach, as it is thought to apply less selective pressure for the development of bacterial resistance than traditional antibiotic-based therapies, which are aimed at killing bacteria or preventing their growth ***(****David et al.[2010] Nature Reviews Drug Discovery, 9:117****).*** In this regard, Dr. Mukhopadhyay has made seminal contributions to understand how some of the cardinal host signaling pathways are hijacked by *Mycobacterium tuberculosis* to downregulate protective immune response mounted by the host (the studies include in vitro cell culture work, mice infection model and TB patient samples). Based on the leads generated from basic bio-medical research, she has further extended her study to design novel drugs and therapeutic interventions useful to control tuberculosis.

The major research studies carried out by Dr. Mukhopadhyay are described below

1a) A novel virulence mechanism of ESAT-6 protein of *M. tuberculosis* to suppress MHC class I antigen presentation and CD8 T cell response of host and to regulate iron homeostasis and identification of drug molecules targeting ESAT-6 protein to increase protective immunity of the host.

The exact mechanism of how ESAT-6 protein manipulates host immune response is not fully known. ESAT-6 is present in *Mycobacterium tuberculosis* but absent in the vaccine strain *M. bovis* BCG. Dr. Mukhopadhyay for the first time demonstrated a novel virulence property of ESAT-6 protein. The study presents a hitherto unknown virulence mechanism by which *M. tuberculosis* may subvert or delay the cytotoxic T cell (CD8) response of the host involving the ESAT-6 protein. Using the yeast two hybrid assay Dr. Mukhopadhyay has screened the human leukocyte cDNA library and identified beta 2-microglobulin (β2M) as an interacting partner of ESAT-6. β2M is an ~12 kDa protein that associates with major histocompatibility complex (MHC)/human leukocyte antigen (HLA)-I (controls MHC class I antigen presentation), CD1 (regulates presentation of lipid antigens to T cells) and HFE (regulates iron absorption) molecules of macrophages. Association of these molecules with β2M is a pre-requisite for their surface expression. Dr. Mukhopadhyay found that ESAT-6 is translocated to the endoplasmic reticulum (ER) of macrophages where it interacts with β2M resulting in reduced surface expression of β2M and HLA molecules and compromised MHC class 1-restricted antigen presentation to CD8 T cells (Figure 1). This is the first report about an interaction between a virulent mycobacterial protein ESAT-6 and host molecule β2M and the effect of this interaction on MHC class I antigen presentation and lipid antigen presentation as well as interference with iron uptake (***Sreejit et al.[2014]PLoS Pathogens,10:e1004446 [citation - 114]***). This work not only identifies a novel interaction but also highlights a novel mechanism of suppression of MHC class I antigen presentation that might plays a crucial role in subverting adaptive immune responses during *M. tuberculosis* infection. Indeed, the presence of ESAT-6:β2M complexes in pleural biopsies of pleural TB patient proves formation of ESAT-6:β2M complex is a virulence mechanism during *M. tuberculosis* infection situations too.

Dr. Mukhopadhyay has further shown that the interaction of ESAT-6 with β2M also results in downregulation of surface expression of HFE; a protein regulating iron homeostasis via interacting with transferrin receptor 1 (TRF1). It was observed that ESAT-6:β2M interaction leads to sequestration of HFE in ER causing poorer surface expression of HFE and HFE:TFR1 complex in macrophage, resulting in increased holotransferrin mediated iron uptake in the

macrophage that could favor longer survival of *M. tuberculosis* in macrophage during infection (***Jha et al.[2020]Journal of Immunology,205:3095***).

When the thermodynamics parameters of ESAT-6 and β2M were validated using Isothermal calorimetry (ITC), it was observed that ESAT-6 interacts with β2M in 1:1 ratio (Figure 2A). Interestingly, Dr. Mukhopadhyay showed that Asp53 residue of β2M is important for the interaction and stabilization of ESAT-6:β2M complexation. The structure-function relationship has been explored which indicated the existence of strong non-covalent hydrophobic interactions between ESAT-6 and β2M in addition to the vital hydrogen bond between aspartate residue (Asp53) of β2M and methionine (Met93) of ESAT-6 (Figure 2B,C). In an attempt to design small molecule inhibitor(s) that can mask the critical Met93 residue of ESAT-6 required for ESAT-6:β2M interaction and thereby renewing class I antigen presentation and CD8 T cell activity, docking based HTVS followed by 16 point screening on MST was carried out which identified two FDA approved drugs, SM09 (Mirabegron) and SM15 (Olsalazine) that specifically mask the Met93 residue of ESAT-6, and prevents interaction of ESAT-6 with β2M. This results in rescuing of ESAT-6-mediated inhibition of class I antigen presentation and CD8 T cell activity (Figure 2D,E). Interestingly, both the drugs reduced the ability of *M. tuberculosis* bacilli to survive inside macrophages (Figure 2F) (***Journal of Immunology[2009]203:1918***).

Leads generated: Dr. Mukhopadhyay has identified two drugs, Mirabegron and Olsalazine as novel anti-TB drugs that have anti-TB therapeutic effects. This is an attempt towards designing of anti-virulence strategies for boosting protective immune responses to fight against tuberculosis. Currently, her group is in an attempt to understand other pleiotropic effects of ESAT-6 to subvert host immune responses..

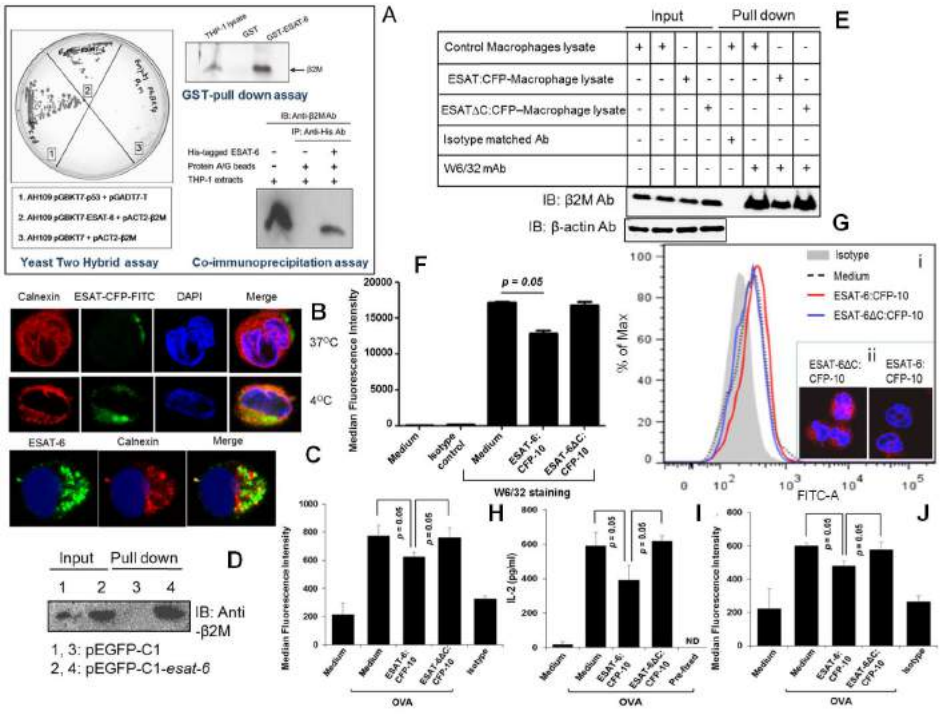

Figure 1. ESAT-6/ESAT-6:CFP-10 protein interacts with β2M and inhibits class I antigen presentation function of macrophages. Yeast two-hybrid assay followed by GST pull-down and co-immunoprecipitation assay indicates a positive interaction between ESAT-6 and β2M (A). Confocal microscopy suggests that exogenously added ESAT-6:CFP-10 (B) as well as intracellularly expressed ESAT-6 (C) gain access into calnexin-positive ER and interacts with β2M present in the ER (D). Less amount of β2M was complexed with HLA class I molecules in ESAT-6:CFP-10-treated macrophages compared to untreated as well as those treated with ESAT-6ΔC:CFP-10 as revealed by co-immunoprecipitation assay using W6/32 (E) resulting in reduced HLA class I-β2M complex formation on THP-1 macrophages (F) increasing the levels of levels of β2M-free HLA class I molecules on the macrophage surface (Gi) as well as intracellularly (Gii). ESAT-6 inhibits cytosolic (H) as well as cross-presented ovalbumin (I) affecting the class I antigen presentation to CD8 T cells (J).

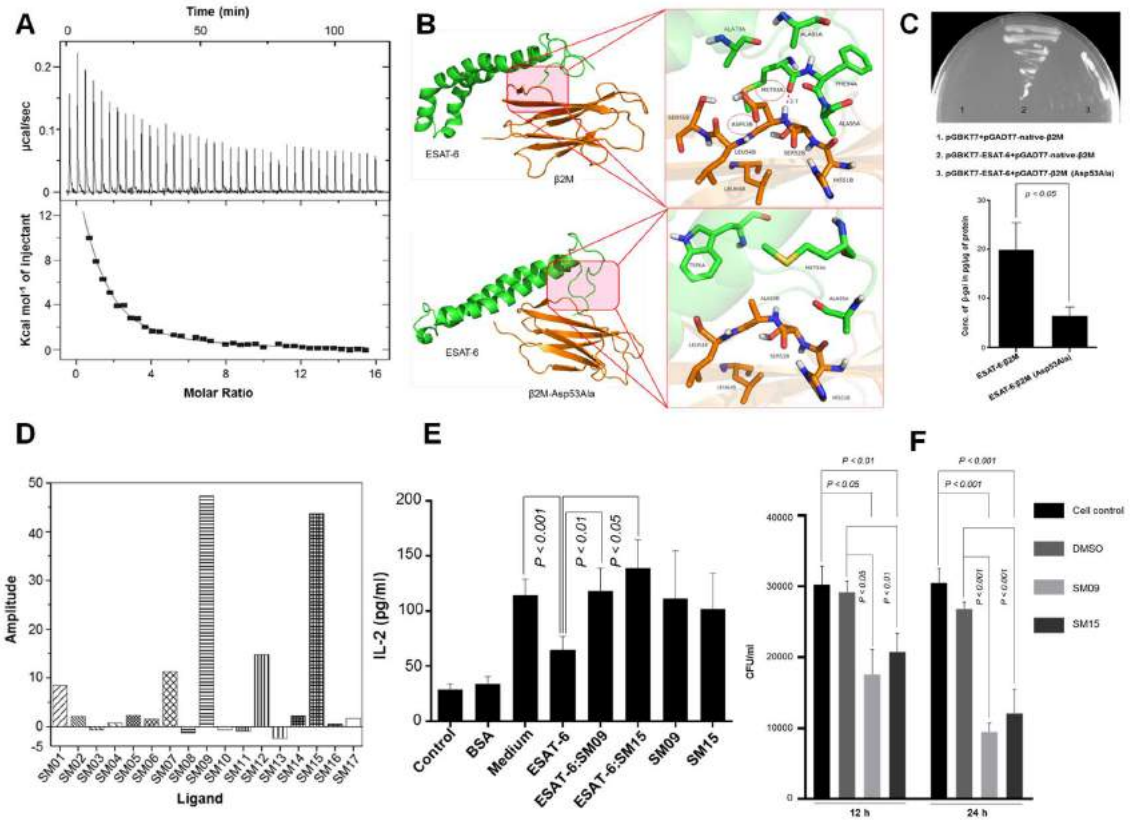

Figure 2. Figure 1. ESAT-6:β2M interaction and identification of small molecules that could inhibit this complexation. (A) ESAT-6:β2M interaction by Isothermal Titration Calorimetry, (B) Key active site residues of the ESAT-6 protein and β2M protein interface. The complex structures were obtained from protein-protein docking followed by 10 ns MD simulations. The interface of ESAT-6 (Met93 of A-chain) and β2M (Asp53 of B-chain), highlighting of the hydrogen bond interactions is shown in pink dotted lines and important residues are shown in stick model i.e. ESAT-6 (green color) and β2M (orange color). The protein structure is represented by chain β2M (orange) and ESAT-6 (green) color. The crucial residues involved in the interaction are circled. (C) Asp53 residue of β2M is crucial for interaction with ESAT-6. A yeast two-hybrid assay was performed to examine the interaction between ESAT-6 and native β2M or mutant β2M (Asp53Ala), (D) Single point screening of the docked compounds for identification of the strong binders with ESAT-6. (E) Mice peritoneal macrophages were pre-treated with ESAT-6 in the absence or presence of SM09 or SM15 for 2 h. Cells were then cytosolically loaded with hypertonic OVA for MHC class I antigen presentation. Next, fixed macrophages were incubated with B3Z T cells. The levels of IL-2 secreted in the culture supernatants were measured by ELISA. Data shown are Mean ± SEM of three independent experiments. (F) PMA-differentiated THP-1 macrophages were infected with H37Rv *M. tb* strain at MOI 1:10 for 4 h and extracellular bacteria were killed by incubating the cells in medium containing amikacin acid for 2 h. Next, THP-1 macrophages were incubated in fresh medium containing 12.5 µM of SM09 or SM15 for 12 h and 24 h time points. Cells were lysed in 0.06% SDS and bacterial CFUs were counted on 7H11 agar plates. Data shown are Mean ± SEM of three independent experiments.

1b) PPE18 as an important virulent factor of *M. tuberculosis* and designing of anti-tuberculosis therapeutics targeting the PPE18-TLR2

In the area of tuberculosis research, Dr. Mukhopadhyay has major leads in understanding the toll-like receptor 2 (TLR2) signaling in the pathogenesis of tuberculosis. (***Hussain Bhat K and Mukhopadhyay S[2015]Future Microbiology,10:853; Ahmed et al.[2015]IUBMB Life, 67:414; Bhat et al.[2012]Journal of Biological Chemistry 287:16930; Parveen et al.,[2013]Journal of Biological Chemistry,288:24956;***). It is known that one of the major immune evasion strategies employed by *M. tuberculosis* is its ability to evoke a deliberate T helper (Th) 2 response (that is critical for its survival) which in turn downregulates the anti-mycobacterial protective Th1 response. Dr Mukhopadhyay has identified that the PPE18 (Rv1196) protein of *M. tuberculosis* is a strong anti-inflammatory protein that activates IL-10 and subsequently inhibits the protective IL-12 and TNF-α cytokines and skews the T cell response towards the non-protective Th2-type. *In silico* docking analyses and mutation experiments indicate that PPE18 specifically interacts with the LRR 11~15 domain of TLR2 to increase IL-10/Th2 response (***Nair et al.[2009]Journal of Immunology,183:6269 [citation - 189]***). She showed that blocking this interaction could increase the protective Th1 response in clinical settings. She further hinted that PPE18 increased SOCS3 which physically interacts with IκBα, inhibiting its phosphorylation at serine 32/36 residues and its degradation. As a consequence, p50/p65 NF-κB and c-rel transcription factors get sequestered in cytoplasm causing downregulation of NF-κB/c-rel-mediated transcription of IL-12 and TNF-α. This study brings to light a novel mechanism of downregulation of NF-κB/rel signaling involving the SOCS3 which is a possible strategy employed by *M. tuberculosis* to suppress host protective Th1 responses (***Nair et al.[2011]Journal of Immunology,186:5413 [citation - 82]***). She further revealed that TLR2-LRR domain can strongly influence macrophage signaling cascades regulating the pro- and anti-inflammatory cytokines profiles. She for the first time hinted that while TLR2 LRR 11~15 domain specific signaling is crucial for activation of p38 MAPK and IL-10, the TLR2 LRR 15~20 is responsible for activation of TNF-alpha and Th1-type response (***Bhat et al.[2012]Journal of Biological Chemistry,287:16930 [citation – 29]; Udgata et al.[2016]Journal of Immunology,197:776 [citation – 15]***). PPE18 was found also to inhibit both MHC class II antigen presentation and the consequent CD4 T cell responses (***Dolasia et al.[2021]European Journal of Immunology,51:603***) The *in vivo* infection studies in mice model clearly indicates an important role of PPE18 during *M. tuberculosis* pathogenesis as mice infected with *ppe18* deleted *M. tuberculosis* had lower infection burden and better

survival compared to mice infected with wild-type strain (***Bhat et al.[2012]PLoS ONE,7:e52601 [citation – 45]***) (Figure 3). This opens up new possibilities to target the TLR2 LRR domains for rational designing of TLR2-immunomodulators to specifically block PPE18-induced anti-inflammatory signaling (that favors *M. tuberculosis* infection) which may serve as novel therapeutics against tuberculosis. Dr. Mukhopadhyay has started exploring this idea in identifying FDA approved drug molecules to specifically block the interaction of PPE18/PPE18 like proteins of *M. tuberculosis* with TLR2 LRR 11~15 domain, thus inhibiting the excess Th2-type response in turn increasing the protective IL-12/Th1-type immune response.

Leads generated: Around 2950 anti-microbial activity compounds were docked and were prioritized according to the docking score, ADME properties and its key active site interactions. Using protein-protein docking and molecular modelling (Schrodinger, LLC), compounds that had high Glide score were shortlisted for specificity towards the ligand TLR2. Dr. Mukhopadhyay eventually went on to identify FDA approved drugs that could constrain the interaction of PPE18 with TLR2 LRR 11~15 domain, which were further confirmed by IL-10 cytokine assay. Three drugs showed promising result in inhibition of IL-10 production during PPE18 triggering as well as during *M. tuberculosis* ex vivo infection assay. The molecules showed promising results in increasing antigen presentation both in mice and human PBMC samples. This study has immense importance to specifically target the TLR2-LRR domain to increase T cell response and especially a Th1-type immune response that is known to be protective against *M. tuberculosis*. These drugs not only will tackle tuberculosis but also other intracellular infections where TLR signaling plays an important role (***Manuscript under submission***). This project is supported by the prestigious TATA Innovation Fellowship (2017 – 2018) by DBT, Govt of India.

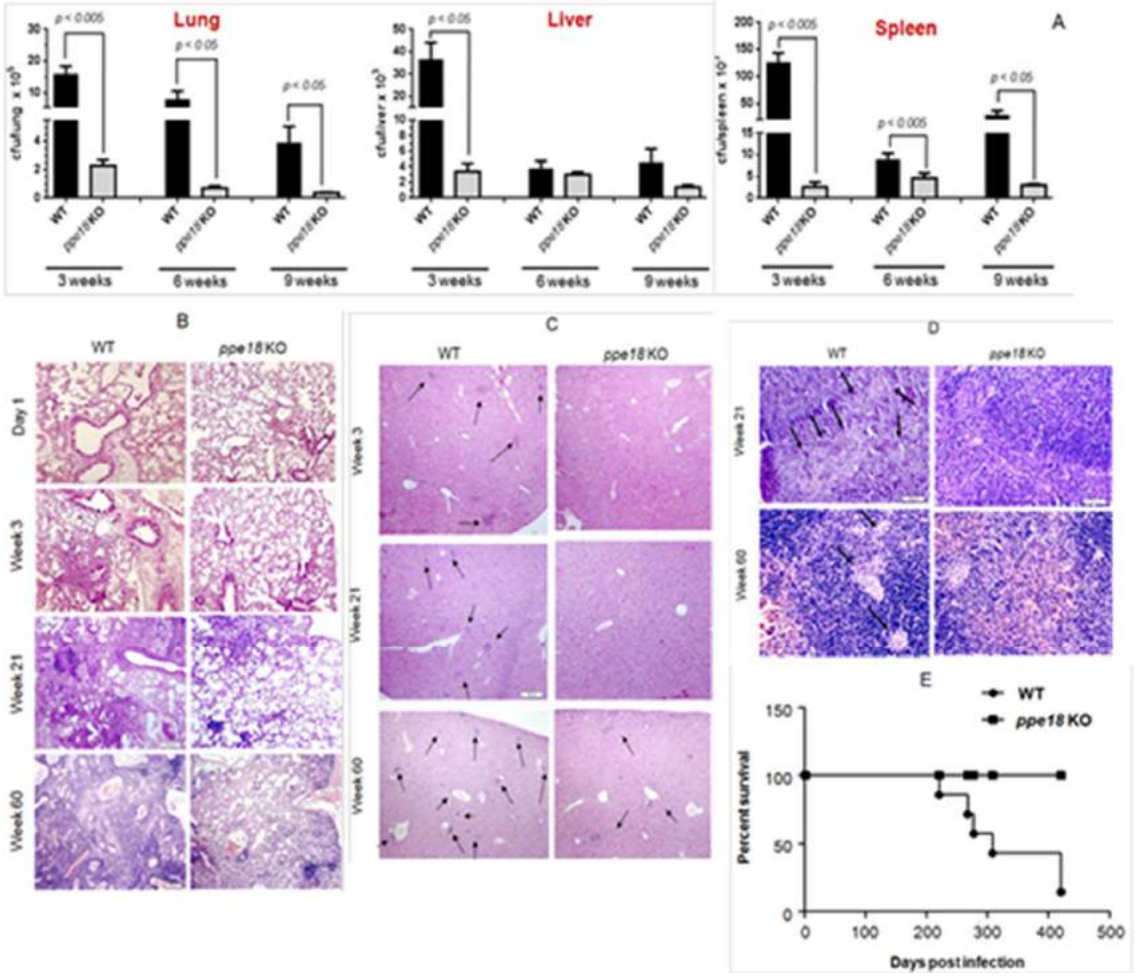

Figure 3. PPE18 protein plays an important role in intracellular survival and pathogenicity of *Mycobacterium tuberculosis* in mice. C57BL/6 mice were infected aerogenically with a low dose of either WT or *ppe18* KO strains of *M. tuberculosis*. At different time points post infection, mice were sacrificed and CFU counts were measured in lung, liver and spleen (A). Data are mean ± SEM of results for five mice per group for each time point. Liver (B), Lung (C) and Spleen (D) sections from mice infected with either WT (left panel) or *ppe18* KO (right panels) strains of *M. tuberculosis* were stained with Hematoxylin and eosin (H&E) at different time points post infection. Photographs of representative sections from 2 mice visualized at 40X magnification are shown. Arrows indicate the sites of lymphocytic infiltration. In another set of experiment, survival of C57BL/6 mice (n = 8) following a low-dose aerosol infection with either WT or *ppe18* KO strains of *M. tuberculosis* was monitored for 420 days post infection (E).

1c) Role of PPE2 protein in mycobacterial virulence

To continue understanding further on the role of PE/PPE family of proteins in modulation of macrophage immune response and mycobacterial virulence, Dr. Mukhopadhyay importantly demonstrated that the PPE2 (Rv0256c) protein of *M. tuberculosis* can strongly inhibit the cytotoxic nitric oxide (NO) production by entering into the macrophage nucleus and binding to the inducible nitric oxide synthase (iNOS, which is responsible for production of NO) promoter region. PPE2 contains the nuclear localization signal (NLS) and also a DNA-binding motif. She observed that PPE2 is secreted by the bacteria and is translocated to the nucleus via the NLS signal present in PPE2. Importantly, PPE2 binds to the iNOS promoter at the GATA transcription factor binding site and inhibits iNOS transcription and nitric oxide production (Figure 4). Ex vivo infection studies further confirmed an important role of PPE2 in the inhibition of iNOS/NO as production of nitric oxide was significantly higher in macrophages infected with *ppe2* deleted *M. tuberculosis* strain as compared to the macrophages infected with wild-type *M. tuberculosis* strain. Thus this mycobacterial protein mimics the typical eukaryotic transcription factor (**which is a novel information revealed from this work**) which is responsible for immediate inactivation of the iNOS transcription, thus saving the bacteria from the cytotoxic attack of NO (***Bhat et al.[2013]Annals of the New York Academy of Sciences,1283:97 [citation – 32]***; ***Bhat et al.[2017]Scientific Reports,7:39706 [citation – 24]***).

In addition to having NLS and DNA binding domain, bioinformatics study reveals the presence of eukaryotic like SH3 domain and a PxxP motif in PPE2. PPE2 interacts with $p67^{phox}$ subunit of NADPH oxidase in the cytosol and hinders the migration of cytosolic subunits $p47^{phox}$ and $p67^{phox}$ from cytosol to membrane, resulting in faulty assembly of NADPH oxidase complex and inhibition of ROS production. Further, to investigate the role of SH3-like domain and PxxP motif in PPE2 mediated ROS inhibition, conserved residues in SH3-like domain to alanine (Y209A, W236A, and P249A) was mutated, also, deleted a PxxP motif (Δ540-543) in PPE2. W236A mutation was shown not to inhibit ROS generation, also, it failed to inhibit PPE2-$p67^{phox}$ interaction. This suggested that W236 residue in SH3 like domain of PPE2 is probably crucial for PPE2-$p67^{phox}$ interaction (Figure 5). These studies suggest that PPE2 may be an important target for the development of novel drugs against *M. tuberculosis*. PPE2 inhibits the production of NO and ROS in a very coordinated manner to diminish oxidative stress. The *in*

vitro and *in vivo* infection studies indeed confirm that PPE2 is important to promote intracellular survival of the bacilli inside host (***Srivastava et al.[2009]Journal of Immunology,203:1218***). Also, PPE2 was shown to be involved in inhibiting myeloid haematopoiesis (***Pal and Mukhopadhyay[2021]Immunobiology, 226:152051***). A new insight suggests that PPE2 could also be playing an important role in regulating the synthesis of Vitamin B_{12} in *M. tuberculosis*. It has been observed that PPE2 could bind to DNA located before operon ppe2-cobq-cobu suggesting, PPE2 protein might be playing a role in the regulation of the ppe2-cobq1-cobu cluster ***(Srivastava and Mukhopadhyay[2021]Biochem Biophys Res Commun,567:166***). This study is important for studying operon organization in Mtb genome. Vitamin B_{12} has fundamental role in bacterial metabolism and gene regulation and if carefully investigated, ppe2-cobq1-cobu cluster and riboswitch together, may present opportunities to translate the basic knowledge of microbial metabolism into effective therapeutic methods. All these studies shed light of mycobacterium's strategy to manipulate innate immune response of host to favor its survival by downregulating production of NO and ROS and myeloid hematopoiesis.

Leads generated: For the first time it has been hinted by Dr. Mukhopadhyay that PPE2 protein of *M. tuberculosis* could downregulate innate immune response of the host and favor its intracellular survival by inhibiting myeloid hematopoiesis as well as production of free radicals (NO and ROS). The present study not only sheds light on the role of NO/ROS in the control of *M. tuberculosis* infection but also discover a very effective manipulation strategy of *M. tuberculosis* to avoid NO/ROS mediated toxicity in macrophages. These studies highlight novel strategies adopted by *M. tuberculosis* to hijack host immune system for its better chances of survival and thus PPE2 can be considered as potent drug target. An International patent has been granted focusing PPE2 (Rv0256c) as a new drug target in tuberculosis (***USA Patent granted in December 10, 2013***).

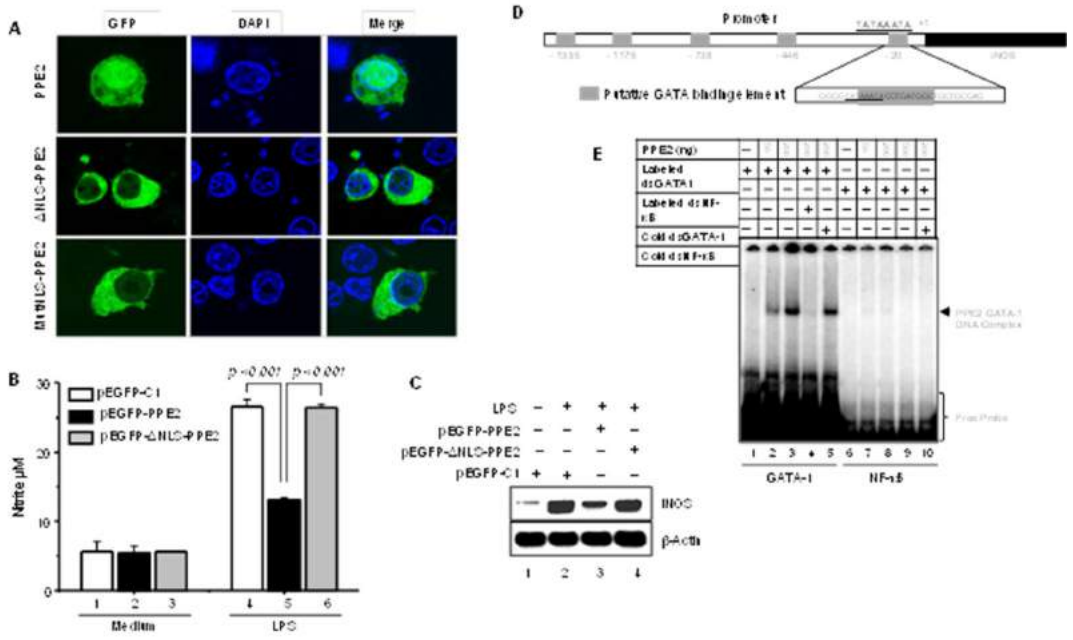

Figure 4. PPE2 protein translocates to host nucleus and inhibits nitric oxide production: (**A**) EGFP-tagged PPE2 protein (green) with mutated NLS failed to translocate into the nucleus. RAW 264.7 macrophages were transfected with either EGFP-tagged wild-type PPE2 (EGFP-PPE2) or PPE2 with truncated NLS (EGFP-ΔNLS-PPE2) or PPE2 with mutated NLS (EGFP-MutNLS-PPE2) and examined by confocal microscopy after at 4 hour post-transfection. Nuclei were stained with DAPI (Blue). (**B**) PPE2 with intact NLS inhibits nitrite accumulation in macrophages. RAW 264.7 macrophages were transfected with either pEGFP-C1 vector (control) or pEGFP-PPE2 or pEGFP-Δ NLS-PPE2. After 8 hours of transfection, the cells were either left untreated and cultured in medium alone or stimulated with 5 μ g/ml LPS. After 48 hours, nitrite accumulation was measured in the culture supernatants using Griess reagent. (**C**) RAW 264.7 macrophages were transfected as described above and after 2 hours post-stimulation with LPS, total RNA was extracted to perform semi-quantitative RT-PCR using *inos*-specific primers. β -actin was used as control. (**D**) A schematic diagram of putative GATA-1-binding consensus elements present in the *inos* promoter. The proximal GATA-1-binding element is overlapped with the TATA box. (**E**) PPE2 interacts with the proximal GATA-1-binding element. Different concentrations of recombinant PPE2 protein were incubated with labelled double stranded (ds) oligonucleotides representing the cognate proximal GATA-1 binding element or NF-κB-binding elements and the DNA-protein complexes were resolved by EMSA. In cold competition reactions, 100-fold molar excess unlabelled double stranded-oligonucleotides were used. Data shown are representative of three independent experiments.

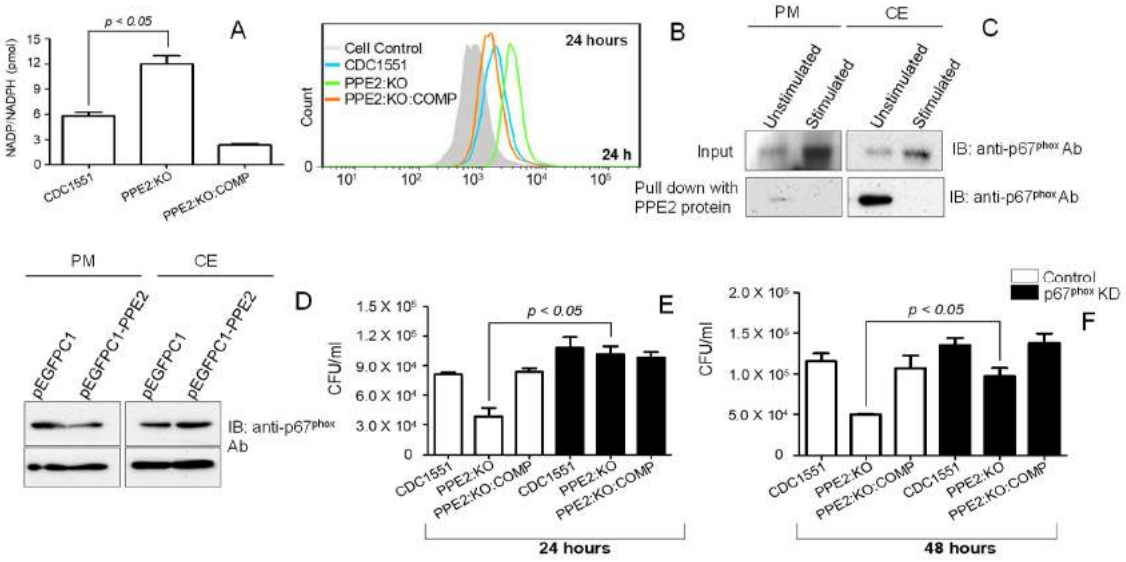

Figure 5. PPE2 protein of *Mycobacterium tuberculosis* suppresses innate immune response of macrophages by inhibiting ROS production and contribute to mycobacterial virulence. A,B. Macrophages were infected with either wild-type (control) or PPE2 deleted M. tuberculosis (PPE2:KO or PPE2 deleted *M. tuberculosis* complemented with PPE2 (PPE2:KO:COMP). NADPH oxidase complex activity (A) and ROS production (B) in macrophages was measured after 24 hours of infection. C. Pull-down experiment from macrophage lysate shows PPE2 interacts with $p67^{phox}$, a component of NADPH. D. Recruitment of cytosolic p67phox at the membrane is inhibited. E,F. Macrophages were infected with either control or PPE2:KO or PPE2:KO:COMP strains and at 24 hours (E) and 48 hours (F), intracellular survival of the bacilli was measured by CFU counting. The data indicate that PPE2 favours intracellular survival of *M. tuberculosis* in macrophages

1d) Role of *M. tuberculosis* PE11 protein in the pathophysiology of the bacilli.

Lipid metabolism plays an important role for the mycobacteria to survive in nutrient limited intracellular conditions and for maintenance of its lipid rich cell wall. The characteristic lipid-rich cell wall is a defining feature of *Mycobacterium* species. The cell wall components affect diverse mycobacterial phenotypes including colony morphology, biofilm formation, antibiotic resistance, and virulence. *M. tuberculosis* lipases/esterases play crucial roles in lipid metabolism to hydrolyse lipids and release fatty acids. The fatty acids act as precursors for the cell wall lipids and provide energy for intracellular persistence of the bacilli. Thus, it is important to study the lipases and lipid metabolism to get an insight of the molecular basis of pathogenicity of *M. tuberculosis* as indicated by Dr Mukhopadhyay (***Rameshwaram et al.[2018]Future Microbiology, 13:1301; Singh et al.[2018]Future Microbiology, 13:689-710***).

In silico analyses identified the presence of around 24 putative genes encoding lipolytic enzymes, including 24 lipid/ester hydrolases belonging to the so-called "Lip" family (LipC to LipZ). These have been annotated as putative esterases or lipases based on the presence of the consensus sequence GXSXG, which is a characteristic feature of members of the α/β hydrolase-fold family. One of these lipases, the LipX (also known as PE11; Rv1169c) was found to be up-regulated during starvation and palmitic acid stress conditions and in macrophages during infection. PE11 belongs to the PE family of genes, specific to pathogenic strains like *M. tuberculosis*, *M. bovis* and clinical strain *CDC1551* but absent in non-pathogenic bacteria, *M. smegmatis*. Upregulation of PE11 in human lung granulomas and induction of B-cell response against PE11 in TB patients indicates that the protein is probably expressed during active TB infection and has important function *in vivo*. Interestingly, *M. tuberculosis* deficient in PE11 failed to grow *in vitro* indicating that the protein is essential for *in vitro* growth of the bacilli and provide clues that PE11 is probably an essential protein for *M. tuberculosis* growth although the detail mechanisms are not well studied.

Expression of Mtb PE11 in *M. smegmatis* (PE11 is absent in *M. smegmatis*), shows a change in colony morphology when compared with *M. smegmatis* harboring the vector control alone (*Msmeg-pVV*) indicating a role of PE11 in remodeling cell wall architecture. While the colonies of *Msmeg*-pVV16 were usual irregular wrinkled acne-like structures, those of *Msmeg-PE11* were found to be rounded, shiny and smooth. Further, the control colonies were dry and fragile

but *Msmeg-Rv1169c* colonies were wetter and stickier (Figure 6A). The Scanning Electron Microscopy (SEM) data indicate that *Msmeg-PE11* cells were significantly wider in diameter as compared to *Msmeg-pVV* cells (Figure 6B). Next the Transmission Electron Microscopy (TEM) analysis showed a poor contrast and hyperstaining of *Msmeg-PE11* compared to *Msmeg*-pVV16 bacteria. This suggests that expression of *PE11* in *M. smegmatis* alters cell wall architecture. The turbidimetric esterase assay using a Tween 20 and Tween 80 as its substrates further confirmed the esterase activity of PE11. It was found that *M. smegmatis* expressing PE11 was able to form profuse pellicles as compared to the control cells (*Msmeg-pVV)*, also, PE11 increased the cell surface hydrophobicity causing an increased tendency of *Msmeg-PE11* to form cellular aggregates possibly due to an increase in the glycopeptidolipid content in the cell wall (Figure 6C and D). *Msmeg-PE11* was more resistant to various environments stressors like SDS, lysozyme, H_2O_2, and low pH (5.5) those mimicking the hostile macrophages environments encountered by the bacilli during infection as well as against antibiotics like ethambutol, rifampicin, isoniazid, ampicillin and vancomycin (Figure 6E). PE11 protein is acting predominantly as an esterase rather than lipase (Figure 6F). Interestingly, when it was quantified the cell wall fatty acids as methyl esters (FAMEs) using a high throughput gas chromatography coupled with mass spectrometry (GC/MS), a similar fatty acid composition in both the strains was observed, except an increased abundance of polar FAMEs in *Msmeg-PE11*. Mycobacterial lipids contain appreciable amounts of myristic (C14), palimitic acid (C16), and stearic (C18) and C16 - C24 monoenoic fatty acids. Expression of Mtb PE11 in *M. smegmatis* caused a noticeable decrease in the amount of linear C18:0 polar fatty acids, along with an increase in the branched chain polar fatty acid content (C18:10-methyl) which may increase the membrane fluidity and the ability of *Msmeg-PE11* to tolerate environmental stress. Mice infected with *Msmeg-PE11* had higher bacterial load, exacerbated organ pathology, weight loss, morbidity and mortality, indicating a potential role of this protein in mycobacterial virulence (Figure 6G and H). Thus, these data suggest that PE11 is actively involved in the cell wall remodeling that may confer increased drug resistance and survival advantages to the mycobacteria inside host (***Singh et al[2016] Scientific Reports, 6:21624 [citation - 80]***).

Leads generated: Based on these leads, Dr Mukhopadhyay is now involved in designing potential small molecule inhibitors that can block esterase activity of PE11, thereby affecting cell wall architecture of the bacilli. Also, the group is studying whether PE11 affects other macrophage protective responses like autophagy and polyamine signaling. This approach is likely to help generation of new anti-tuberculosis cell wall targeting drugs and/or to allow a

better efficiency of the currently available tuberculosis drugs, by either shortening of treatment duration or by lowering the treatment regimen.

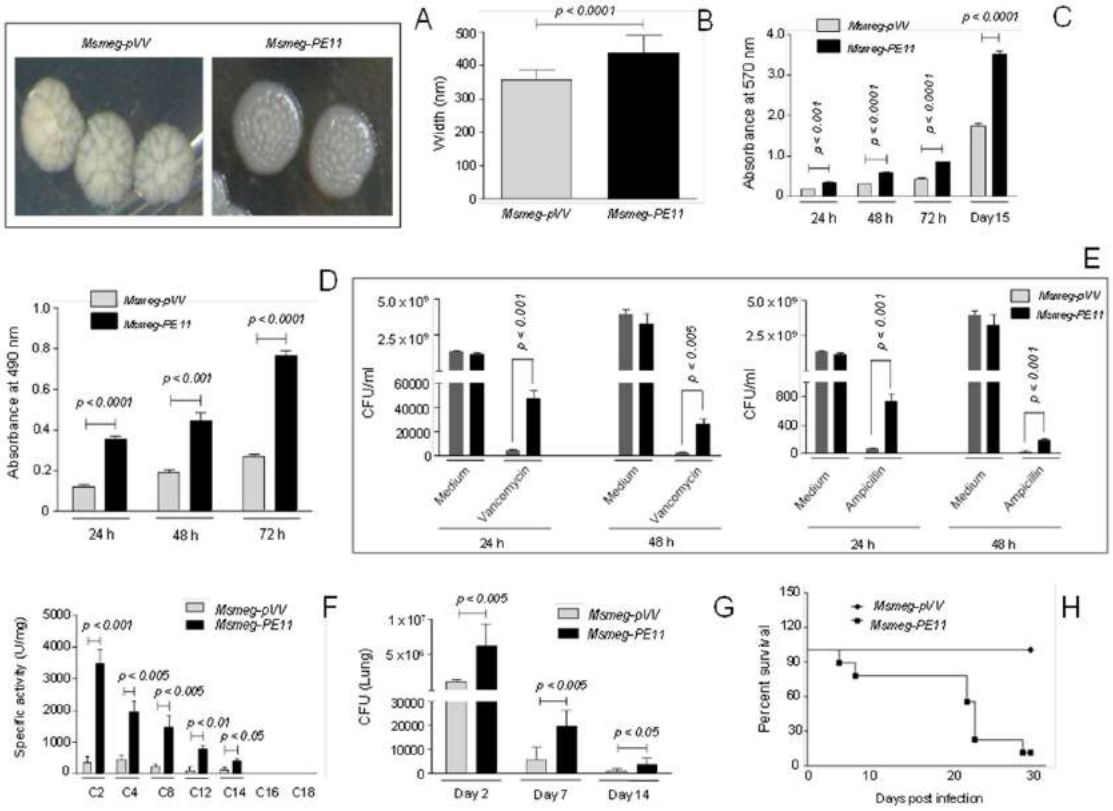

Figure 6. A novel role of PE11 protein in mycobacterial cell wall remodeling and virulence. A. Expression of PE11 in *M. smegmatis* (*Msmeg-PE11*) alters colony morphology when compared with *M. smegmatis* harboring the vector control alone (*Msmeg-pVV*). B. PE11 increases width of *M. smegmatis.* C. PE11 increases biofilm formation in *M. smegmatis*. D. Cells from *Msmeg-PE11* retain more congo red dye compared to *Msmeg-pVV* . E. *Msmeg-PE11* shows more resistant to antibiotics compared to *Msmeg-pVV*. F. PE11 shows esterase activity. G. PE11 confers a growth advantage to *M. smegmatis* in a mouse model of infection. H. Expression of PE11 reduces survival of infected mice

1e) PknG protein of *M. tuberculosis* targets the Rab7l1 signaling of macrophages to inhibit phagosome-lysosome fusion and favor bacterial survival in macrophages

Phagosomal maturation, a complex and orchestrated process regulated by numerous factors is one of the key processes used by macrophages to kill the intracellular pathogens and pathogens have acquired strategies to ensure their survival by modulating phagosomal maturation process. Dr. Mukhopadhyay for the first time identified the novel role of Rab7l1 in phagosomal maturation process and efficient killing of *Mycobacterium tuberculosis*. Interestingly, she observed that the Rab7l1 signaling is targeted by the *M. tuberculosis* PknG protein to maintain its longer persistence. Based on the yeast-two hybrid and co-immunoprecipitation assay, she observed that PknG of *Mycobacterium* sp. interacts with the human Rab GTPase protein, Rab7l1. PknG is shown to translocate to the trans-golgi where it directly interacts with inactive form of Rab7l1 (Rab7l1-GDP) inhibiting its GTPase activity and recruitment to phagosome for induction of phagosomal maturation (Figure 7). Localization of Rab7l1-GTP to phagosomes was found to be critical for the subsequent recruitment of other phagosome-lysosome markers like EEA1, Rab7, and LAMP2 during infection. Thus, PknG prevents phagosome-lysosome fusion and favors bacterial survival inside human macrophages by interfering with the Rab7l1 signaling process (Figure 7). This study is useful to understand the host-pathogen interactions and virulence process of *M. tuberculosis* Thus, designing of small molecule inhibitors that can block interaction of PknG with Rab7l1 is likely to be promising in the field of Mycobacterial drug research and designing of anti-tuberculosis therapeutics. Part of this work is published (***Pradhan et al[2018]Journal of Immunology, 201:1421[citation - 25]***). Currently, she is studying the detail signaling pathway involved in the recruitment of Rab7l1-GTP to phagosome and how PknG interferes with this process to inhibit phagosomal recruitment of Rab7l1. Also she is` studying the role of Rab7l1 in regulating various macrophage immune-effector functions like cytokine signaling, apoptosis, autophagy and antigen presentation (***manuscript under revision in Journal of Immunology***).

Leads generated: For the first time, Dr. Mukhopadhyay has hinted about the specific role of Rab7l1 in phagosome-lysosome fusion and the PknG protein of *M. tuberculosis* targets the Rab7l1-signaling to inhibit phagosome-lysosome fusion. This study hints at unique strategies of mycobacteria to interfere with Rab7l1 function to favor its survival inside macrophages. This information is helpful in understanding host-pathogen interaction and mycobacterial virulence mechanism.

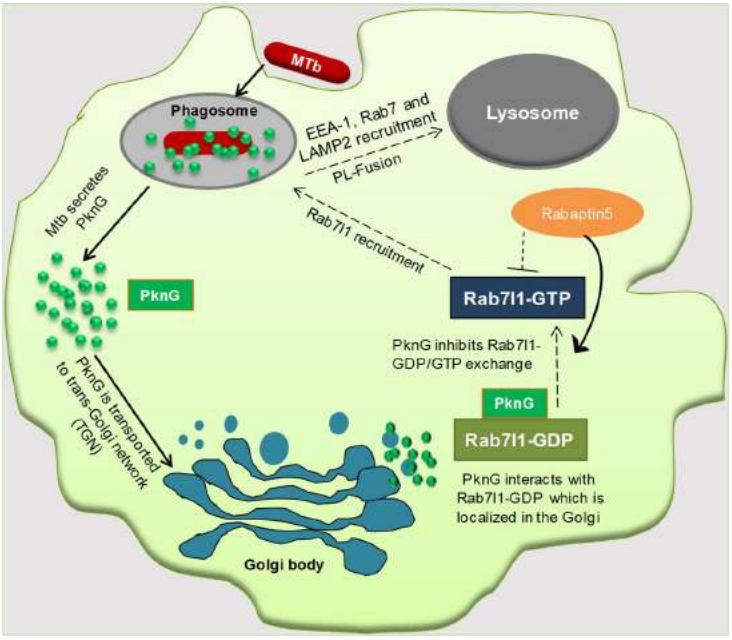

Figure 6. Model mechanisms by which PknG of *M. tuberculosis* inhibits phagosome-lysosome fusion. PknG is secreted out of *M. tuberculosis* and reaches to cytosol. PknG is transported to trans-golgi network (TGN) where it interacts with Rab7l1-GDP and inhibits Rab7l1-GDP to Rab7l1-GTP transition. PknG inhibits the recruitment of Rab7l1-GTP to phagosome and block the consequent recruitment of EEA1, Rab7 and LAMP2 and inhibits phagosome-lysosome fusion.

Objective 2. ROS inhibit IL-12 production causing immunosuppression and antioxidant (NAC) as a Th1-based adjuvant to tackle tuberculosis

Reactive oxygen species (ROS), generated during the innate immune response are considered to be important antimicrobial agents. However, in exceptional cases like tuberculosis, overproduction of ROS 'per se' do not kill Mycobacterium bacilli but rather are involved in exacerbating the disease, which hint that ROS may actually favor pathogen survival by directly suppressing host's immune responses. Dr. Mukhopadhyay's work for the first time demonstrated that excessive ROS produced by chronically activated macrophages (during certain pathophysiological conditions like tuberculosis) can actually backfire to suppress host's protective immune responses. She demonstrated that excessive ROS inhibits IL-12 (a T helper 1 (Th1) cytokine) induction that consequently suppress Th1 response via p38-MAPK-calmodulin-c-rel signaling pathway (***Khan et al.[2006]Blood, 107:1513 [citation - 48]); Boddupalli et al.[2007]Free Radical Biology and Medicine,42:686***). These may augur well to understand the basis of immunosuppression observed in diseases like cancer or infections where IL-12/Th1-dominated immune response is important for protection. Thus, her work provides novel cues for cure and prevention of a large spectrum of disorders including infectious, metabolic, and neoplastic diseases. Further, she for the first time observed that glutathione redox balance can directly affect macrophage cytokine response, mainly the IL-12. Increase in the redox glutathione level by GSH donors N-acetyl-L-cysteine (NAC) increases IL-12 and Th1 T cell responses crucial for protective immunity against tuberculosis and other intracellular pathogens but at the same time decreases TNF-alpha production. She has demonstrated that NAC at 3 mM concentration could increase bacillus Calmette-Guérin-induced IFN-gamma production by PBMCs from patients with active tuberculosis and shifts the anti-bacillus Calmette-Guérin immune response toward the Th1 type. (***Alam et al.[2010]Journal of Immunology,184:2918 [citation - 60])***).

Her collaborative efforts also helped to demonstrate unique proinflammatory properties of resistin (***Gene[2003] 205:27 [citation - 205; Biochem. Biophys. Res. Commun.[2005]334:1092 [citation – 713]; Endocr. Metab. Immune Disord. Drug Targets[2011]11:23 [citation – 52]***) that explained possible molecular link between obesity and inflammation.

Leads Generated: The results indicate that antioxidant like NAC can be used as Th1 adjuvant to boost immune system of patients infected with *M. tuberculosis* or other intracellular pathogens through specific activation of IL-12

Objective 3. Use of PPE2 protein of *Mycobacterium tuberculosis* as a therapeutic to treat inflammation and tissue injury

Modulating the inflammatory mediators in the initial stages of tissue injury would provide faster healing. Protein or peptide-based drug candidates are appreciated in targeting the specific mechanisms and likely to show fewer adverse effects than conventional chemical moiety-based drugs. Successful development of these protein/peptide-based molecules for the tissue injury would impact largely in further advancement of better and faster therapy in tissue injury. Role of mast cell in inflammation has been studied extensively. Once activated, mast cells release/degranulate anaphylactic mediators/compounds into the local microenvironment which promotes extravasation of leukocytes and plasma, causing redness, swelling and pain. Therefore, reduction of mast cell activity or mast cell population could be an excellent strategy to treat inflammation and its related disorders. Removal of mast cells will provide a better and a broad spectrum relief, but to the best of knowledge, at present there are no pharmacological drugs available to limit mast cell population.

Earlier studies by Dr. Mukhopadhyay have documented that PPE2 protein of *Mycobacterium tuberculosis* (Mtb) can induce anti-inflammatory phenotype downregulating pro-inflammatory molecule like nitric oxide (NO)/reactive oxygen species (ROS) and TNF-alpha (***Bhat et al.[2017]Scientific Reports,7:39706; Srivastava et al.[2009]Journal of Immunology,203:1218***). Also, an interesting work by Dr. Mukhopadhyay document that PPE2 inhibits mast cell population (***Pal and Mukhopadhyay[2021]Immunobiology,226:152051***). In Mtb infection, PPE2 is likely to reduce levels of host protective pro-inflammatory molecules like NO/ROS and Mast cell-mediated inflammatory reaction which can favor bacterial survival, These same properties of PPE2 can be exploited to dampen the effects of extreme inflammation observed in situations like tissue injury. PPE2 is non-toxic, inhibits transcription from the promoter of stem cell factor which is important for mast cell maintenance and migration, and downregulates expression of various markers associated with inflammation like

β-hexosaminidase, MCP-3, MCP-4, and TNF-alpha (***Manuscript under revision in EMBO Molecular Medicine***). Thus, it is possible that PPE2 can be used as an effective therapeutic to manage tissue injury.

Dr. Mukhopadhyay's work indicate that PPE2 is able to reduce the number of infiltrating mast cells and decreased inflammation and tissue damage in mice caused by formalin injection (Figure 8). When 0.02 ml of 5% formalin was injected via a sub-planter route into the right hind paw of BALB/c mice of 6-8 weeks age group and after the development of complete symptom, i.e. paw edema, recombinantly purified PPE2 (rPPE2) was administered through intraperitoneal route and paw edema was measured using vernier caliper, it was found that mice treated with rPPE2 had a significant reduction in the paw edema as compared to PBS/vehicle control (Figure 8A). Diclophenac sodium (10 mg/kg) was used as a controlled drug and was administered via the intraperitoneal route. The paw sections revealed that PPE2 treatment reduced the inflammation and tissue damage in paw samples (Figure 8B). After 21 Days, PPE2 showed almost complete abduction of inflammation and its symptoms when compared with Diclophenac and PBS control group (Figure 8C) which indicate that along with anti-inflammatory activity, rPPE2 has a tissue-protective activity too. Tissue sections of mice treated with rPPE2 showed a significant reduction in mast cell population when compared to PBS control (Figure 8D) which might be one of the major factors causing the reduction of inflammation in paw tissues. For easy cellular delivery, next she designed PPE2-derived synthetic peptide and checked its efficacy to reduce tissue inflammation. The peptide also showed an excellent effect in reducing formalin-induced paw edema which was correlated with decrease in mast cell population and mast-cell induced inflammatory molecules (Figure 8E-G). An international and National patent has been filed through BCIL based on these results (**). Presently, she is in touch with Bharat Biotech International Ltd, Hyderabad for commercialization of the product.

** **Mukhopadhyay S**, Pal R and Battu MB. Therapeutic composition for Inflammation/Tissue Injury. Indian Patent has been filed on January 7, 2020 (Priority date – January 8, 2019); Patent No. 201941000876

The US patent application has been filed on January 8, 2020 at the US Patent Office (USPTO) and the application number accorded is '16737012'.

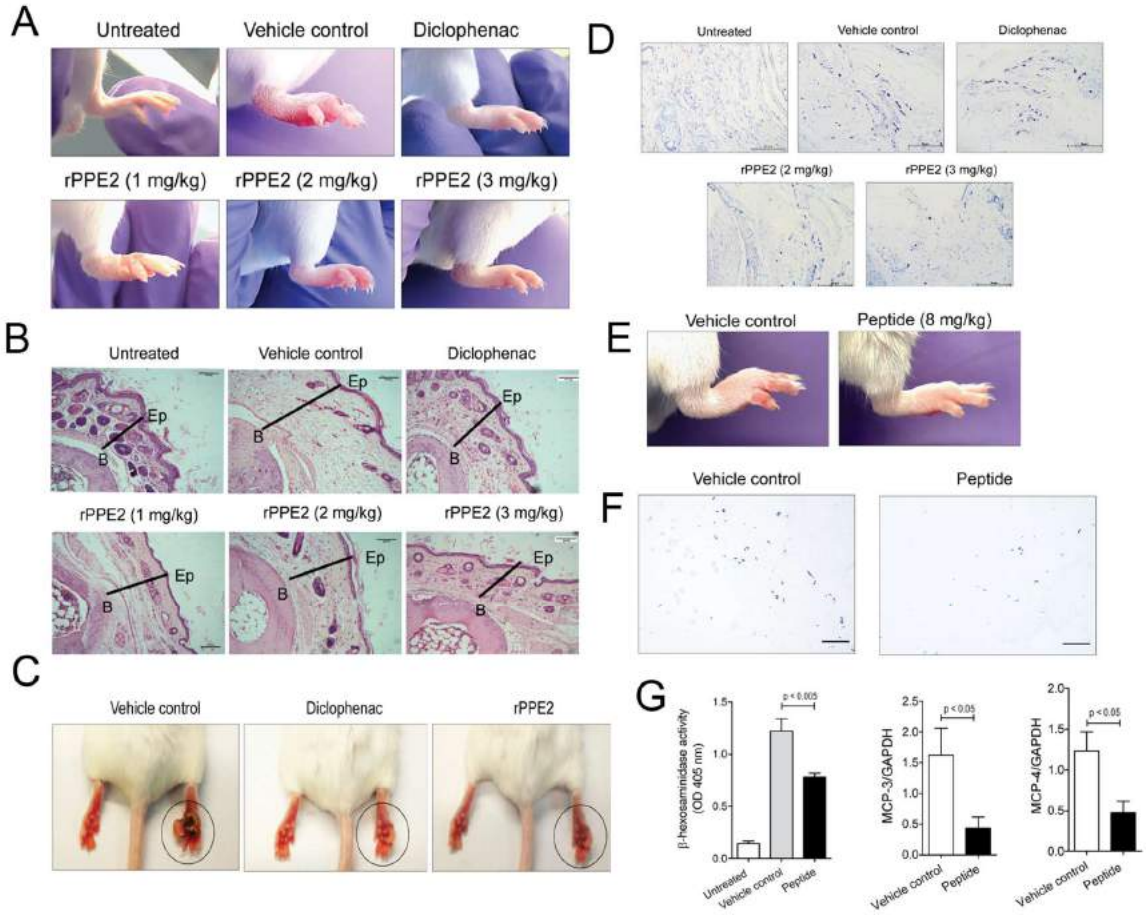

Figure 8. PPE2 and PPE2-derived synthetic peptide reduces Formalin-induced inflammation and tissue injury. BALB/c mice were injected with 0.02 ml of 5% formalin via sub-planter route into the right hind paw and were treated with either Diclophenac or recombinantly purified PPE2 protein via intra-peritoneal route one hour after injection of formalin. Same volume of PBS was injected in the right hind paw as vehicle control. Mice were examined for paw swelling and redness for three hours post-treatment of PPE2/Diclophenac. (A) Representative photographs of right hind paw three hours of post-treatment. (B) Three hours post-treatment, mice were sacrificed and the paw sections were prepared and stained with hematoxylin and eosin. Photographs of representative sections were visualized at 40X magnification. The arrow represents thickness/edema (B = bone; E = epidermis). (C) Tissue injury was observed at day 21 and representative photographs of right hind paw after 21 days of treatment were taken. (D) PPE2 treatment reduces mast cell population in inflamed paw tissues. (E) PPE2-derived synthetic peptides also reduces paw edema, swelling and redness and (E) mast cell population in the paw tissues. (F) The peptide inhibits various inflammatory markers. Data shown are mean ± SD of 5 mice per group.

To summarize, major areas where Dr Mukhopadhyay's research has direct implications in translational research are as follows:

- Identified novel virulence mechanism of ESAT-6, PPE2 and PknG proteins and new generation drug targets (ESAT-6, PPE2, PknG) for treatment of tuberculosis.
- Identified two novel repurposed drugs (Mirabegron and Olsalazine) for the therapy of tuberculosis
- Identified that specific regional interaction of TLR2-LRR domain with PPE18 protein of *M. tuberculosis* can regulate subsequent T-cell cytokine profile and accordingly designing of anti-TB therapeutics targeting TLR2 LRR 11~15 domain.
- Identified an important role of PE11 in remodeling of mycobacterial cell wall architecture that present opportunities to translate this basic knowledge into effective therapeutic methods.
- Identified novel immunoregulatory role of free radicals which can dictate type of T-cell response and development of anti-oxidants as therapeutics to activate host immune response.
- PPE2 in the treatment of inflammation/tissue injury and scar-less and faster wound healing

Based on the achievements and potential research contribution of Dr. Mukhopadhyay in the field of Tuberculosis and Inflammation Disease Biology, she may be considered for the **Sun Pharma Science Foundation Research Awards, 2021 under Medical Sciences - Basic Research**. This would be a tremendous encouragement to a woman scientist.

Signature: 

Name: Sangita Mukhopadhyay

Date: 24.09.2021

Dr. SANGITA MUKHOPADHYAY
Staff Scientist-VII and Group Leader
Laboratory of Molecular Cell Biology
Centre for DNA Fingerprinting and Diagnostics
Uppal, Hyderabad-500 039, India.