b. In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not exceed 3000 words)

In brief, Dr Mukhopadhyay's work provide us important clues about important virulence factors of *Mycobacterium tuberculosis* (ESAT-6, PPE18, PPE2, PE11 and PknG), novel mechanism of pathogenesis of *M. tuberculosis* (based on studies in cell culture, animal model and clinical settings) and designing of anti-tuberculosis (TB) drugs and anti-virulence strategies to combat tuberculosis. Also, she identified novel immunoregulatory role of oxidative free radicals which is responsible for downregulation of immune response during infection and designing of N-acetyl cysteine (NAC) as an important anti-oxidant to improve Th1-based T cell protective immune responses against tuberculosis. Her publications have higher citations with a total citation of 2522 and h-index of 23.

List of ten best papers

1. Sreejit G, Ahmed A, Parveen N, Jha V, Valluri VL, Ghosh S and **Mukhopadhyay S***. The ESAT-6 protein of *Mycobacterium tuberculosis* interacts with beta-2-microglobulin (β2M) affecting antigen presentation function of macrophage (2014). ***PLoS Pathogens*** 10: e1004446. *(Impact factor – 6.218) (Citations-114)*

Important contributions: *M. tuberculosis* is multifactorial and it employs several virulent proteins to modulate/suppress host's protective immune responses. Though it is known that the CD8 cytotoxic T cell responses are suppressed and/or delayed during tuberculosis in human, the reason is not very clear. To the best of our knowledge, for the first time Dr. Mukhopadhyay demonstrated a novel mechanism by which ESAT-6 protein of *M. tuberculosis* plays important role to inhibit class I antigen presentation and CD8 T cell function as realized by the citation number. She demonstrated existence of an important interaction of *M. tuberculosis* ESAT-6 protein with human beta-2 microglobulin (β2M) which in turn prevents formation MHC-1:β2M complex and the class-I antigen presentation thereby undermining the protective CD8 T cell response of the host. The study is continued in clinical samples where a strong interaction between ESAT-6 and β2M is found in sera samples indicating the probability of involvement of ESAT-6 in downregulating CD8 T cell function in human tuberculosis. Based on the urgent need of identification of new targets and designing of novel anti-tuberculosis (TB) drugs, information described in this manuscript is extremely important as the study hinted a novel drug target, ESAT-6:β2M. Identification of small molecule inhibitors that interact with ESAT-6 is likely to prevent interaction of ESAT-6 with β2M allowing β2M to interact with MHC-1 molecules and provide better class 1 antigen presentation for effective killing of the pathogens. This is an attempt for designing of novel anti-virulence strategy in TB.

2. Jha V, Rao RN, Janardhan S, Raman R, Sastry GN, Sharma V, Rao JS, Kumar D and **Mukhopadhyay S*.** (2019). Uncovering structural and molecular dynamics of ESAT-6:β2M interaction: Asp53 of human β2-microglobulin is critical for the ESAT-6:β2M complexation. ***Journal of Immunology*** 203:1918-1929. *(Impact factor – 5.4) (Citations-4)*

Important contributions: To continue the study published in PLoS Pathogens (2014), Dr. Mukhopadhyay next planned to identify small molecule inhibitors that can block interaction of ESAT-6 protein of *M. tuberculosis* with β2M which in turn could increase CD8 T cell-based protective immune response of the host, She identified two drugs (SM09 [Mirabegron] and SM15 [Olsalazine]; repurposed drugs) that specifically mask the methionine residue of ESAT-6 which is vital for the interaction with Asp53 residue of human β2-microglobulin (β2M) and thereby prevents interaction of ESAT-6 protein with β2M. This helps β2M to interact with MHC class I and HFE molecules of the macrophages increasing class I antigen presentation and modulation of iron regulation helping the host to reduce infection. Both SM09 and SM15 caused reduced survival of the bacilli inside macrophages. Thus Dr. Mukhopadhyay has identified two drugs which can be repurposed as anti-tuberculosis drugs.

3. Jha V, Pal R, Kumar D and **Mukhopadhyay S***. ESAT-6 protein of *Mycobacterium tuberculosis* increases holotransferrin-mediated iron uptake in macrophages by downregulating surface hemochromatosis protein HFE (2020). ***Journal of Immunology*** 205: 3095-3106. (Impact factor – 5.4)

Important contributions: In further continuation of study to understand the virulence property of ESAT-6 protein, Dr Mukhopadhyay identified a novel mechanism by which *M. tuberculosis* acquires iron by manipulating the holotransferrin-mediated iron uptake in macrophage. In the macrophages, holotransferrin-mediated iron uptake is one of the ways of acquiring iron from the extracellular environment and Hereditary hemochromatosis protein (HFE) is one of the key protein in this process. In endoplasmic reticulum, HFE interacts with beta-2-microglobulin (β2M) which is an important event for the proper folding of HFE and its subsequent surface-localization. At the cell surface, HFE interacts with Transferrin receptor-1 (TFR-1) and forms a HFE-TFR-1 complex. Only the free form of TFR-1 can bind to the iron bound-holotransferrin and facilitate iron intake in the cells and that is how the cells maintain their intracellular iron levels via the HFE-TRF-1-holotransferrin system. During infection, *M. tuberculosis* secretes a protein, ESAT-6, which goes to the endoplasmic reticulum of the infected cell and binds with β2M. ESAT-6-β2M interaction creates a deficit of β2M in the endoplasmic reticulum. In the β2M deficit, HFE's folding is greatly impaired and unfolded HFE remains sequestered in the endoplasmic reticulum and is unable to reach the cell surface. In the absence of HFE at the surface, TFR-1 remains free to interact with iron bound-holotransferrin. As more of the, holotransferrin binds with the TFR-1 more iron is taken up by the macrophages resulting in the surge in the intracellular levels

of iron in the presence of ESAT-6 protein. Intracellular accumulation of iron is found to be less in macrophages infected with ESAT-6 deficient *M. tuberculosis* indicating a direct role of ESAT-6 protein in iron uptake. Among many other things, *M. tuberculosis* requires iron for carrying out its own metabolic processes even when it is residing inside the host macrophages. Iron alone is the co-factor of more than 40 enzymes of the mycobacterial metabolism. During infection conditions, bacilli requires iron for own metabolism as well as for the multiplication. Therefore, our finding suggests that, *M. tuberculosis* targets the holotransferrin-mediated iron uptake pathway in macrophages for iron acquisition during infection by utilizing ESAT-6 protein. This is one of the mechanisms by which *M. tuberculosis* increases the iron uptake which eventually helps in its better persistence inside host macrophages.

4. Nair S, Ramaswamy PA, Ghosh S, Joshi DC, Ghosh S, Pathak N, Siddiqui I, Sharma P, Hasnain SE, Mande SC and **Mukhopadhyay S***. The PPE18 of *Mycobacterium tuberculosis* interacts with TLR2 and activates IL-10 induction in macrophage (2009). ***Journal of Immunology*** 183: 6269-6281. *(Impact factor – 5.4) (Citations-189)*

Important contributions: To the best of our knowledge, Dr Mukhopadhyay for the first time hint about the role of TLR2 LRR domain in regulation of inflammation and cytokine signaling leading to alteration in immune response during infection and *Mycobacterium tuberculosis* PPE18 protein targets the TLR2 11~15 LRR domain to specifically activate IL-10 cytokine and a dominant non-protective Th2-type response that favors the bacteria to persist successfully in the host. PPE18 was found to inhibit class II antigen presentation (***Dolasia et al.[2020]European Journal of Immunology,51:603).*** Importance of PPE18 in *M. tuberculosis* pathogenesis was prominent based on the observations that infection of mice with PPE18-deleted *M. tuberculosis* resulted in reduced persistence of the bacilli resulting in decreased organ pathology and better survival of mice (***Bhat et al.[2012]PLoS ONE, 7:e52601***), further strengthening the importance of TLR2-LRR signaling in regulation of cytokine balance and Th1-Th2 immune environment – crucial for *M. tuberculosis* survival. Also the role of PPE18-TLR2 signaling in *M. tuberculosis* infection in human was emphasized (***Nair et al.[2009]Journal of Immunology, 183:6269***). Based on these hints, proposal has been initiated to design small molecule inhibitors that specially block interaction of PPE18 with TLR2 11~15 LRR domain to activate the anti-mycobacterial Th1-type response for induction of better protective immunity, which was supported by TATA Innovation Fellowship by DBT, Govt of India. This research paper has immense importance (as indicated by citation numbers) to specifically target the TLR2-LRR domain to modulate immune response not only to tackle tuberculosis but other infectious as well as pathophysiological disorders where TLR signaling plays an important role.

5. Nair S, Pandey AD and **Mukhopadhyay S***. The PPE18 protein of *Mycobacterium tuberculosis* inhibits NF-κB/rel-mediated proinflammatory cytokine production by upregulating and phosphorylating suppressor of cytokine signaling 3 protein (2011). ***Journal of Immunology*** 186: 5413-5424. *(Impact factor – 4.34) (Citations-82)*

Important contribution: This is a follow up work of earlier studies carried out by Dr. Mukhopadhyay which was published in *Journal of Immunology* (2009) where she reported that the PPE18 protein of *M. tuberculosis* can activate IL-10 cytokine and Th2 response with simultaneous inhibition of IL-12 and TNF-alpha cytokines – an immune environment that favors survival of *M. tuberculosis* inside host. IL-12 and TNF-alpha cytokines are known to play important role in mediating cytotoxicity against various intracellular pathogens including *M. tuberculosis*. The detail knowledge of pathways involved in inhibition of IL-12/TNF-alpha cytokines by PPE18 is useful in designing of appropriate therapeutics to activate induction of these cytokine to increase protective immunity against *M. tuberculosis*. In this manuscript, she made a critical contribution in discovering a novel signaling network that regulates NF-κB/rel activity and IL-12/TNF-alpha induction involving the SOCS3 in addition to the classical IKK-pathway. This pathway is involved directly to inhibit the pro-inflammatory signaling (IL-12/TNF-alpha) and the protective T-helper-1 response. She further indicated that *M. tuberculosis* exploits this signaling pathway to inhibit the anti-mycobacterial protective response skewing the immune response towards the non-protective T-helper-2 type to suit its intracellular life style. In the follow up work, Dr Mukhopadhyay also shows an important contribution of PPE18 protein in inhibiting MHC class II antigen presentation and CD4 T cell function (***Dolasia et al. [2021] European Journal of Immunology 51:603-619***) and Inflammasome activation (Ongoing study).

6. Khan N, Rahim SS, Boddupalli CS, Ghousunnissa Padma SS, Pathak N, Thiagarajan D, Hasnain SE and **Mukhopadhyay S***. Hydrogen peroxide inhibits IL-12 p40 induction in macrophages by inhibiting c-rel translocation to the nucleus through activation of calmodulin protein (2006). ***Blood*** 107: 1513-1520. *(Impact factor – 16.6) (Citations-48)*

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 attempts to understand the molecular basis of such situation which established an immunoregulatory role of ROS. She found that excessive ROS could directly inhibit IL-12 induction as well as Th1 response to induce immunosupprssion (***Khan et al.[2006]Blood; Khan et al.[2007]Free Radical Biology and Medicine***). These may augur well to understand the basis of immunosuppression observed in related diseases like cancer or various intracellular infections where IL-12/Th1-

dominated immune response is important for protection. Thus, Dr. Mukhopadhyay's work provides novel cues for cure and prevention of a large spectrum of macrophage-associated disorders including infectious, metabolic, and neoplastic diseases (***Alam et al.[2010]Journal of Immunology***).

7. Alam K, Ghousunnissa S, Nair S, Valluri VL, and **Mukhopadhyay S***. Glutathione-redox balance regulates c-rel-driven IL-12 production in macrophages: possible implications in antituberculosis immunotherapy (2010). ***Journal of Immunology*** 184: 2918-2929 *(Impact factor – 5.4) (Citations - 60)*

Important contributions: In this manuscript, Dr. Mukhopadhyay for the first time has 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 protective Th1 type. These results indicate that redox balance of glutathione plays a critical role in regulating IL-12 induction in native macrophages and 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.

8. Srivastava S, Battu MB, Khan MZ, Nandicoori VK, **Mukhopadhyay S***. *Mycobacterium tuberculosis* PPE2 protein interacts with $p67^{phox}$ and inhibits reactive oxygen species production (2019). ***Journal of Immunology*** 203: 1218-1229. *(Impact factor – 5.4) (Citations-9)*

Important contributions: The free radicals like reactive oxygen species (ROS) are known to be toxic to *M. tuberculosis* and *M. tuberculosis* adopts strategies to inhibit ROS although the mechanisms are not well known. In this manuscript, Dr. Mukhopadhyay for the first time hints that a PPE family protein, PPE2 inhibits ROS production in activated macrophages and helps in better survival of the bacilli inside macrophages. PPE2 is shown to inhibit NADPH oxidase activity by directly interacting with one of the NADPH oxidase family protein $p67^{phox}$, preventing its translocation to the membrane and forming NADPH oxidase complex. PPE2 is shown to be a secretory protein as shown *in vitro* bacterial culture as well as in active TB patients' sera, Thus PPE2 suppresses innate defense system of host and contributes to virulence of the bacilli. This information will help to improve our knowledge of host-

pathogen interactions during *M. tuberculosis* infection which is crucial for designing effective anti-TB therapeutics targeting the PPE2.

9. Pradhan G, Shrivastva R and **Mukhopadhyay S***. Mycobacterial PknG targets the Rab7l1 signaling pathway to inhibit phagosome-lysosome fusion (2018). ***Journal of Immunology*** 201: 1421-1433. (Impact factor – 5.4) (Citations-25)

Important contributions: 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. Dr. Mukhopadhyay's group for the first time identified the novel role of Rab7l1 in phagosomal maturation process and killing of *Mycobacterium tuberculosis*. Surprisingly, they observed that the Rab7l1 is targeted by the *M. tuberculosis* PknG protein to maintain its longer persistence. The mycobacterial protein PknG is shown to directly interact with inactive form of Rab7l1 (Rab7l1-GDP) inhibiting its GTPase activity and recruitment to early phagosome. This results in inhibition of recruitment of subsequent markers like EEA1, Rab7 and LAMP that are required for phagosome maturation, thereby causing blocking of phagosomal maturation. Thus, designing of small molecule inhibitors that can block interaction of PknG with Rab7l1 is likely to be important for discovery of new anti-*M. tuberculosis* drugs and designing of therapeutics.

10. Singh P, Rao RN, Reddy JR, Prasad RB, Kotturu SK, Ghosh S and **Mukhopadhyay S***. PE11, a PE/PPE family protein of *Mycobacterium tuberculosis* is involved in cell wall remodeling and virulence (2016). ***Scientific Reports*** 6: 21624. (Impact factor – 4.379) (Citations – 80)

Important contributions: The role of the unique proline-glutamic acid (PE)/proline-proline-glutamic acid (PPE) family of proteins in the patho-physiology and virulence of *Mycobacterium tuberculosis* is not clearly understood. One of the PE family proteins, PE11 (LipX or Rv1169c), specific to pathogenic mycobacteria is found to be over-expressed during infection of macrophages and in active TB patients indicating its role in mycobacterial virulence. In this study, we report that *M. smegmatis* expressing PE11 (*Msmeg-PE11*) exhibited altered colony morphology and cell wall lipid composition leading to a marked increase in resistance against various environmental stressors and antibiotics. The cell envelope of *Msmeg-PE11* also had greater amount of glycolipids and polar lipids. *Msmeg-PE11* was found to have better survival rate in infected macrophages. Mice infected with *Msmeg-PE11* had higher bacterial load, exacerbated organ pathology, weight loss, morbidity and mortality, indicating a role of this protein in mycobacterial virulence. Thus PE11 plays an important in remodeling *M. tuberculosis* cell wall architecture and can be a potential drug

target. We currently are studying the importance of PE11 in modulating macrophage defense process like autophagy and M1/M2 signaling cascades using wild-type and PE11 knock-out *M. tuberculosis* strain. Also, we are designing novel drug targeting PE11 which can be useful to treat tuberculosis either as a standalone therapy or as an adjunct to current DOTs based regime against *M. tuberculosis*

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