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)

Total Citations: 2854, h-index: 24; i10-index- 28 (as per Google Scholar)

List of ten best papers

1. Pal R, Madhu Babu Battu MB and **Mukhopadhyay S*** (2022). Therapeutic application of PPE2 protein of *Mycobacterium tuberculosis* in inhibiting tissue inflammation. ***EMBO Molecular Medicine***, e14891. doi: 10.15252/emmm.202114891 (*Impact factor - 14*)

Important contributions: Inflammation is a generic immune response generated to protect the body against harmful agents such as bacteria and viruses. The actual problem arises when the inflammatory response eventually starts damaging healthy cells, tissues, and organs. There are anti-inflammatory drugs available on the market but are associated with side effects during prolonged usage. Therefore, there is a need to develop anti-inflammatory drugs/molecules with better efficiency and the least/no side effects. Dr Sangita Mukhopadhyay has unraveled a novel biological, PPE2 protein of *M. tuberculosis*, which can be used as an anti-inflammatory drug. She demonstrated that a single dose of 3 mg/kg of recombinantly purified PPE2 protein was better effective in subsiding the acute and chronic inflammation/tissue injury in mice paw as compared to the commercially available anti-inflammatory drug Diclophenac used at 10 mg/kg. Stem cell factor (SCF) secreted by fibroblast is important for migration of mast cells to the site of injury and its survival. PPE2 exerts its anti-inflammatory activity mainly by downregulating SCF production in the fibroblasts by binding to *scf* promoter (as it has nuclear localization signal and DNA binding motif [***Bhat et al [2017] Scientific Reports, 7:39706]***) and diminishes the *in situ* mast cells population at the site of injury. Reduction of mast cells in the tissues results in reduced mast-inflammatory mediators and cues of inflammation, and hence suppresses inflammation. Interestingly, Diclophenac does not have any effect on mast cell inhibition. Unlike Diclophenac, PPE2 did not show any liver and kidney toxicity. PPE2-derived synthetic peptide imparts similar anti-inflammatory activity by suppressing the mast cell population. **Since mast cells are the first ones to sense the tissue aberration, their depletion prevents mast cell-associated inflammatory avalanche and inhibits the inflammation-mediated damage to tissue. There are no commercially available mast cell inhibitors at present. Drugs available for neutralization of one or a few mast cell mediators are often limited due to a lack of cell specificity. Dr Mukhopadhyay designed novel non-steroidal anti-inflammatory biologic to allow scar-less wound healing, cancer and tissue inflammation which has significant impact in reducing morbidity and mortality of humankind.**

2. Bhat KH, Srivastava S, Kotturu SK, Ghosh S and **Mukhopadhyay S***. The PPE2 protein of *Mycobacterium tuberculosis* translocates to host nucleus and inhibits nitric oxide production (2017). ***Scientific Reports*** 7:39706 *(Impact factor – 4.996) (Citation - 36)*.

Important contributions: A unique mechanism of *M. tuberculosis* (Mtb) pathogenesis was identified out of this work by Dr Mukhopadhyay where PPE2 was shown to be associated with increased intracellular infection burden. This is a follow up studies of the USA patent granted in 2013. In this work, she has studied in detail the mechanism by which PPE2 protein of *M. tuberculosis* supports the survival of mycobacteria. Dr Muklhopadhyay has demonstrated that PPE2 is a unique protein mimicking the eukaryotic transcription factor having a nuclear localization signal (NLS) and a DNA binding domain. PPE2 is translocated into the nucleus from cytoplasm where it interacts with GATA-1 binding site of *iNOS* promoter and inhibits nitric oxide (NO) production. Lungs of mice infected with non-pathogenic *M. smegmatis* bacteria expressing Mtb PPE2 (Msmeg-PPE2) had lesser amount of iNOS as compared to lungs of mice infected with *M. smegmatis* harboring the vector control (Msneg-pVV16) which was correlated well with better survival of the Msmeg-PPE2 bacilli in mice. Ability of PPE2 to bind to host DNA further raises interesting questions about modulation of other host gene activities by PPE2 which is under investigation (Ongoing project). Thus, inhibition of its nuclear entry and/or binding to DNA can be targeted to develop novel therapeutics against mycobacterium. An US Patent has been granted focusing PPE2 (Rv0256c) as a new drug target in tuberculosis. In addition to the cytotoxicity effect, nitric oxide is a signaling molecule that plays a key role in the pathogenesis of inflammation. This work for the first time not only highlights the importance of PPE2 as virulent factor of *Mycobacterium tuberculosis* that inhibits NO production, but also to use PPE2 as a therapeutic to inhibit NO and thus in the treatment of inflammation.

3. 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 - 5.43) (Citation - 23)*

Important contributions: While working in detail with PPE2 protein, Dr. Mukhopadhyay for the first time demonstrated that PPE2 also inhibits free radicals like reactive oxygen species (ROS) in activated macrophages and helps in better survival of the bacilli inside macrophages. She explained that PPE2 inhibits 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. Thus PPE2 suppresses innate defense system of host by inhibiting both NO and ROS. Though this is a unique strategy of the bacilli to inhibit host defense system, the same property of PPE2 is considered

to use PPE2 as therapeutic to inhibit higher ROS production happened during extreme case of inflammation.

Thus, Dr Mukhopadhyay for the first time highlights that PPE2 protein has a pleiotropic effect in abrogating host innate defense (*Inhibition of NO [Bhat et al[2017]Scientific Reports], ROS [Srivastava et al[2019]Journal of Immunology] and Mast cells [Pal et al[2021]Immunobiology]*) to favor Mtb survival. These studies helps to improve our knowledge of host-pathogen interactions during *M. tuberculosis* infection which is crucial for designing of effective anti-TB therapeutics targeting the PPE2. Also, it provides a clue to use PPE2 as a therapeutic against inflammatory disorder that is associated with higher NO/ROS and Mast cells (Indian and US patents are filed).

4. 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 – 25.669) (Citation - 48)*

Important contributions: Reactive oxygen species (ROS), generated during the innate immune response and inflammation 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 attempts to understand the molecular basis of such situation which for the first time establishes an immunoregulatory role of ROS. She found that excessive ROS could directly inhibit IL-12 induction as well as Th1 response to induce immunosuppression (***Khan et al.[2006]Blood 107:1513; Khan et al.[2007]Free Radical Biology and Medicine 42:686***). These may augur well to understand the basis of immunosuppression observed in related diseases like cancer, and disease associated with excess inflammation and various intracellular infections. Thus, Dr. Mukhopadhyay's work provides novel cues for cure and prevention of a large spectrum of disorders including infectious, metabolic, and neoplastic diseases. This manuscript highlights the importance of anti-oxidants to boost the immune system to fight against these disorders.

5. 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 - 5.43) (Citation - 64)*

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. She has demonstrated that NAC at 3 mM concentration could increase bacillus Calmette-Guérin (BCG)-induced IFN-gamma production by PBMCs from TB patients and shifts the anti-BCG immune response toward the protective Th1 type. These results indicate that redox balance of glutathione plays a critical role in regulating IL-12 in macrophages and antioxidant NAC can be used as Th1 adjuvant to boost immune system of TB patients and other infections.

Manuscript 4 and 5, published by Dr. Mukhopadhyay for the first time indicated an immunoregulatory role of free radicals especially reactive oxygen species (ROS) in establishing Immunosuppression through inhibition of IL-12/Th1-type immune response. These may augur well to understand the basis of immunosuppression observed in related diseases like cancer and infections. Thus, Dr. Mukhopadhyay's work provides novel cues for use of anti-oxidant for cure for these health disorders and tuberculosis.

6. 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 - 5.43) (Citation - 214)*

Important contributions: To the best of the knowledge, Dr Mukhopadhyay for the first time hints about the role of TLR2 LRR domain in regulation of inflammation and cytokine signaling leading to alteration of immune responses during infection with *Mycobacterium tuberculosis* (Mtb) as well as various other pathogens. PPE18 protein is shown to interact with TLR2 11~15 LRR domain that specifically results in activation of IL-10 cytokine and a dominant non-protective Th2-type response that favors the bacteria to persist successfully inside the host. PPE18 also inhibits class-II antigen presentation (***Dolasia et al.[2020]European Journal of Immunology, 51:603).*** Expectedly, mice infected with PPE18-deleted *M. tuberculosis* resulted in reduced persistence of bacilli resulting in decreased organ pathology and better survival of mice indicating a virulent role of PPE18 in Mtb pathogenesis (***Bhat et al.[2012]PLoS ONE 7:e52601***). This manuscript not only focusses the importance of PPE18 in activation of Th2-response and Mtb pathogenesis but strengthens the importance of TLR2 LRR signaling in regulation of inflammation by regulating cytokine balance and Th1-Th2 immune environment. Based on these hints, project has been initiated

to design small molecule inhibitors that specially block interaction of PPE18 with TLR2 11~15 LRR domain to block non-protective Th2-type response with simultaneous upregulation of anti-Mtb 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 infections as well as inflammation-associated pathophysiological disorders where TLR signaling plays an important role.**

7. 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 - 5.43) (Citation - 95)*

Important contribution: This is a follow up work of earlier studies carried out by Dr. Mukhopadhyay which was published in *Journal of Immunology[2009]183:6269* where she reported that PPE18 protein of *M. tuberculosis* activates 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 Th1 response. 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).

Thus Dr Mukhopadhyay for the first time report important virulence properties of PPE18 which acts in pleiotropic manner (activation of non-protective IL-10 *[Nair et al[2009]Journal of Immunology],* inhibition of protective IL-12 and TNF-alpha *[Nair et al[2011]Journal of Immunology];* inhibition of MHC class-II antigen presentation *[Dolasia et al[2021]European Journal of Immunology]* and inhibition of Inflammasome *[ongoing study]*) to inhibit host protective responses that support survival of Mtb [*Bhat et al.[2012]PLoS ONE*]. This leads to designing of 2 FDA approved drugs to be

repurposed as anti-TB drugs targeting TLR2 LRR 11~15-PPE18 which is supported by TATA Innovation Fellowship.

8. Bhat KH, Chaytanya CK, Parveen N, Varman R, Ghosh S and **Mukhopadhyay S***. Proline-proline-glutamic acid (PPE) protein Rv1168c of *Mycobacterium tuberculosis* augments transcription from HIV-1 long terminal repeat promoter. (2012). ***Journal of Biological Chemistry*** 287:16930-16946. (Impact factor – 5.485) (Citation - 36)

Important contributions: While working with TLR2-mediated regulation of inflammatory signaling, Dr Mukhopadhyay identified that interaction of protein with TLR2 15~20 LRR domain can trigger nuclear factor-κB (NF-κB) signaling and TNF-alpha production unlike TLR2 11~15 LRR domain that triggers IL-10/Th2-type immune response indicating the importance of TLR2-LRR signaling in the regulation of inflammation/immune respoonse. Though the manuscript is in the context of occurrence of accelerated HIV type 1 (HIV-1) infection in people infected with *Mycobacterium tuberculosis* (Mtb) where one of the Mtb protein PPE17 was found to interact with TLR2 15~20 LRR domain resulting in downstream activation of NF-κB augmenting HIV-1 LTR trans-activation, this is an important study to understand the role of TLR2-triggered signaling in regulation of inflammatory immune response. **This study thus, not only helps in understanding the mechanism of pathogenesis of HIV-1 during *M. tuberculosis* co-infection but also indicates that the site of interaction on TLR2 dictates the downstream signaling events leading to activation of NF-κB and inflammation important for increased transactivation of HIV during *M. tuberculosis* co-infection.**

9. N. Parveen, R. Varman, S. Nair, G. Das, S Ghosh and **S.Mukhopadhyay* (2013)**. Endocytosis of *Mycobacterium tuberculosis* heat shock protein 60 is required to induce interleukin-10 production in macrophages. ***Journal of Biological Chemistry*** 288:24956-24971 *(Impact factor - 5.485) (Citation - 60)*

Important contributions: While continuing studies on importance of TLR2 signaling in *M. tuberculosis* virulence, Dr Mukhopadhyay has decoded another important fact published in the ***Journal of Biological Chemistry [2013]*** which suggests that cellular localization of heat shock protein 60 of *M. tuberculosis* (Mtbhsp60) post interaction with TLRs dictates the type of polarization in the innate immune responses in macrophages. The results indicated that IL-10 activation by Mtbhsp60 was restricted predominantly to TLR2-mediated signaling as opposed to TLR4 that induced a minute quantity of IL-10. However, TNF-alpha induction was higher when Mtbhsp60 interacted with TLR4 receptors. Thus, this study hints at the role

of TLR2-driven endocytosis in activation of non-protective IL-10 signaling with simultaneous downreguation of IL-12 as opposed to TLR4-driven signaling that mainly retains Mtbhsp60 on the surface triggering increased production of IL-12 and TNF-alpha. These findings explain how interaction with TLRs can differently dictate type of immune-response polarization and inflammatory signaling induced during innate activation of macrophages. These findings is useful in devising strategies to manage inflammatory signaling as well as to regulate macrophage innate responses to engineer host's protective immunity against *M. tuberculosis* and other intracellular pathogens that target TLR and also to manage various TLR-associated diseases.

10. 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 – 7.464) (Citation - 152)*

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 the 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. She demonstrated existence of an important interaction between *M. tuberculosis* ESAT-6 protein with human beta-2 microglobulin (β2M) which in turn prevents formation of MHC-1:β2M complex and the class-I antigen presentation thereby undermining the protective CD8 T-cell response of the host. The study was continued in clinical samples where a strong interaction between ESAT-6 and β2M was found in sera samples indicating the probability of involvement of ESAT-6 in downregulating CD8 T-cell function during active 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 this study hinted about 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-I antigen presentation for effective killing of the pathogens, which was further explored by Dr Mukhopadhyay (*Jha et al[2019]Journal of Immunology*).

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In summary, Dr Mukhopadhyay is working on applied Immunology, Infection Biology, host-pathogen interactions, signal transduction and host-directed immunotherapy

She has worked extensively to understand regulation of cytokine signaling and inflammatory responses in macrophages and immune evasion strategies of *M. tuberculosis (J. Immunol[2009]; J. Immunol[2011]; J. Immunol[2016]; PLoS ONE[2012]; Eur J Immunol[2020]; PLoS Pathog[2014]; J. Immunol[2018]; J. Immunol[2020]* etc] which laid an excellent foundation for carrying out translational research to design novel and repurposed drugs to treat tuberculosis - which is the mission of 'Pradhan Mantri TB Mukt Abhiyan' policy.

Dr Mukhopadhyay designed effective immunomodulator to boost immune responses and host-directed immunotherapy to control TB.

She highlighted important immunosuppressive effects of ROS and use of anti-oxidants to boost immune responses (*Blood[2006]107:1513; J. Immunol.[2010]184:2918).*

Also, she designed non-steroidal anti-inflammatory biologic to treat inflammatory disorders like tissue injury/inflammation (*EMBO Molecular Medicine [2022*]), wound healing and melanoma cancer (*Indian and USA Patent filed and Commercialization through BCIL and NC TRAC under process*) and inflammatory bowel disease (*ongoing projects*) which has significant impact in reducing morbidity and mortality of humankind.