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संजीव खोसला, पीएच.डी.
निदेशक
Sanjeev Khosla, Ph.D.
Director

सीएसआईआर–सूक्ष्मजीव प्रौद्योगिकी संस्थान
(विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार)
सैक्टर 39–ए, चंडीगढ़–160036 (भारत)

CSIR-INSTITUTE OF MICROBIAL TECHNOLOGY
(Ministry of Science & Technology, Govt. of India)
Sector 39-A, Chandigarh-160036 (India)

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To,

The search-cum-selection committee
Sun Pharma Research Award 2021.

It gives me immense pleasure to nominate Dr. Ashwani Kumar for the Sun Pharma Research Award 2021 in Medical Sciences (Basic Research). The nominee has been working in the field of TB pathogenesis for more than twenty years. His laboratory has primarily focused on two dimensions of TB pathogenesis, first being\ phenotypic drug tolerance displayed by the genetically sensitive bacterium, and the second relates to the pathways involved in killing of intracellular Mtb. Besides these, the nominee is also helping in the progress of the TB field through the development of novel tools and by studying the physiology of the lungs in relation to Microbiome. I have listed below the major contributions of the nominee.

- **Mycobacterial biofilms in drug tolerance.** The nominee's laboratory has demonstrated that intracellular thiol reductive stress induces mycobacterial biofilm formation and that cellulose is a major component of the extra polymeric substance of Mycobacterial biofilms [**Nature Communications** 2016]. Before this study, mycolic acids were believed to be the most important constituents of the mycobacterial biofilms (**Cell 2005**). It was believed that short-chain mycolic acids are hydrolyzed and released to act as the greasy material that holds the Mtb biofilms through the hydrophobic interactions (**Molecular Microbiology** 2008). These views were contrary to the rest of bacterial biofilms, wherein polysaccharides, DNA, and structural proteins are the major components. However, the nominee's laboratory has demonstrated that polysaccharides are the primary component of the extracellular matrix. The nominee's laboratory has provided several pieces of evidence to conclude that mycolic acids only contribute to initial interaction between mycobacterial cells and do not contribute to extracellular material (**Microbial Cell 2019**). This view is now widely accepted. Next, the nominee's laboratory has shown that cellulose is a universal component of mycobacterial biofilms. Furthermore, the nominee has demonstrated that Mtb forms biofilms inside the lungs to exhibit drug tolerance and evading the host immune system [**Nature communications** 2021]. Additionally, the administration of cellulase aids antimycobacterial agents in killing Mtb *in vivo*. These findings demonstrated that **pulmonary TB is a classical biofilm infection and represent path-breaking discovery**. Unfortunately, it took over 125 years of research to understand the true nature of the mycobacterial infection. These results also change the way we look at TB disease. These observations also explain why TB is a chronic disease and why we need multiple drugs to treat TB. These findings also challenge the dogma that Mtb is an obligate intracellular pathogen. Given the demonstration of cellulase as a potential adjunct therapeutic molecule, a new race for developing molecules capable of disrupting biofilms has started. Such molecules could someday be used along with antimycobacterial for shorter treatment of TB!!

- **Host pathways involved in the killing of intracellular Mtb.** The role of signaling gases in killing intracellular Mtb cells has remained poorly understood in the field. Nominee's laboratory has delineated the function of carbon monoxide in autophagy and elucidated the molecular basis of

दूरभाष / Tel.: +91-172-2690785 / 2690684
फैक्स / Fax : +91-172-2690585
ई-मेल / E-mail : director@imtech.res.in
वेबसाईट / Website : www.imtech.res.in

Interferon-gamma (IFNG) mediated activation of macrophages and killing of intracellular Mtb [**Autophagy. 2018**]. His laboratory has also demonstrated that endogenous hydrogen sulfide could induce maturation of phagosomes containing Mtb cells and killing of intracellular Mtb through upregulation of autophagic flux. Importantly, host GAPDH plays a vital role, independent of its enzymatic activity in this process [**Autophagy. 2021**]. Overall, both the manuscripts are breakthrough studies in defining the signaling associated with gaseous signaling mediated clearance of intracellular pathogens. These studies also open up new avenues of research in developing the new generation of hydrogen sulfide and carbon monoxide donors. Such donors could be used as an adjunct therapy and used in addition to the chemotherapeutic regimen to shorten therapy length.

- **Molecular basis of Mtb reactivation.** During the post-doctoral fellowship, the nominee has unraveled the hypoxia sensing mechanism by Mtb through the DosRST system [**Proceedings of National Academy of Sciences. 2007**]. This was a landmark study in the TB field and attracted more than 350 citations. The sensing of oxygen to reactivate regrowth has remained poorly understood. The nominee's laboratory has demonstrated that the virulence factor SenX3 is an oxygen sensor and regulates mycobacterial regrowth in response to oxygen exposure of the hypoxic culture [**Antioxidants & Redox Signaling** 2015]. Thus, the nominee has delineated a plausible mechanism explaining the oxygen-induced reactivation of tuberculosis in humans after many years of latent infection.

- **Tools for understanding intracellular Mtb physiology.** The nominee's laboratory has adapted the genetically encoded biosensor Peredox to measure the $NADH:NAD^+$ ratio of Mtb *in vitro* and during the infection of macrophage cells. The nominee has demonstrated that the Mtb cells residing in macrophages display metabolic heterogeneity, explaining the intracellular Mtb tolerance. They have shown that the activation of resting macrophages with interferon-gamma results in higher NADH:NAD+ levels in resident Mtb cells [**Frontiers in Cellular and Infection Microbiology 2016**]. **In addition, they have made these reporter strains are readily available for use and distributed this resource to more than 50 laboratories across the world.** Furthermore, his laboratory has recently developed a new tool for the measurement of intracellular metabolic state. This probe has been named PHR-mCherry. Using this probe, the nominee's laboratory has demonstrated that Mtb cells residing inside the phagosome do not face metabolic stress. In contrast, those residing inside the phagolysosomes and autolysosomes face metabolic stress (**mBIO, 2021**). I believe that these tools will be instrumental in our understanding of mycobacterial physiology and will open up new avenues of research. These tools could also lead to discovenes such as new metabolic pathways that may be unique to Mtb and the discovery of metabolic gradients across the mycobacterial cells.

- Besides understanding Mtb's biology inside the host, the nominee is also interested in understanding lung tissues' physiology. The nominee has initiated research to understand the role of the Microbiome in lung development and function. In the context of alveolarization of the lung after birth which takes place in two phases, but whether these affect the Microbiome of the lung is not known. Nominee's group has mapped the changes in the lung microbiome's composition and diversity during developmental stages in mice. The nominee's group has shown that the diversity of the lung microbiome increases during 2-3 weeks and 5-6 weeks of development of lungs in mice. This coincides with the age at which the alveolarization takes place [**Microbiome.** 2017]. These findings suggest that alveolarization directly affects the Microbiome of the lungs.

As can be seen from his numerous scientific accomplishments, Dr. Kumar has emerged as a leader in the biology of infectious diseases. I nominate Dr. Kumar with the highest possible enthusiasm and wish him luck for future endeavors.

(Sanjeev Khosla)