Department of Immunology

Director: Stefan H. E. Kaufmann

4. *Mycobacterium tuberculosis*: Biology, Biochemistry and Omics

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Mtb can persist throughout the lifetime of its mammalian host. We seek to understand the mechanisms that enable Mtb to resist and endure stresses presented by the intracellular milieu, host immunity and anti-TB therapy with the following three approaches.

First, using a holistic systems biology approach, in partnership with two multidisciplinary consortia (http.www.systemTB.org and http.www.broadinstitute.org), we have established Mtb's transcriptional regulatory network under hypoxia, a condition that predominates in human TB granuloma [1]. The outcome of this study revealed a diverse role of transcription factors in initiating the response to hypoxia with a concomitant reprogramming of metabolic pathways involved in lipid catabolism, lipid anabolism and biogenesis of cell wall lipids. In a parallel study, an atlas of the comprehensive proteome of Mtb [2], in absolute terms of both composition and dynamics during dormancy and reactivation [3], was successfully established. Currently, we are trying to integrate the data obtained from different omics approaches to construct regulatory networks of Mtb infection of the host cell *ex vivo* [4]. We are also extending our work on Mtb proteome and transcriptome analyses of lung granulomas from patients with active TB.

Second, we are studying the cellular respiration and energy metabolism of Mtb during its transition into hypoxic/anoxic state [5]. We predicted that mycofactocin, a unique ribosomally derived electron carrier [6], might fulfill the role of alternate reducing equivalents to energize respiration and oxidative phosphorylation. Consistently, targeted mutagenesis of these gene clusters and phenotypic analyses of the mutant/s thereof confirmed the role of mycofactocin in redox homeostasis and hypoxia/anoxia survival. In addition, mycofactocin is required to maintain NADH/NAD^+ and intracellular ATP levels during Mtb growth at lower dissolved oxygen concentration. Therefore, understanding the mycofactocin-dependent regulatory mechanism may pinpoint vulnerabilities, which could help in developing interventions to treat latent infections and significantly reduce the risk of reactivation of TB.

Third, we are repurposing our established murine models recapitulating the immunopathophysiology of human pulmonary TB for testing the efficacy of anti-TB drugs. In this regard, we are actively engaged with academic and industry laboratories under the umbrella of the Innovative Medicines Initiative (IMI) to discover, develop, and evaluate new TB drugs in humanized mice (see Section 2) and NOS2 KO mice models [7] presenting hypoxic granulomas similar to human granulomas (solid versus caseous types). The outcome of such studies will be mathematically modeled and compared with other model/s (e.g. Cornell hypoxia model) to determine the efficacy of anti-TB drugs.

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