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The bacillary and macrophage response to hypoxia in tuberculosis and the consequences for T cell antigen recognition

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Abstract

Mycobacterium tuberculosis is a facultative anaerobe and its characteristic pathological hallmark, the granuloma, exhibits hypoxia in humans and in most experimental models. Thus the host and bacillary adaptation to hypoxia is of central importance in understanding pathogenesis and thereby to derive new drug treatments and vaccines.

Keywords: Tuberculosis; Hypoxia; T cells; Antigens; Macrophage; Lipid droplets

1. Introduction

Since tuberculosis (TB) was declared a global health emergency in 1993 [1] a number of important control efforts have led to a fall of TB-associated mortality and the saving of 45 million lives [2]. However, up to a third of the world's population is estimated latently infected with *Mycobacterium tuberculosis* (Mtb), serving as a reservoir for many of the estimated 9·6 million people who developed TB worldwide in 2014, leading to 1·5 million deaths. Thus, TB now ranks alongside HIV as a leading cause of death worldwide, and the rate of HIV-TB co-infection worldwide in 2014 was 12% [2].

Mtb is transmitted by the cough of an infected person (aerosolized) and inhaled into the alveoli of a new host. This process can lead to three possible outcomes: i) a minority develop active primary progressive TB disease and develop a detectable but ineffective acquired immune response (immune sensitization), ii) the majority develop latent TB infection that is contained throughout their life by an effective acquired immune response, and iii) a small proportion of those latently infected develop post-primary TB as a result of reactivation of their latent infection, which can be triggered by immune suppression such as HIV-1 infection [3]. Latent Mtb infection (LTBI) is defined solely by evidence of immune sensitization by mycobacterial proteins: a positive result in either the

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tuberculin skin test (TST) or an *in vitro* interferon gamma release assay (IGRA), in the absence of clinical signs and symptoms of active disease [4]. However, TST and IGRA do not distinguish latent TB from active disease, and neither have high accuracy to predict subsequent active tuberculosis [5]. Better understanding of the biology of Mtb and of LTBI is necessary in order to develop better diagnostic methods and treatment options. However, the interplay between Mtb and the human host is incompletely understood.

Conventionally, LTBI is conceived as Mtb remaining in an inactive, stationary phase in the granuloma as a stable latent population of bacilli capable of surviving under stressful conditions generated by the host [6]. Alternatively, viable non-replicating persistent Mtb reside within alveolar epithelial cells in the lung, with reactivation being associated with the upregulation of resuscitation promoting factors within MTB and the escape of newly dividing microorganisms into alveoli and bronchi [7]. Recent advances in imaging technologies such as computed tomography (CT) combined with positron emission tomography (PET) have aided the evolution of a concept that LTBI encompasses a diverse range of individual states extending from sterilizing immunity in those who have completely cleared the infection via an effective acquired immune response, to subclinical active disease in those who harbor actively replicating bacteria in the absence of clinical symptoms, through to active TB disease with clinical symptoms [8,9]. Thus, it has been proposed that Mtb infection may be better viewed as a continuous spectrum of immune responses, mycobacterial metabolic activity, and bacillary numbers. In this model the impact of HIV infection can be conceptualized as a shift towards poor immune control, higher mycobacterial metabolic activity, and greater organism load, with subsequently increased risk of progression to active disease [3,8–11].

Direct measurement of lesional oxygen tension in rabbits [12], and indirect measurements in non-human primates and humans using hypoxia-sensitive probes demonstrate many TB lesions *in vivo* are hypoxic [13]. Hypoxia is only one of the many different stresses Mtb encounters in the granuloma and *in vitro* and animal models are limited in the extent to which they recapitulate the multifactorial environment created by the host to arrest mycobacterial growth. Nonetheless, many conceptual advances have been achieved in recent years in our understanding of mycobacterial physiology under low oxygen conditions, particularly in the areas of gene regulation, metabolism, and energy homeostasis.

2. *M. tuberculosis* and hypoxia: *in vitro* studies of bacterial response and adaptation

The existence of a coordinated and inducible response of Mtb to low oxygen conditions was initially revealed by Wayne and colleagues, culminating in the now widely employed *in vitro* "Wayne" model of hypoxia-induced dormancy [14]. In this system, bacteria grown in liquid medium in sealed tubes with limited head space gradually deplete oxygen supplies, leading to a non-replicating state of persistence (NRP) characterized by reduced metabolism and increased drug tolerance. In this state cellular viability can remain unchanged for weeks to months, with synchronized replication resuming following culture reaeration. The inferred similarities between bacteria grown *in vitro* under hypoxic conditions and clinical cases of latent infection have made the Wayne model a key tool for investigating the molecular basis of mycobacterial dormancy. A key caveat is that many of these studies were performed using laboratory strains of Mtb that have been passaged aerobically over many years, these findings therefore need to be revisited using recent clinical isolates.

2.1. Gene regulation, hypoxia sensing

Early work on gene expression analysis of Mtb undergoing hypoxic challenge identified a suite of almost 50 genes that were significantly and consistently upregulated relative to aerobic controls. Further work identified that this regulon was controlled by a transcription factor subsequently named DosR (Dormancy Survival Regulator), the activation of which was mediated through two classic two-component system-type transmembrane sensor histidine kinases, DosS and DosT [15]. Activation of DosS and DosT in turn is still the subject of some debate, however strong evidence suggests they sense cellular redox status and dissolved oxygen concentration, respectively, via their heme prosthetic groups [16]. Genes within the DosR regulon are involved in multiple processes including central metabolism, energy generation and gene regulation; however the majority are of unknown function. Interestingly, despite its dominance of gene expression under hypoxia, multiple studies have demonstrated that genetic inactivation of *dosR* results in a relatively mild loss of viability under hypoxia *in vitro* (2–3 logs decrease in CFUs after 30–50 days incubation) [17–19] and varying responses *in vivo* in multiple animal models [20]. Further evidence suggests these effects may be dependent on the exact hypoxia model, strain, animal model, and growth media used [17,18,20,21]. Furthermore, upregulation of the DosR regulon is not specific to hypoxic challenge (it is also activated by NO and CO [22,23]; nor is it uniquely controlled by DosST sensing there is significant cross-talk with other TCS regulons [24]. Nonetheless, DosR and its regulon are modestly upregulated in sputum from active TB cases [25], supporting a role for the dormancy survival response in infection.

Outside of the DosR response, other transcriptional regulators have been identified as playing significant roles under hypoxic conditions, although precise functions have not been determined [25,26]. Galagan and colleagues used ChIP-Seq data from strains overexpressing various transcription factors to develop a detailed map of regulatory interactions in Mtb under hypoxia [26]. They identified Rv0081, itself part of the DosR regulon, as a major regulatory hub controlling multiple hypoxia-relevant processes. Interestingly, the *Mycobacterium smegmatis* homologue of the Rv0079 - a gene within the same operon as Rv0081 - has also been shown to have functional importance in this bacterium during hypoxia in stabilizing ribosomes in the 70S form, in contrast to the higher order structures seen in many enteric bacteria grown under similar