COMMENTARY

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Mechanisms of mycobacterial transmission: how does *Mycobacterium tuberculosis* enter and escape from the human host

“Novel approaches are needed to further elucidate these critical events in the pathogenesis of tuberculosis.”

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In 1882, while lecturing on his discovery of the cause of tuberculosis, Robert Koch said “If the importance of a disease for mankind is measured by the number of fatalities it causes, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera and the like.” Although Koch’s identification of *Mycobacterium tuberculosis* (Mtb) as the cause of tuberculosis heralded many future discoveries and treatments, Mtb remains responsible for 1.5 million deaths annually. Therefore, more research is needed on all stages of Mtb infection. This commentary provides a framework for understanding two central questions in Mtb biology at the extremes of the infectious life cycle: how does Mtb enter the body and how does it escape?

How is tuberculosis spread?

The tubercle bacillus is spread person-to-person almost exclusively by aerosolized particles. The size of infectious droplets from Mtb in infected patients ranges from 0.65 (small) to >7.0 μm (medium–large) [1]. While small Mtb aerosol particles are expected to transit past the nasopharyngeal or tracheobronchial region to be deposited in the distal airways, larger

particles can be trapped in the upper airway or oropharynx where they can potentially lead to tuberculosis of the oropharynx or cervical lymph nodes [2]. Despite the apparent ease with which Mtb is spread – how else could nearly 2 billion humans be infected currently? – and the strong epidemiologic evidence linking airborne transmission to close personal contacts, directly demonstrating aerosol transmission of Mtb has been exceedingly difficult [3]. Indeed, Riley *et al.* needed to expose hundreds of Mtb-susceptible guinea pigs to air from a ward of Mtb-infected patients continuously for months to demonstrate airborne transmission of Mtb [4], indicating that aerosolization and transmission of Mtb is a rare event. This observation is corroborated by epidemiologic and modeling data indicating that the likelihood of transmission of Mtb is proportional to the duration of exposure to an infectious person and inversely proportional to the volume of space within which exposures occur [5].

What causes active disease?

The simplest model of human interaction with Mtb is represented by the binary outcome ensuing

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from exposure of a naive individual to Mtb: development of either asymptomatic latent infection or active disease characterized by fever, weight loss and a bloody cough. Latently infected individuals can either remain asymptomatic throughout their lifetime or develop active disease at a remote time from the primary infection in a process known as reactivation. Epidemiologic studies have shown that only 5% of otherwise healthy individuals will develop active disease when first exposed to Mtb, thus leading to the fundamental question in Mtb biology of why so few acute infections prove symptomatic? One straightforward explanation relates to inoculum dose: multiple studies have demonstrated a direct correlation between the number of bacilli in an infected person's sputum and the likelihood that contacts will develop symptomatic active tuberculosis. Likewise, animal studies show that a lower initial Mtb inoculum in wild-type animals results in a quiescent but persistent infection, whereas a higher inoculum leads to active disease, pneumonia and death. Since a coughing, actively infected person typically produces only a few aerosolized bacteria [3], his or her contacts likely will inhale a low number of bacteria, skewing the probability to development of latent infection rather than active disease. As well as the importance of inoculum size, environmental factors such as proximity and duration of the contact as well as micronutrient or relative vitamin D deficiency have been associated with susceptibility to tuberculosis. Furthermore, being immunocompromised at the time of infection due to either host genetics or acquired deficiencies can also increase a person's likelihood of developing active disease from an initial infection. In addition, it has been recently proposed [2] that trapping of larger particles in the upper airway may induce a protective immune response. Finally, as described below, direct translocation of bacteria across the epithelium overlying mucosa-associated lymphatic tissue (MALT) might also dictate whether acute or latent infection occurs.

How does tuberculosis cross the airway mucosa?

The current paradigm of primary tuberculosis infection is that small airborne particles distribute to the terminal alveoli, where resident alveolar macrophages or tissue dendritic cells ingest the airway bacteria. Subsequently, infected macrophages or dendritic cells migrate to draining lymph nodes, activate adaptive immunity and then return to the initial site of infection where a

granuloma forms [6]. However, prior to reaching the terminal alveoli, some small and large particles likely become trapped along the mucosa, where they can interact with noninnate immune cells between and above MALT. Oropharyngeal MALT, widespread in childhood but regressed in adults, includes nasal-associated lymphatic tissue, the tonsils and adenoids of Waldeyer's ring, and bronchus-associated lymphatic tissue. Interestingly, MALT is prevalent during a vulnerable period of early childhood and adolescence when tuberculosis manifests in more severe and disseminated forms. Overlying MALT are both primary epithelial cells and specialized cells called microfold cells (M cells), the latter whose function in both the airway and GI tract is to ingest and transcytose foreign antigens [7,8]. It has previously been demonstrated that airway epithelial cells can mediate dissemination of Mtb from the airway [9], and a functional role for M cells in Mtb entry was recently established [10], extending observations made over 15 years ago [11]. M cell depletion prior to airway infection results in fewer Mtb recovered from draining lymph nodes during nasal or airway infection and protection from long-term Mtb-mediated mortality in mice [10]. Not tested in these experiments is the impact of Mtb transcytosis by M cells on adaptive immunity, nor how inoculum dose affects the immune response. Since M cells targeting has recently been proposed as a method for inducing vaccine responses in MALT as well as systemically [12], M cells are prominent in nasal-associated lymphatic tissue [13] and intranasal vaccination with BCG provides enhanced protection from Mtb infection [14], it is also possible that low level or repeated infection (i.e., with <10 bacteria) in the upper airway results in direct delivery of Mtb to APCs in MALT in a pathway that stimulates a more robust protective immune response than when Mtb are ingested by alveolar macrophages directly. Thus, both inoculum dose and particle size could influence the outcome of disease such that individuals whose M cells transcytose either paucibacillary or larger Mtb particles develop latent disease or even remain completely uninfected. However, if the initial dose of Mtb transcytosed by M cells in the upper airway mucosa overwhelms the primary local response, then drainage of Mtb to local lymph nodes could result in lymphatic disease, as is seen in scrofula. Likewise, if the particles are very small, they might bypass epithelial cells altogether and reach the terminal alveoli to be ingested by airway phagocytes.

How does tuberculosis escape from the lung?

The success of any bacterial pathogen ultimately depends on its ability to multiply and infect new hosts. Many pathogenic bacteria interact with hosts by secreting virulence molecules like proteins and lipids. A classic example is the production of cholera toxin by *Vibrio cholerae*, which enhances the pathogen's spread from person-to-person by promoting profuse watery diarrhea. Mtb also employs sophisticated means of dissemination, including mediating caseation, access to the bronchial tree and cough-mediated airborne transmission.

Role of tissue destruction & caseation

Recently, it was proposed that the transition of airway tuberculosis from the initial, asymptomatic infection to symptomatic, necrotic and cavitory disease occurs in three stages [15]. In this model, the first stage reflects early infection of alveolar macrophages by Mtb and activation of cell-mediated immunity, followed by immune control with granuloma formation. In some individuals, a second stage ensues, represented by accumulation of Mtb antigens and host lipids in the airway, leading to bronchial obstruction and obstructive lipid pneumonia. Finally, depending on the host response, the third stage is characterized by either cavitory or fibrocavitary disease with communication to the outside world. The mechanisms accounting for each stage, and in particular, the formation of obstructive, lipid pneumonia are not well known, though some studies have identified critical roles for host matrix metalloproteinases in tissue destruction and dissemination [16].

Cough in tuberculosis transmission

Although cough is a well-known hallmark feature of active pulmonary tuberculosis, very little is known about the pathogenesis of infectious cough [17]. Cough may be a natural consequence of lung inflammation and host

production of prostaglandins, bradykinin and other inflammatory mediators that activate afferent neuronal C-fibers in the lung mucosa [18]. In addition, cavity or cyst formation itself might induce mechanical activation of either rapidly adapting receptors or slowly adapting stretch receptors that can sensitize the lungs to cough triggers [19,20]. Conversely, cough itself, perhaps triggered by secreted mycobacterial factors, could lead to Mtb aerosolization and/or cavity formation. In this model, a granuloma or region of caseous necrotic pneumonia could be induced to form a cavity by very high mechanical forces (intrathoracic pressures as high as 300 mm Hg and expiratory velocities as high as 800 km/h) generated by a strenuous cough [18], forcing weakened extracellular matrix and elastic tissue [16] to stretch into a cavity. Thus, while cough may be a major route of aerosolization and spread, it may precede and/or overlap with the cavitory disease stage.

Conclusion

Though significant progress has been made to understand the innate and adaptive responses to Mtb infection since Koch's discovery of tuberculosis, both how Mtb penetrates the mucosa to initiate infection and how it reverses the process to escape remain poorly understood. Novel approaches are needed to further elucidate these critical events in the pathogenesis of tuberculosis.

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