How Does Mycobacterium tuberculosis Establish Infection?

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(See the article by Barrios-Payán et al, on pages 1194–205.)

On 16 January 1924, Allen Krause gave an address entitled “The spread of tuberculous infection in the body” before the Bronx County Medical Society, under the auspices of the New York Tuberculosis (TB) Association [1]. He said “few of us, it would seem, think of the possibility of a more general distribution of infection as perhaps the mode of tuberculosis; of bacilli nesting in many places throughout the body, once entrance is made, and causing only minimal or microscopic, in other words, unnoticed changes in many tissues, with only a single visible focus, or a few here and there, to indicate infection; with, maybe, a rather free and frequent moving about of small numbers of germs along the various avenues of dissemination and a consequent repeated new focalization that is widespread, the early focus of relatively many bacilli and of native tissue reaction remaining the only visible process and numerous minute foci of few bacilli remaining concealed with their growth kept in abeyance by the immunity of the body, as long as this holds.” Nearly 90 years later, in the study by Barrios-Payán et al [1a] reported in this issue of The Journal, multiple sensitive techniques were used to probe the presence, location, and viability of Mycobacterium tuberculosis (Mtbt) in immunocompetent individuals in Mexico. All subjects died from reasons unrelated to TB and had no evidence or history of clinical TB. The authors provide evidence of latent Mtbt infection not only in the lungs but also in the liver, spleen, and kidneys of these individuals. As expected from a TB-endemic setting, a majority of these individuals were latently infected, with Mtbt DNA demonstrable in the lungs of approximately 70%. Of importance, almost all of the individuals also had bacterial DNA in the spleen, kidney, and/or liver. Whereas Mtbt DNA was present in only extrapulmonary locations in many subjects, none showed infection restricted to only the lungs. Different individuals were infected with different strains of Mtbt. Thus, dissemination from the lungs and infection in multiple organs appear fundamental to the establishment of Mtbt infection and, as Krause postulated, may indeed be “the mode” of spread of Mtbt in the body.

Although the BCG-vaccination status of the subjects is not provided, BCG vaccination is routine in Mexico. Barrios-Payán et al were unable to detect Mycobacterium bovis BCG DNA in any specimen. Reactivation of BCG in HIV-positive patients occurs, but it is far less frequent than reactivation of latent Mtbt. BCG has been shown to be attenuated for dissemination in animal models [2–4]. Moreover, latently infected individuals exhibit immune responses to ESAT6. Although the mechanism underlying attenuated dissemination of BCG is unclear, ESAT6, which is one of the RD1-encoded proteins secreted via ESX-1, may play a role in bacterial dissemination from the lungs [2–5]. ESAT6 is a pore-forming toxin that causes cytolysis of both type 1 and type 2 alveolar epithelial cells in vitro, and it is also an adhesin which binds to laminin [5, 6]. Laminin is synthesized by pneumocytes and is a major component of the basement membrane on which these cells rest. It is possible that Mtbt organisms replicating in alveolar epithelial cells use ESAT6 for anchoring onto the basolateral laminin-expressing surfaces and cause damage to the cells and the basement membrane, thus participating in their dissemination via the alveolar wall.

While the presence of >1 clinical strain in TB patients was reported earlier, Barrios-Payán et al provide evidence for latent infection with multiple Mtbt strains in the same individual [7, 8]. Thus, adaptive immune responses elicited by the first infection (which was driven to latency) could not inhibit a second or even a third strain of Mtbt from infecting and disseminating. Even robust immune responses, which in most individuals can prevent reactivation of
latent Mtb infection throughout the lifetime, cannot protect against new infection. Development of vaccines should include consideration of the importance of both eliciting sterilizing immunity that can eliminate latent bacteria and/or protect against new infection. This is important because the risk for reactivation will exist as long as latent infection lurks anywhere in the body.

Barrios-Payán et al also show that latent infection with Mtb is present in endothelial and/or epithelial cells in every organ tested, in the absence of inflammatory responses or granuloma formation. While the role of macrophages has been studied extensively, little attention has been paid to infection of non-phagocytic cells in TB. Infection with Mtb is initiated by the few bacilli in a droplet inhaled into an alveolus. Adaptive immune responses are elicited roughly 4–5 weeks later in humans (and rabbits) and approximately 2–3 weeks later in mice. Studies of primary infection cannot easily be done in humans and are challenging even in animal models because of the very low numbers of bacteria present at early time points. Recent studies report >20,000-fold bacterial replication in the lungs of Mtb-infected mice before immune responses are generated; this replication occurs in a “nonmigrating compartment” that does not induce proliferation of naive CD4+ T cells [9]. Dissemination of Mtb from the lungs to other organs precedes the development of immunity [10, 11]. Is it possible that the early infection and replication occurs in alveolar epithelial and endothelial cells, which vastly outnumber the alveolar macrophages? Do the bacteria then spread by hematogenous routes to the endothelial cells in different organs? Evidence exists in guinea pigs for dissemination of Mtb from the site of inoculation to the liver, spleen, kidney, bone marrow, and elsewhere within hours, as does evidence for dissemination of free bacteria [12–14]. Perhaps at the very early stages of infection, Mtb organisms replicate inside alveolar epithelial and endothelial cells and disseminate systemically, even as they replicate in the lymph nodes to reach the required antigenic threshold for eliciting immune responses [9]. Invasion and replication in nonphagocytic cells would be advantageous since these cells lack the killing mechanisms of macrophages. Moreover, replication in these cells may provide the bacteria with an opportunity to modify their phenotype to better disseminate systemically and/or to enhance survival in phagocytic cells that eventually migrate to the site of infection [13]. Infected epithelial/ endothelial cells may also regulate the initiation of adaptive immune responses [15, 16].

The extensive replication and dissemination of Mtb that results in seeding of the body is eerily silent, with no discernible signs and symptoms; the site of this bacterial replication and the means by which the bacteria disseminate after infection are unknown. Other nonmotile, gram-positive bacteria use cell-wall/surface proteins called adhesins to establish infection in the cells of the relevant mucosal barrier and to disseminate across the barrier [17–19]. Adhesins enable the bacteria to attach/invade/translocate across the epithelial and endothelial cells directly or by binding to 1 or more components of the host extracellular matrix [17–21]. Adhesins can also regulate the early immune responses via induction of chemokines and cytokines from cells of the mucosal barriers [22–24]. Some adhesins of Mtb have been identified in recent years, and two, HBHA and ESAT6, contribute to Mtb infection and dissemination from the lungs in vivo [2–5, 14, 25–28]. A third adhesin, PknD, also a laminin-binding protein of Mtb, contributes to invasion of the human brain microvascular endothelial cells that form the blood-brain barrier [27]. Considering its ability to disseminate and infect the epithelial and endothelial cells in multiple extrapolmonary locations, Mtb may use different adhesins for establishing infection in different extrapolmonary sites. While the role of adhesins in infection is an active area of investigation for other gram-positive bacteria, studies of Mtb adhesins are still in their infancy [20, 21].

This ability to quietly disseminate and then lie dormant is responsible for the success of Mtb in establishing the huge reservoir of latently infected humans: estimates are that one-third of the global population, or approximately 2 billion individuals, carry a latent infection with Mtb. Although our knowledge of TB pathogenesis is increasing rapidly, there are still many gaps in our understanding of the simple question, How do the few Mtb organisms that are inhaled establish infection?

Notes

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