The secret trumps, impelling the pathogenicity of tubercle bacilli

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A B S T R A C T

Confrontation between invading microbial pathogens and host defense systems involves intricate cellular and molecular interactions. Here we discuss the virulence factors as trumps, overriding the contest in favor of the tubercle bacillus (Mycobacterium tuberculosis). It evolved a number of molecular constituents, which can interfere with antigen presentation and Toll receptor function, thus impairing immune defenses. It also evolved stress responses, which can drive its cell cycle into a non-replicating, low metabolic mode. Although the low counts of latent bacilli prevent their direct detection, we contend that they retain a capacity to survive for long periods in foamy macrophages and within the necrotic parts of lung granulomas. We attributed significance to drainage of M. tuberculosis by the alveolar fluid: while out-flow is responsible for the clearance, the reverse-flow has an important capacity to re-infect the lungs and to transmit the infection to new recipients. We consider the cycling between replicating and latent organisms to be a continuous process, which is a departure from the concept of long-lived dormant organisms, with a capacity to resuscitate. These aspects impinge also on the actions of isoniazid (INH) chemotherapy and on the topography of human lung lesions. Eventually, fibrosis of the connective tissue of the lungs is known to encapsulate lung lesions, thus limiting the impact of both outward and reverse drainage. In conclusion, the novelty of our views on M. tuberculosis-host interactions rests in the dynamic perception of M. tuberculosis latency and its evolutionary importance for the pathogenesis of tuberculosis.

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R E S U M E N

El enfrentamiento entre los patógenos invasivos y los sistemas defensivos del huésped implica interacciones celulares y moleculares. En el presente artículo se discuten los factores de virulencia como triunfos, favoreciendo el éxito de la contienda a favor del bacilo tuberculoso (Mycobacterium tuberculosis). Éste desarrolla un número de constituyentes moleculares que pueden interferir con la presentación antígenica y la función Toll receptor, deteriorando las defensas inmunes del huésped, así como respuestas al estrés que entlentecen su ciclo celular hasta convertirlo en no replicante. Aunque el recuento bajo de bacilos latentes previene su detección directa, postulamos que retienen cierta capacidad de sobrevivir dentro de macrófagos espumosos y en las partes necróticas de los granulomas pulmonares. Mientras que el circuito natural del fluido alveolar hacia las vías respiratorias superiores es el responsable de la eliminación de bacilos, su retorno para generar aerosoles de forma fisiológica también implica la posibilidad de que con el tiempo los bacilos puedan reinfectar de forma endógena los pulmones y transmitir la infección a nuevos individuos. Consideramos, pues, la tuberculosis latente como un proceso continuo, en contraposición al concepto de la existencia de bacilos largamente durmientes y con capacidad de resucitar. Creemos, además, que la fibrosis del tejido conectivo de los pulmones, capaz en ocasiones de encapsular lesiones pulmonares, es la responsable de frenar el drenaje y la diseminación de bacilos, limitando el ciclo reinfecutivo. En conclusión, la novedad de nuestra visión radica en la percepción dinámica de la latencia de M. tuberculosis y sus consecuencias sobre la patogénesis de la tuberculosis.

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Los triunfos secretos que dan fuerza a la patogenicidad del bacilo tuberculoso

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“Behold, I tell you a mystery: we shall all be changed, in the twinkling of an eye.
For our earthly bodies that can die, must be transformed into heavenly bodies that cannot perish but will live forever.”
1 Corinthians, 15:51-53.

Evolution of virulence by adapting the bacterial life cycle

The long history of co-evolution between Mycobacterium tuberculosis and our human ancestors is pertinent to the recent 200th birthday anniversary of Charles Darwin. It has been deduced that an ancestor of M. tuberculosis was able to infect our predecessors, the Australopithecus. M. tuberculosis adapted itself efficiently to the physiology of man and took advantage of it, for evolving its host pathogenicity and transmittance. The intricate outcome of this co-evolution made the life cycle of the bacillus highly sophisticated to an extent that still frustrates current attempts for the global eradication of tuberculosis (TB). The menacing epidemiological figures suggest that one third of mankind carry latent M. tuberculosis infection (latent TB infection [LTBI]). There are 9 million cases of active TB and 1.8 million TB-related deaths per year. Unlike other microbial pathogens, the virulence of M. tuberculosis is not caused by any overtly cytopathic constituents, but rather by the intricate action of immunomodulatory cytokines. It has previously been suggested, that they act as “decoys” to provoke adaptive macrophage and immune reactions, pretending to be protective, but in effect causing host pathology. Here, we postulate, that the “secret trump” of M. tuberculosis for winning over the infected host involves an adaptation of its life cycle. We further discuss how the unraveling of the underlying mechanisms could be mandatory for turning the tables in favor of the host.

Mycobacterium tuberculosis constituents involved in pathogenicity

M. tuberculosis infects the host by the inhalation of small diameter infected aerosols of “droplet nuclei” into alveoli. After being phagocytosed by the alveolar “resident macrophages”, several constituents of M. tuberculosis cell walls can mediate a number of different strategies, by which the bacilli can avoid their destruction inside a phagolysosome. M. tuberculosis can inhibit the phagolysosome fusion by: increasing the pH,10 disturbing the ATPase pump,10 secreting the ESAT-6/CFP-10 (early secreted antigenic target 6/culture filtered protein 10) complex11 or by the autophagy mechanism. Then, bacilli can grow until the death of the macrophage itself. This outcome is manifested by necrosis, which prevents the bacilli being killed by macrophage apoptosis. However, the burst of bacilli, released from the infected macrophages into the stressful extracellular milieu, instantly curtails their growth. Alternatively, a number of constituents of intracellular M. tuberculosis can interfere with the host defense mechanisms. Thus, Man-lipoarabinomannan (Man-LAM) and 19 kDa lipopglycoprotein can inhibit the antigen presenting functions and TLR-mediated inflammatory responses of infected cells.24,25

Granuloma and tumour necrosis factor-alpha functions

Blood derived monocytes and neutrophils are attracted to infected sites by the continuous production of tumour necrosis factor-alpha (TNF-α) by the infected resident macrophages (Fig. 1). The most prominent cellular response is represented by the phagocytosis of M. tuberculosis by macrophages. The induced accumulation of macrophages leads to granulomatous lesions (Fig. 1). While preventing the dissemination of bacilli, they are also an ideal milieu for the bacilli to grow. This has been called the “Citadel paradox”, in analogy to the Citadel of 18th century (Barcelona), which had an architecture pretending to defend against foreign invaders, but in fact serving against the potential rioting of its own residents. Sustained TNF-α signaling is required to maintain the local chemokine gradients for holding the cells in close apposition, which favors the activation of infected macrophages. Although M. tuberculosis infected TNF-knock-out mice also develop granulomas, this requires a high bacillary concentration and results in larger and more necrotic structures. TNF-α apparently can induce the granulomatous response even with a lower bacillary concentration, thus helping to save the host integrity.

The role of neutrophils, natural killer cells and dendritic cells, before specific immune response onset

Neutrophils have both mycobactericidal32 and regulatory anti-inflammatory activities33 but their role is not fully understood. However, interferon-gamma (IFN-γ) producing natural killer cells can activate or lyse macrophages, containing other intracellular microbial infections,34 but do not curtail the TB infection. Monocytes...
in the vicinity of infection develop rapidly into dendritic cells (DCs) and emigrate to regional lymph nodes, thus favorable to the host by presenting antigen to T cells. However, infected DCs could also be the “Trojan horse” spreading the bacilli haematogenically from lymph nodes to distant organs. Gradually, antigen stimulated CD4 Th1 cells will proliferate and will be attracted to the lung granulomas to activate infected macrophages through the secretion of IFN-γ. When immune responses to M. tuberculosis infection had been established and tubercle bacilli become trapped in granulomas, there is notable bacillary killing. However, not all bacilli get killed and some will enter a stationary, non-replicating, latent phase and will become “hidden” and extremely resistant to host immunity generated stress (Fig. 2), conditions which are bactericidal toward replicating M. tuberculosis.

Closing the ring by foamy macrophages

Foamy macrophages (FMs) develop as a consequence of macrophage necrosis and the accumulation of apoptotic short-lived neutrophils. In FMs, accumulate the cellular debris and generate lipid bodies in their cytoplasm. They can phagocytose from the extracellular milieu tubercle bacilli which had already become non-replicating (or latent) under the influence of the stress inflammatory process; this is represented by acidic pH and intermediate oxygen and nitrogen radicals. Oxidation of extravasal LDLs accumulate in macrophages and develop into FMs. In mice, these cells are drained into lung alveoli, building an external ring around the granulomas, because of limited alveolar space.

Reverse passage of alveolar fluid

Particles are drained out from the parenchyma of lungs in humans, towards the upper bronchial tree and then into the gastrointestinal tract. This process could be protecting the host by draining replicating M. tuberculosis and latent M. tuberculosis, organisms out of the body, in actively and latentely infected individuals. However, there is also a reverse flow, as induction of aerosols with the inhaled air could lead to re-infection of the lungs (Figs. 1 and 2). This process could not be prevented by T cell immunity, because that develops only after the emigration of the infected DCs to the draining lymph nodes.

“Continuous re-infection” origin of latent Mycobacterium tuberculosis

The continuous re-infection of lungs through the reverse flow of alveolar fluids has been integral to the previously formulated “dynamic hypothesis” of LTBI pathogenesis. Drained bacilli from infected hilar lymph nodes through the thoracic ducts to the right atrium are pumped back to the lung across the pulmonary artery. The mandatory role for a cycle between replicating and latent organisms in LTBI is supported also by the knowledge that T cells in LTBI recognize predominantly antigens expressed by replicating M. tuberculosis bacilli. Hence, the “dynamic hypothesis” differs from the traditional concept, which assumes that latent M. tuberculosis remain dormant for many years, while retaining a potential to resuscitate into active TB.

Chronic production of new granulomas has been demonstrated by the production of small new lesions (0.25 to 2.5 mm of diameter) in minipigs (with pulmonary structure similar to human’s). Furthermore, lesions of about 2 mm of diameter, which are too small to be detected by a routine chest X-ray, have been identified in human lungs, using High resolution CT (data not published). However, direct evidence for the “hidden trump” of M. tuberculosis bacilli, represented by their ability for continuous low-grade infection of aerosols would require more sensitive detection techniques.

Continuous re-infection of macrophages apparently proceeds despite pronounced T cell immunity, which accompanies LTBI. As existence of an extracellular stage seems integral for the re-infection concept, one can speculate, that antibodies against surface expressed antigens might have a protective potential for LTBI.

Fibrotic processes in the lungs

The fibrotic process is important for stabilizing cellular accumulation in granulomas. With an early onset, TNF-α and chemokines attract new macrophages and neutrophils to alveoli. Here, new macrophages take up apoptotic bodies containing bacilli, or (in larger proportion) the extracellular bacilli released from necrotized macrophages. Epithelial and endothelial cells and fibroblasts also participate in this process. These cells build a cellular architecture involving fibrin, proliferation of transforming growth factor-beta (TGF-β) stimulated fibroblasts and production of mainly type III collagen. The TGF-β anti-inflammatory response may counterbalance excessive local Th1 reactions. In guinea pigs and minipigs the structure of granulomas containing necrosis is also stabilized by collagen. In these animals, TGF-β transforms fibroblasts to myofibroblasts which organize collagen fibers, leading to sphere-like structures that help to control the mechanical stress, induced by lung respiration. The necrotic tissue of granulomatous lesions in guinea-pigs and minipigs is linked to the accumulation of the apoptotic cells and phosphatidylserine-rich lipid bodies from destroyed FMs. Phosphatidylserine accumulation, retaining calcium and phosphate from the local blood transudate leads to calcification, particularly when the inflammatory response is reduced. The mineralization process at alkaline pH is an important starvation, hypoxic and osmotic stress inducer, trapping latent M. tuberculosis extracellular bacilli in necrotic tissues.

A different type of fibrotic process, encapsulating the granulomas using a net of intralobular septa, takes places in larger mammals, including humans. Fibroblasts producing type I collagen in these septa proliferate around the granuloma, when stimulated by TGF-β. This process named as the “double patron” of fibrosis, was observed by Canetti in necropsies of M. tuberculosis-infected subjects without active TB, whom he classified as a “benign” progression of TB infection (Fig. 1).
A strong fibrotic process also takes place around the granulomas in the hilar lymph nodes to curtail further drainage to the right atrium.

Isoniazid chemotherapy needs to be long-term

Slow metabolism is one of the most important, fundamental features of *M. tuberculosis* bacilli. It leads to a slow cell division cycle and thus favors bacterial persistence. Since INH chemotherapy is bactericidal only at a certain stage of the cycle, it has to be delivered for a long period (Fig. 3). However, INH by its bactericidal action and by inducing a stress response in bacilli also reduces the occurrence of infected DCs and of the induction of T cell mediated surveillance of new infected cells. Nevertheless, *M. tuberculosis* has a possibility of returning and re-growing in the lungs, if INH is discontinued. The longer the INH treatment period, the lower the probability of bacillary re-growth: a 6 months treatment has about 60% efficacy and 9 months 90% efficacy. During that period, latent *M. tuberculosis* would be removed, returned to the parenchyma, but could not re-grow in the presence of INH. Drainage of macrophages through the alveolar fluids and their removal through the gastrointestinal tract would hinder the return of viable bacteria to the lung parenchyma.

Association of tuberculosis with the topography of the lungs

The upper lobes of the lung are known to be a predilected site for cavitary lesions, harbouring extracellular bacilli in adult patients with reactivated TB. Reactivation of latent *M. tuberculosis* bacilli when reaching an upper lobe, could involve a number of different mechanisms, none of which is fully understood. These could be related to increased speed of bacillary growth, caused by: a) high oxygen pressure; b) the more discrete net of capillaries; and c) less acidity in upper lobes; all these factors may be somehow unfavourable to immune surveillance. A burst of inflammation with high IFN-γ/TNF-α ratio in response to bacterial replication would then interfere with the development of fibrosis. Lung lesions would develop into cavities, under influence of increased levels of plasmin, generated from plasminogen, trapped at the bacillary cell wall. The stronger mechanical ventilation of the upper lobes, leading to liquefaction, will impair the fibrotic structure of granulomas. According to the “damage framework” concept of infectious diseases, the development of TB could be favoured by either a too strong or too weak host responses to the infection. In the first instance, an excessive IFN-γ level could interfere with the production of fibrin, thus inducing liquefaction. This can lead to extracellular growth of the *M. tuberculosis* bacilli, enhancing the local inflammatory response and tissue destruction. Eventually, large cavities eroding the bronchi would drain out massive numbers of bacilli, thus generating highly contagious aerosols (Fig. 4).

In immunosuppressed patients however, bacilli grow diffusely, without predilection to the upper lobe, inducing weak inflammation and less liquefaction and the aerosols are less contagious. Malnutrition interferes with the development of both innate and acquired immunity, because they require a large supply of nutrients; consequently, this is a significant factor that favors TB incidence, particularly in poor-resource countries.

It has previously been proposed, that the evolutionary advantage of tubercle bacilli rests in efficient transmission, rather than in killing of the infected host. From this angle, infection of immunocompetent individuals appears to be to the best advantage of the tubercle bacilli.
Moreover, the predilection to the lungs seems mandatory for the host. This encapsulates the lesions and a mineralization process, leading to fibrosis of the connective tissue of the lungs. Eventually, a reverse flow is harnessed in favor of the pathogen through continuous re-infection of the lungs. Eventually, a mineralization process, leading to fibrosis of the connective tissue of the lungs. Eventually, a reverse flow is harnessed in favor of the pathogen through continuous re-infection of the lungs.

**Conclusions**

Persistence of infection with *M. tuberculosis* leading to tuberculosis symbolically resembles the “mysteries”, eluded to in the 1 Corinthians (see motto). We discussed here the trumps played by the bacillus and the tenacity of the infected host’s resistance. The bacterial response to the stress, encountered with the infected host cells drives the *M. tuberculosis* cell cycle into a non-replicating (latent, dormant) mode and a low metabolic rate. Latent bacilli retain a capacity to induce necrotic granulomas in the lungs and to survive embedded in the necrotic tissue for long periods. The flow of draining fluids in cases of active TB re-infects the original host and also transmits the infection to new susceptible hosts. Though the host immune reactions are not capable to destroy the bacilli, the host tries at least draining them out by the flow of alveolar fluids. However, this reverse flow is harnessed in favor of the pathogen through continuous re-infection of the lungs. Eventually, a mineralization process, leading to fibrosis of the connective tissue of the lungs counters the pathogen. This encapsulates the lesions and a limits both outward and reverse drainage. However, continuous re-infection of the surviving *M. tuberculosis* persists. In conclusion, the trumps used in the intricate contest between *M. tuberculosis* and humans have been perfected over millions of years of evolution. Better understanding of the formidable natural resilience of the *M. tuberculosis* bacillus in relation to humans gives at least some clue, why it evaded so far elimination by the various used strategies. Better understanding of these factors is mandatory for developing more effective means of control for the many, still vulnerable populations around the world.

**Conflict of interest**

The authors declare they have not any conflict of interest.

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