The evolution of TB depends on the complex interaction between the host and MTB.

‘Once the players have finally left, once time has devoured them, surely the ritual will not have ended.’

*Chess*

Jorge Luis Borges

*Untitled*
Ivo Saglietti
Photography
MTB can remain silent, as a latent infection for long periods of time.

‘You live in another time, you are the owner of a closed space as a dream.’

To a Cat
Jorge Luis Borges

Landscape
Fidelio Ponce
Oil on cardboard; 61 × 61 cm
Collection of the National Museum of Fine Arts,
La Habana, Cuba
The answer is simple: time. Time is crucial in MTB evolution and is therefore one of the reasons for its success.

‘...riders on the storm...’

Riders on the Storm
The Doors

**Untitled**
Lidzie Alvisa
Sculpture, timer, pins, cardboard
MTB

MTB is an old ‘connu’ of humankind. Indeed, it seems that *Australopithecus* was already infected by this bacterium (1). Such an intimate and long-standing host-bacterium relationship is unique and has intrigued, and continues to intrigue, numerous researchers. This interest arises particularly due to the fact that, although it remains loyal to its host, at the same time it is one of its major killers. Indeed, it is estimated that MTB has already killed 1 billion people. How could that happen if, in normal prey–predator ecology systems, such massive mortality should have led to the disappearance of the predator? (2) The answer is simple: ‘time’. Time is crucial in MTB evolution, and is therefore one of the reasons for its success. Overall, MTB is a slow-growing organism, and this is probably the key aspect of its nature.

Is dormancy the key to MTB’s success?

Although MTB can be considered to be a tough bacterium — it has probably the thickest bacterial cell wall possible — this doesn’t make it invincible. Indeed, it has recently been discovered that just 15 minutes of contact with the surfactant from the alveolar fluid is enough to reduce its thickness by 50% (3). As such, it cannot resist calcification (4) around it or damage induced by UV light and desiccation, thus making its survival outside the human body impossible. Although this could explain why it becomes dormant and persists for a significant time in tissues, it is not sufficient to explain one of the keys to MTB’s success, namely its ability to remain in the body for many years. All bacteria have the ability to become dormant (5). Indeed, this is a general mechanism that is triggered whenever a bacterium detects stressful conditions around it. Despite this, lungs have a sophisticated method for constantly cleaning the alveolar surfaces to ensure that gas exchange is maintained. Thus, these surfaces are constantly cleaned by macrophages, with phagocyte foreign particles, and by the alveolar fluid produced by the epithelial cells (6). Both these mechanisms are well-orchestrated by the mechanical dynamics of the lung, which tend to constantly flush anything out of the lung like a balloon. In the light of this scenario, MTB’s ability to become dormant, or non-replicating,
cannot therefore explain why it is able to persist in the lung for such a long period of time.

It may be supposed that the bacilli are able to remain within necrotic tissue, residing therein for a long time in a dormant state. However, the pioneers of TB research have shown that such necrotic tissue becomes calcified in humans and that this calcification process kills the bacteria (4). The best option for MTB is therefore to be removed from the lungs during the cleaning process. A similar process is involved in the lifecycle of the alveolar macrophages, which, after becoming filled by lipid bodies, are subject to purely mechanical forces and suffer the same fate as the alveolar fluid, namely the definitive expulsion from the body via the gastrointestinal duct. However, MTB has an escape route: the fact that alveolar fluid is the origin of aerosols indicates that the bacilli have an opportunity to return (7).

**Importance of the aerosol route of infection**

At this point, we should discuss another key factor that could be crucial for MTB: the aerosol route of infection. However, it soon becomes clear that not even this factor is crucial. Thus, although this route does not expose the host to any risk factor, it has a clear disadvantage for MTB, which can only multiply inside the alveolar macrophages (6). In this regard, any simple respiratory virus is a more efficient pathogen as it only needs to infect the epithelial cells of the pharynx in the host. MTB is also handicapped because there are criteria needed to be met in order for MTB to reach the alveolar space: that it needs to be encapsulated in a very small aerosol particle and not to be smashed against the bronchial wall and to avoid being emerged inside the alveolar fluid and thus, becoming drained up to the bronchial escalator. Again, even if MTB succeeds in reaching the alveolar space, the surfactant present there constantly attempts to dissolve its cell wall.

**MTB needs to be phagocytosed**

Eventually, however, some bacilli are phagocytosed. This process results in another scenario, namely the presence of bacilli inside an alveolar macrophage. A great deal of information is available regarding the complex panoply of ligands that facilitate the phagocytosis of the macrophage (8). However, if the macrophage is not sufficiently activated, all the complexities related to the ability of the bacilli to avoid forming a phago-lysosome complex, and to start their intracellular growth— a fact that seems to be closely related to their ability to avoid the acidification of this compartment— come into play. This latter process appears to be related to a
group of proteins secreted by the bacilli in the region RD1, the most notorious being the complex between CFP-10 and ESAT-6 (9). At this stage, the ability of MTB to avoid apoptosis also appears to be important, although this has not yet been well-established or characterized. In any case, and as a result of purely mechanical factors, the macrophage is eventually unable to withstand the growth of bacilli inside it and is destroyed, thus releasing the bacilli into the external milieu, where they are phagocytosed by other macrophages.

**Is the success of MTB related to its growth rate?**

In light of the above, the slow growth-rate of MTB appears to be the key factor behind its survival ability. Indeed, it can take up to 6 days on average for the macrophage to be destroyed (10). During this time it continues to perform its duties, namely to clean the surface of the alveoli, thus moving freely around this vast surface (an area half the size of a tennis court). This is essential as, when the first macrophage bursts and releases around 30 bacilli, the 20 macrophages that potentially become infected do not remain together but keep moving around the lung for 6 more days, thereby helping to disseminate the infection. Even when the macrophages finally detect the danger and secrete chemokines, they do so individually. The fact that the immune response triggered is cell-based is important as, during the early stages of infection in a specific area of the lung, there is an indication that there is a huge window during which the bacilli can grow, because they do so in individual macrophages, which cannot be targeted by specific lymphocytes (or other actors of innate immunity such as the NK cells). The immune response only becomes effective once these cells clump together to form a granuloma, probably as a result of an additive effect due to the chemokine production. This process, which has been very well-studied in a mouse model, although it is seldom taken into account, allows the bacilli to increase in number from 100 to 100,000 CFU in the space of two weeks, without causing any lesions in the lung (10).

So, once more, slowness is crucial, even if from a microbiological point of view it would appear to be detrimental and is caused by the presence of a single copy of RNA polymerase (11). When studied more carefully, however, it can be seen that this slowness does not trigger any danger signals to the macrophage, thus delaying the chemokine production, and it also confines the infection to isolated cells for as long as possible, thus avoiding granuloma formation and triggering the cell-based immune response. Moreover, this slowness probably also explains why antibodies are poorly stimulated by MTB (12) and therefore it could be the reason that cellular immunity appears to be sufficient to control the infection in 90% of
cases (13). Again, this value of 90% appears to be key as it is the degree of bacillary destruction caused by the cellular immune response once the activation of the infected macrophages has started (14).

And a little bit of necrosis...

And now we come to the other crucial point, namely the induction of necrosis. This factor differentiates MTB from a very similar bacterium, namely the M. avium complex, which has an even thicker cell wall than MTB and grows as slowly as it can possibly be. The difference between these two bacteria is the inability of M. avium to induce macrophage necrosis. Indeed, this is one of the greatest mysteries of MTB: what causes this necrosis? It seems apparent that necrosis of the macrophage (or simply the avoidance of apoptosis) occurs once there is a certain number of bacilli inside it (15). Although this is not relevant at an individual level as they are simply drained by the cleaning mechanism of the lung, if this happens in a granuloma, where a higher amount of chemokines (i.e. TNF) accumulates and larger numbers of different macrophages become necrotic, this process could accelerate the destruction of neighbouring infected macrophages, even though they may contain fewer bacilli. This process could also be accelerated by the sudden onset of the immune response and the mass eruption of neutrophils, which die in a matter of hours and also release toxic factors (reactive oxygen and nitrogen intermediates) that could induce the necrosis of the macrophage. Indeed, this process could lead to the liquefaction of lesions if it occurs in the upper lobe, where the higher oxygen pressure increases bacillary growth slightly and delays the immune response. Once the granuloma has formed, this process would be even more intense due to the larger number of macrophages undergoing necrosis and the presence of more intense mechanical forces (a characteristic of the upper lobes), thereby decreasing the fibrotic capacity and promoting liquefaction (16). Once liquefaction takes place, the bacilli have more opportunities to grow as they do so extracellularly, thereby enlarging the lesion dramatically (from mm to cm) and causing erosion of the bronchi and massive drainage to the open air, enabling the infection of other subjects.

But what triggers this necrosis? Is there any specific MTB antigen that causes necrosis? Or could there be a specific toxin? The answers to these questions remain unknown. For a long time this process was thought to be related to the presence of cord factor, but today we have found in credible findings that this molecule is also present in M. avium and other mycobacteria. It appears that ESAT-6 could be the key antigen (17) despite the fact that M. kansasii also has it (18) and does not
contribute any success, even if it has a thick cell wall and also a slow grower. In this regard, it appears that cellular immune response is not very important in the control of *M. kansasii* infection (19). Further effort should therefore be dedicated to investigating this aspect, including a comparison of the antigenic composition of both *M. avium* complex and MTB. All in all, the intrinsic factors that make MTB so effective can be summarised in terms of its slow growth and its ability to induce slight necrosis, or simply avoid apoptosis.

To be even more accurate, it is a combination of MTB's slowness and human weakness, which had ruined our expectations in the 1970s, namely our expectations for immediate results. A six-month regimen takes too long for patients to be cured although patients would normally feel better from the medication in two weeks time.

**REFERENCES**


