A Dynamic Reinfection Hypothesis of Latent Tuberculosis Infection

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Abstract

Background: It has been traditionally postulated that individuals, once infected by *Mycobacterium tuberculosis*, will retain throughout their entire lifetime latent bacilli which will remain dormant in old lesions. This bacillus would then be the source of a later reactivation of active tuberculosis (TB), with the aid of resuscitation factors. Unfortunately, the presence of these bacilli can only be predicted by indirect immunological methods, such as the tuberculin skin test (TST) or T cell interferon–gamma release assays. Other evidence shows that a 9-month isoniazid treatment of TST+ individuals converting to TB reduces the incidence of TB by approximately 90%.

Questions: Taking into account this widely accepted framework, I suggest that there are at least three relevant questions to answer: (1) How can dormant bacilli remain in the lungs for an entire lifetime, taking into account constant cellular turnover and the healing of damaged tissues? (2) What provides the resuscitation factor to dormant bacilli, assuming that these latent bacilli are indeed present inside old lesions? (3) Why can a 9-month treatment with isoniazid eliminate dormant bacilli? As isoniazid is active only against growing bacilli, and thus is only able to destroy them after reactivation of latent bacilli, this treatment should have to be provided for life if the traditionally accepted postulate is correct.

Hypothesis: For a better understanding of latent TB infection. I propose a hypothesis that describes a dynamic scenario of constant endogenous reinfection with *M. tuberculosis* which explains the efficacy of the current standard of treatment. If this hypothesis is true, new strategies for the management of TB may arise.

Abbreviations: INH: Isoniazid; LTBI: Latent tuberculosis infection; NO: Nitric oxide; TAG: Triglycerides; TB: Tuberculosis; TST: Tuberculin skin test

Infection 2009; 37: 80–86
DOI 10.1007/s15010-008-8087-y

Background

Latent tuberculosis infection (LTBI) is a major public health problem for several reasons: (1) it represents a massive reservoir for developing active tuberculosis (TB); (2) the different diagnostic methodologies (namely the tuberculin skin test, TST, or T cell IFN–gamma release assays, TIGRA) are logistically difficult and/or expensive [1, 2]; (3) its treatment is expensive, toxic, and very long and, therefore, its implementation is difficult. All these factors create a negative disposition in all physicians who, in addition, cannot find a clear rationale indicating the nature of LTBI and its treatment. In fact, the presence of latent bacilli in LTBI is difficult to demonstrate [3]. The only clear evidence for LTBI control, validated by empirical practice and accumulated over more than 50 years of constant practice, is that a 9-month treatment with isoniazid of the TB contacts TST+ avoids the incidence of TB by approximately 90% [4].

The traditional line of thought has been that individuals with LTBI retain latent bacilli for their lifetime and that these remain dormant and reside within old lesions in the upper lobes of the lung. Reactivation of these dormant bacilli leads to TB, aided by resuscitation factors as well as the high oxygen pressure in the upper lobes, both of which enhance bacillary growth and diminish immune responses [5, 6] (Figure 1).

Discussion

Three very relevant questions arise when this widely accepted context is taken into account.

How Can Dormant Bacilli Remain in the Lungs for an Entire Lifetime?

Alveolar macrophages are known to have a 3-month lifetime [7]. Their progenitors pass through the capillaries in the

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Received: February 27, 2008 · Revision accepted: September 10, 2008
Published online: March 23, 2009

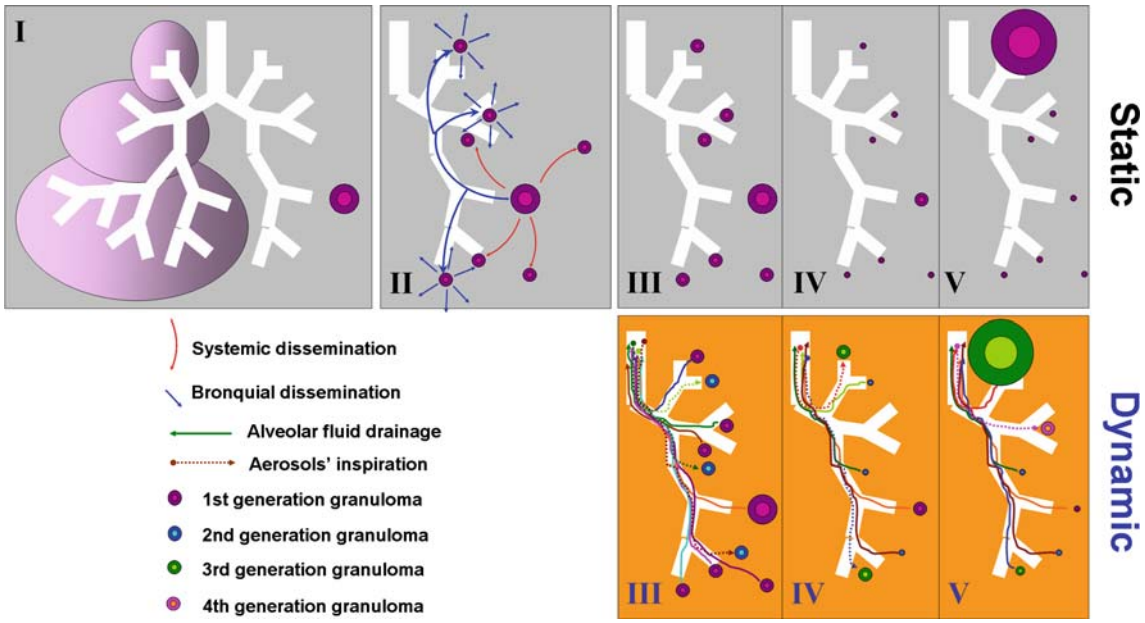

Figure 1. Latent tuberculosis infection (LTBI) and the generation of active tuberculosis (TB). Comparison between the traditional "static" theory and the dynamic hypothesis. Once the initial lesion is generated (I), there is a bronchial (blue arrows) and systemic (red arrows) dissemination that generates new secondary granulomas. This process is stopped once the specific immunity is established (III). From that point onwards (IV), lesions remain where dormant bacilli reside; these have the ability to reactivate their growth after a long time (V). In the dynamic hypothesis, there is a constant drainage of non-replicating bacilli towards the bronchial tree (solid arrows), but also inspired aerosols (dotted arrows) can return the bacilli to generate new granulomas (III–IV). This process implies the induction of different generations of granulomas. In this process, if one of these reinfections takes place in the upper lobes, it has the opportunity to induce a cavitary lesion.

form of monocytes and are progressively transformed in the parenchyma in order to provide it with a good coverage. They must face and neutralize the enormous amounts of particles and microorganisms that are constantly inspired. It is difficult to understand how a macrophage could host bacilli for years in a static manner. In actual fact, the host tends to clean up the lesions in order to replace the original parenchyma and its function [8], which is why a complex net is deposited by the fibroblasts at an injury site—to allow the site to be rebuilt. Only when a lesion is very large can it be maintained for a long time; it may then remain, becoming fibrosed and calcified for years or even for the individual's entire lifetime [8]. This is the scenario of those patients who actually suffered of TB then treated with an artificial pneumothorax or pneumoplasty that allowed the establishment of such non-functional sites [9].

In contrast, it has been suggested that the latent bacilli could survive outside the lesions, in fibroblasts or epithelial cells [10]. In this case, the scenario is less understandable as these cells have an even shorter half-life than macrophages.

What Provides the Resuscitation Factor to Dormant Bacilli?

Taking into account the scenario of a large fibrosed and calcified site, which is not the scenario in LTBI but rather in an old TB lesion site, it has been demonstrated that a small percentage of bacilli may be recovered after being removed and cultured in a rich medium [11]. However, this is an artificial context. Assuming that these bacilli can survive for a long time, the question is how resuscitation factors can be introduced into these sites to reactivate the non-replicating bacilli, as resuscitation factors are only produced by already growing bacilli [12].

Why Can a 9-month Treatment with Isoniazid Eliminate Dormant Bacilli?

It is well known that isoniazid (INH) is only active against growing bacilli [13]. Assuming the presence of these dormant bacilli in fibrosed lesions and also that they can reactivate, how could a 9-month treatment targeted against growing bacilli have any efficacy? If INH is to be given in anticipation of the moment of bacilli growth, the treatment should be given for life.

Lessons from the Experimental Models

Some years ago, in the experimental murine model of TB, it was demonstrated that after the acquisition of the immune response, the granulomas were surrounded by a large ring of foamy macrophages (FM) [14, 15]. These cells are usually present during the resolution phase of any kind of pulmonary lesion [16]. The FM were located in the alveolar spaces, indicating that they were at the end of

their life cycle as FM, after being filled with lipid bodies derived from the phagocytosis of dead cells, get into the alveolar spaces [17]. Some of the FM observed carried single acid-fast bacilli at the time they could hardly be seen in the center of the granuloma (where the bacilli were in the acute, pre-immune phase). These bacilli were able to start growing again inside the FM, thus beginning a new lesion. This development resulted in the progressive pulmonary infiltration that finally killed the mice, although it allowed the bacillary concentration to be controlled for months [14, 15].

"Slowness" as a Persistence Factor

It has been demonstrated that this bacillary control is not a consequence of a constant reactivation and destruction of the bacilli but, rather, of the slower metabolism adopted by most bacilli entering the stationary phase [18]. This slower metabolic activity confers some resistance to stress relative to that shown by active growing bacilli of the pre-immune phase [19]. This state, combined with the fact that the bacteria, after suffering a stressful condition, require a proportionally longer period of progressive growth acceleration (lag phase) before logarithmic growth (log phase) [20] as well as the usual slow metabolism of *Mycobacterium tuberculosis* [21], makes this reactivation process extremely long. The prolonged duration of the infection could therefore be explained as a consequence of a balanced situation between the host and the pathogen, which would allow the survival of both for a long time.

Infected Aerosols and Constant Endogenous Reinfection

Murine alveolar spaces are smaller than those of larger hosts, such as guinea pigs or humans [22]. This clarifies why FM accumulation in the alveoli is less extensive in the latter than the former and, consequently, why the opportunity to reactivate in the pulmonary parenchyma and to induce progressive infiltration in the latter is rare. Those FM carrying acid-fast bacilli would be drained to the upper bronchial tree by the alveolar fluid and finally swallowed into the stomach, where they would be destroyed [23]. Interestingly, in this process the bacilli are included in the alveolar fluid and thus can take part in the aerosols generated in the upper bronchial tree. The function of these aerosols is to condition the inspired air, i.e., providing it with greater humidity and temperature and thus avoiding any potential damage in the alveolar space [24]. This is a frequently forgotten fact – i.e., that the generation of infected aerosols has a higher chance of inducing constant endogenous reinfections than the generation of new exogenous LTBI cases.

FM Cells Allow Bacillary Escape and Survival

Foamy macrophages cells allow the bacilli to "escape" from the granulomas. This mechanism also explains why TB patients with no cavitary lesions can also generate infective aerosols [25]. It is not clear how the bacilli can reach the sputum in these clinical forms because there is no clear connection between the lesions and the bronchi, as there is in the case of patients with cavities. Interestingly, FM are able to maintain a stressful environment that keeps the bacilli in a non-replicating state [15, 26]. This state allows the bacilli to confront future stressful environments more effectively than the one that is developing. Such hostile situations can be found in the alveolar fluid itself [27] or when the fluid is exhaled within an aerosol droplet into the open-air environment where it faces dryness or UV light [28].

A traditional postulate has been that the TB cavitary form is an effective way to drain the bacilli [29, 30]. The moderately higher infection rate (two-fold on average) of TB cavitary vs. non-cavitary forms suggests a relative lack of infective effectiveness, given that the bacillary concentration detected in the sputum is 10–1,000-fold higher in the former [31]. This is probably due to the high proportion of active growing bacilli in the former, which in turn gives bacilli a lower capacity to resist the stressful conditions in the open air.

In addition, the large number of lipid bodies present in these cells and a constant interaction with the bacilli-containing phagosomes [26] enable the bacilli to accumulate lipids in their interior in the form of triglycerides (TAG), thus providing them with a future carbon (energy) source in the event of starvation or as a means to accelerate bacillary growth in a new infection site [32]. TAG are accumulated in response to an elevated ratio of carbon:nitrogen [33], which occurs inside FM and adipocytes, where a prolonged presence of non-replicating *M. tuberculosis* bacilli that accumulate intracellular lipophilic inclusions (ILIs) has also been reported [34]. In fact, the accumulation of TAG has been recently related to the Beijing strains, which have an enhanced dissemination capacity [35]. Finally, when FM are destroyed in the alveolar fluid, the lipid bodies also contribute to an increased surfactant concentration, thereby reducing the superficial tension and increasing the amount of aerosol [36].

The Dynamic Hypothesis of LTBI

All of this information and the fact that *Homo sapiens* and *M. tuberculosis* have been evolving together for 3 million years [37] makes it possible to postulate an evolution of LTBI in which both organisms have adapted to each other extremely well.

From the point of view of the host, it is imperative to generate a strong immune response against the growing bacilli [38]. Growing bacilli represent the most dangerous form, as an increasing concentration induces a massive tissue destruction that may be fatal. However, growing bacilli are the easiest to kill, as they badly tolerate stressful environments [19, 39]. Once the growing bacilli

are controlled and most bacilli are in a non-replicating state, they are drained by the FMs to the alveolar fluid that will be finally swallowed and digested in the stomach.

The bacilli try to hide in the intraphagosomal milieu, which is the best site for growth, and to avoid the effect of the components of the alveolar fluid on their lipid cell walls. Interestingly, the humoral response has been found to be very weak in LTBI [40, 41]. Contrary to what is traditionally accepted, antibody-mediated immunity has been shown to have a protective role against *M. tuberculosis* infection [42–44]. Thus, the weakness of this response in LTBI leads the host to the only alternative – inducing a cellular immunity less immediate than the antibody-mediated one [45], giving a better chance for constantly reinfecting the host. The bacilli then grow as much as possible until they cause necrosis of the macrophages, although sometimes they induce their own apoptosis. This implies an increased inflammatory response and the attraction of macrophages and neutrophils. The former will provide sites in which to grow, and the latter will be a massive source of cell membranes to induce FM, which will also be destroyed at this point [17, 26]. All this cellular debris will be mixed with extracellular bacilli immersed in a stressful milieu and become the first source of the non-replicating population [46].

The time for non-replicating bacilli to drain from the necrotic tissue to the alveolar space is when the immune response commences and bacillary growth is limited. In this case, the metabolic slowness of the bacilli and the certainty that consistently stressful conditions will be present in the FM allow the bacilli to maintain their non-replicating status, enabling them to accumulate lipids (such as TAGs) in order to achieve a better level of preparedness for future reactivation and to be able to resist further stressful conditions. The production of nitric oxide by FM and activated macrophages may even induce local immunosuppression by suppressing T effector cells [15, 17], a process that can be also crucial for maintaining the viability of the drained bacilli.

Non-replicating bacilli can become part of the aerosols and again reinfect the host with the inspired air. The time required for dendritic cells to phagocytose the new growing bacilli at the new site and to present the antigens to the lymph nodes is a ''privileged'' period for the bacilli [47] as gives them the time to maintain their concentration in the host; this concentration is of course at lower levels than those during primary infection, but it is still high enough to consistently repeat the process. However, if reinfection takes place in a conducive site, such as the upper lobes where the high oxygen pressure allows a quicker bacillary growth and the less acidic conditions and less irrigated zone reduce the immune response, rapid growth can take place that also induces a massive inflammatory response [6]. The amount of necrotic tissue generated in this process is then too large to be perfectly structured by the fibroblast, leading to liquefaction and cavitation of the lesion (Figure 1).

LTBI: Once Infected Always Infected?

The dynamic hypothesis describes LTBI as a complex cycle in which many factors play a part. This cycle occurs within the context of an immune host, and it also depends on aspects associated with fluids and aerosol mechanics. This complexity makes it reasonable to assume that the frequency of endogenous reinfection will decrease with time. LTBI will probably remain for a long period, but not for an entire lifetime as is usually thought, an interpretation that closely matches the probability of the incidence of TB after infection [47]. At the present time, this hypothesis can be carefully proved using the new LTBI diagnostic tools. TIGRA techniques support the concept that growing bacilli are constantly present in LTBI, as postulated in the dynamic hypothesis. TIGRAs may detect the IFN-γ released by effector lymphocytes (with an approximate half-life of 3 days) after identifying macrophages that present antigens (included in the ESAT-6 complex) produced by growing bacilli [2, 38].

The decrease of the TIGRA value with time in LTBI patients could be demonstrated in very long-term longitudinal studies. This technique would reflect the progressive decline in the reinfection process. This reinfection process can be masked by the immune memory, which can persist for at least 2 years after bacillary eradication [48], or by external reinfection. Obviously, the interpretation of these experiments should also take into account other factors, such as the fact that endogenous reinfection incidents must be quite separate in time and will probably generate ''peaks'' and transient boosts of immune response before a constant negativization will take place.

What is Needed to Demonstrate the Dynamic Hypothesis?

As mentioned above, the TIGRA test assay provides support to this hypothesis as it constantly detects effector T cells against antigens produced by growing bacilli. Equally, the mechanism by which non-replicating bacilli can reach the alveolar fluid through FM has been well demonstrated in TB experimental models in mice and guinea pigs [26] as well as in humans [49]. What has not yet been demonstrated is the presence of these bacilli in the bronchial aerosols or of small pulmonary lesions, not visible on chest X-rays, in individuals subject to LTBI. Empirical evidence of these would definitively demonstrate the dynamic hypothesis and transform it into a theory.

One approach to addressing the first point would be to detect bacilli in samples of condensed exhaled air [50] from individuals with LTBI. It is well known that these persons are unable to infect other people, meaning that the bacillary concentration must necessarily be very low. In this case, very sensitive techniques, including the detection of specific DNA, should be used.

The demonstration of small undetectable lesions through chest X-rays in individuals subject to LTBI would require a very sensitive imaging technique. However, even in this case, the confirmation of their etiology would be impossible – for ethical reasons. This is why I believe that any demonstration of such undetectable lesions will require the use of LTBI experimental models. The only well-established data obtained to date have been obtained after a low dose infection in rhesus monkeys [51], pigs [52], and mini-pigs [53]. In all these models, there is a control with a low bacillary concentration in the tissues (around 10e2–10e3 cfu) and an immune response that resembles what is obtained in the case of LTBI subjects. The study of these models should provide evidence on the ability of the INH treatment to avoid the constant induction of new small lesions. The existence of these lesions and the ability of INH treatment to reduce their number have been demonstrated [53]. However, it is not yet clear if the granulomas are generated from the dissemination caused at the acute phase of the infection or because the INH treatment removes the threat of constant reinfection. It will also be important to demonstrate that this constant reinfection is not caused by systemic dissemination. This is a crucial as this process has not been demonstrated in humans [54], and empirical evidence of this process would be extremely important for the validation of the model.

Implications of the Dynamic Hypothesis

The first implication of this hypothesis is that the gold standard treatment of LTBI can be explained (Figure 2). A 9-month treatment allows the host to drain all non-replicating bacilli and restore the lung parenchyma, thereby avoiding the risk of endogenous reinfection in 90% of cases. This suggests a scenario in which the cycle required to maintain LTBI can be attacked in different ways. The possibilities include researching for drugs or vaccines able to disrupt the mechanisms that allow the non-replicating phase to begin (e.g. through interfering with the storage of TAG), manipulating the cycle of the FM, adapting the drugs to the environment created in the FM, and manipulating the properties of the alveolar fluid. At the present time, it seems likely that a combined treatment may be necessary to shorten the LTBI treatment, probably by including mycobacterial drugs, therapeutic vaccines, the induction of antibody-mediated immunity, the use of passive serum therapy [44], and even the use of anti-inflammatory drugs [17] or substances able to control the superficial tension of the alveolar fluid [36] or lipid body accumulation in the macrophages [17].

In the epidemiological field, this hypothesis also supports the view that reinfection may be the norm. An old theory postulated that once infected, a person had the infection for life [47] and, consequently, there was no possibility of reinfection. Therefore, traditionally it has been considered that TB in previously infected people was a consequence of endogenous reactivation [55]. Molecular epidemiology has disproved this rather extreme hypothesis [56]. Hence, the natural history of LTBI would imply the removal of non-replicating bacilli after some years and, thereby, the disappearance of specific immunity and the possibility of being reinfected again.

Another intriguing factor has been introduced with this hypothesis. As the induction of a cavitary lesion in the upper lobes requires reinfection at this specific site, there is an implied existence of a factor that is not related to any kind of host immune response. This is a probabilistic question linked to fluid and aerosol mechanics that also requires further study.

Summary

- A traditional view of LTBI supports the persistence of dormant latent bacilli in old lesions that are able to reactivate their growth during the entire lifetime of the host, with the assistance of resuscitation factors.

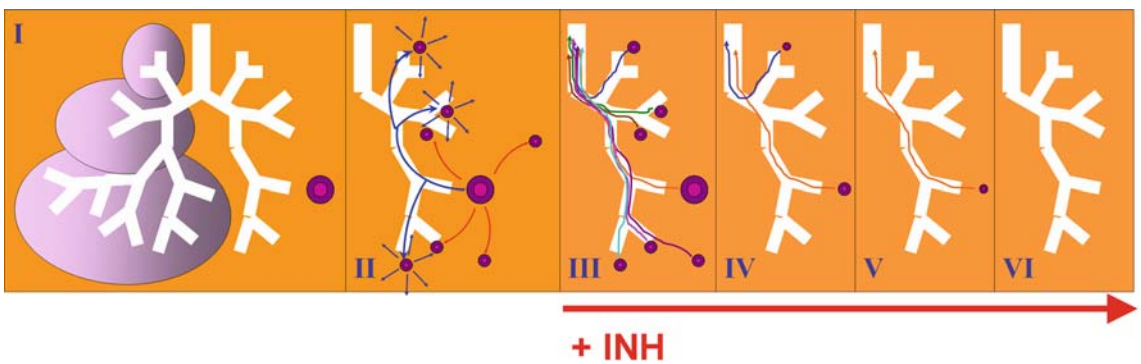

Figure 2. Mechanism to explain the success of isoniazid treatment in the LTBI according to the dynamic hypothesis. This treatment allows the drainage of the non-replicating bacilli, but once they come back with the inspired aerosols to the parenchyma, the bacilli have no chance to reactivate. In this case, the lesions disappear with time, and the opportunity for the bacteria to reach the upper lobes and generate the cavitary lesion is avoided.

- The traditional view does not take into account the general lack of pulmonary lesions in the chest X-rays of LTBI patients, progressive cellular turnover, and the regenerative processes involved in reconstituting the injured tissues.
- The traditional view does not explain why 9-month isoniazid treatment has a 90% efficacy in the treatment of LTBI.
- The dynamic hypothesis suggests that LTBI is caused by the constant endogenous reinfection of latent bacilli, and it is the only hypothesis that can explain the efficacy of the current form of treatment.
- The dynamic hypothesis is supported by the constant drainage of bacilli in non-replicating state from the granulomas to the alveolar fluid, thus making reinfection through self-inspiration of the host' infected aerosols a feasible explanation.

Acknowledgments

This is project FIS 01/3104. I am very grateful to my PhD students, Cristina Vilaplana, Evelyn Guirado, Olga Gil, and Neus Caceres, who constantly challenge me with their awkward questions and for whom I constantly try to find explanations and develop responses that can provide some sense to the LTBI process. They patiently try to follow my lucubrations and, what is even more important, they do all the ''dirty work'', putting up with the extreme difficulties and lengthy duration of the experiments we conduct with the aid of the technicians with the aim of making things clearer. They also have the patience required to read innumerable versions of my writings and to try to make them more understandable. I gratefully acknowledge them and their absolute dedication to the study of LTBI and TB, because without them none of these thoughts would exist!

Conflict of interest statement. The author declares that he has no competing interests.

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