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RUTI: A new chance to shorten the treatment of latent tuberculosis infection

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Summary Treatment of latent tuberculosis infection (LTBI) requires a long period of chemotherapy (9 months), which makes treatment-compliance extremely difficult. Current knowledge of latent bacilli and of the lesions with which they are associated suggests that these bacilli survive in granulomas with a central necrotic core and an outermost layer of foamy macrophages (FM) that represent an important immunosuppressive barrier. The presence of FM, which is especially strong in mice, explains not only the kinetics of the drainage of dead bacilli, debris and surfactant, but also how latent bacilli can escape from the granuloma and re-grow in the periphery, particularly in the alveolar spaces where they can disseminate easily.

RUTI, a therapeutic vaccine made of detoxified, fragmented *Mycobacterium tuberculosis* cells, delivered in liposomes, was used to assess its effectiveness in a short period of chemotherapy (1 month). The rationale of this therapy was first to take advantage of the bactericidal properties of chemotherapy to kill active growing bacilli, eliminate the outermost layer of FM and reduce local inflammatory responses so as to avoid the predictable Koch phenomenon caused by *M. tuberculosis* antigens when given therapeutically. After chemotherapy, RUTI can be inoculated to reduce the probability of regrowth of the remaining latent bacilli.

RUTI has already demonstrated its efficacy in controlling LTBI in experimental models of mice and guinea-pigs after a short period of chemotherapy; these experiments in animals showed the induction of a mixed Th1/Th2/Th3, polyantigenic response with no local or systemic toxicity. Local accumulation of specific CD8 T cells and a strong humoral response are characteristic features of RUTI that explain its protective properties; these are particular improvements when compared with BCG, although the regulatory response to RUTI may also be an important advantage.

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Further experiments using bigger animals (goats and mini-pigs) will provide more data on the efficacy of RUTI before starting phase I clinical trials.

Introduction: what is a latent bacillus?

Despite this being a simple question, finding an appropriate answer is crucial to demonstrate the validity of any new treatment against latent *Mycobacterium tuberculosis* bacilli; latent bacilli are responsible for the long period of treatment currently required for sterilizing lesions infected with *M. tuberculosis*.

The nature of latent bacilli and their ability to survive "in vitro" in low pO_2 conditions, and even in anaerobiosis,[1] has been widely studied. To survive in low pO_2, bacilli appear to acquire a state of nonreplicant persistence (NRP).[2] Huge lesions with intragranulomatous necrosis (IN) that are induced in tuberculosis (TB) may develop such an extreme anaerobic environment,[2] supporting the idea that latency induced by low pO_2 occurs in lesions. However, direct measurements have never demonstrated the presence of anaerobiosis in TB lesions and factual support for the hypothesis is lacking. Furthermore, IN is a "living" tissue, full of collagen fibers, that also needs a physiological environment for survival. Anaerobiosis, therefore, appears unlikely in such a setting. Even if such atmosphere with low pO_2 be induced in calcified lesions, experimental models[3] demonstrate that the period in which the bacilli may survive in this environment is definitely finite. Moreover, microaerobiosis is usual in the host tissues[4] and, therefore, not only latent bacilli but also actively growing cells may adapt to this environment. Furthermore, experiments in knock out (KO) mice lacking functional genes essential for triggering a competent immune response demonstrated that active growing bacilli can be found inside massive lesions with abundant IN, expected to have a low pO_2.[5,6]

Some authors defined latent bacilli as those present in the tissues of mice treated with chemotherapy for a long time, whose presence was not detected in cultures until a few months after the end of the treatment; this process was favored by the administration of cortisone. This experimental model, characterized a long time ago, is known as the Cornell model.[7] In the Cornell model bacilli do not have to resist an extremely low pO_2. However, patients with latent tuberculosis infection (LTBI) face circumstances different from those in the Cornell model.[8] Usually, people with LTBI display a cell-mediated and antibody-mediated immune response that controls progression of the infection in the initial focus of infection and in the local draining lymph nodes (the "Ghon complex") by inducing a strong granulomatous infiltration,[9] a process which does not happen in the Cornell model. Besides, It is currently accepted that the population obtained with the Cornell model is special and is not considered to be a good model of latent bacilli, but rather of "persistent" bacilli.[10,11] Persistence implies that a special population of bacilli subsists the period of chemotherapy, probably through acquiring tolerance to antibiotics,[11,12] although its mechanism remains poorly understood.

Other authors focused on the hypothesis that latent bacilli have to adapt to a lack of nutrients, thus resembling the bacterial population found in the steady state of conventional liquid cultures.[13] Thus the term "dormant" was established to define bacilli that were "in a state of low metabolic activity and unable to divide or to form a colony without a preceding resuscitation in liquid medium, which may either occur spontaneously or require the provision of compounds (growth factors) present in the supernatant of growing cells".[13,14] These authors considered the in vitro "dormant" form as the equivalent to the one obtained in vivo with the Cornell model, representing an extreme form of viability, close to the death of the cell. However, the exquisitely fastidious requirements needed for regrowth of these "dormant" bacilli are difficult to reconcile with the conditions faced by latent bacilli in vivo, bacilli which are, however, able to reactivate after a long period of time to induce TB disease.

Finally, there is the observation made a long time ago that the bacillary population obtained from the lungs of chronically infected mice has a greater resistance to heat stress (53 °C) than the bacillary population in the acute phase.[15] The same author submitted "in vitro" cultures, young (exponential phase) and old (steady-state phase) to the same conditions and obtained a higher resistance to heat stress in the latter. This observation led to the conclusion that a slower growth rate made bacilli from the chronic phase more resistant to stress. Consistent with this hypothesis, Muñoz-Elias et al.[16]