EDITORIAL

Therapeutic Vaccines Against Tuberculosis: A Glowing Future

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Linking the adjective "glowing" with tuberculosis (TB) is undoubtedly a provocative exercise, given that the outlook in the battle against this disease is not promising, as revealed by a telling statistic: a third of the world's population (2500 million people) has latent tuberculosis infection (LTBI). Furthermore, the number of individuals with LTBI is growing steadily, and there are some 90 million new cases annually, resulting in 9 million cases of active TB and 2.5 million deaths each year.[1] A vaccine developed in Spain called RUTI represents a new hope for LTBI control in the future (there is a play on words, incidentally, between the name of the vaccine and the word "rutilante," meaning "glowing," in the title of the original article in Spanish: "Vacuna terapéutica antituberculosa: un `rutilante´ futuro). On 23 April 2007—St Jordi´s Day in Catalonia, traditionally celebrated by gifts of books and roses—RUTI was inoculated for the first time in 6 healthy volunteers, thereby launching the first initiative that combines chemotherapy and immunotherapy to reduce the duration of standard LTBI treatment from between 6 and 9 months[2] to just 1 month plus the inoculation of 2 doses of RUTI.

RUTI was clinically developed following significant efforts aimed at developing a more rational view of the mechanisms involved in generating LTBI. Our ultimate aim was to lay the groundwork for future developments in this field in terms of a dynamic hypothesis that refutes the static hypothesis that has traditionally been used to explain the origins of LTBI.[3]

The dynamic hypothesis is, in fact, the only hypothesis capable of explaining why 6 to 9 months´ treatment with isoniazid is capable of preventing LTBI from developing into TB, thus removing the requirement for the complicated physiological mechanisms proposed to support the static hypothesis. The proposed physiological mechanisms center on the generation of what might be called reinforced granulomas, once infection and the initial diffusion of bacilli have taken place. These granulomas would be capable of physically restricting the diffusion of the bacilli by developing a fibrotic tissue that, in turn, creates an anaerobic environment.[4] A second assumption is that the granulomas potentially remain unchanged in the tissue for many years.[5,6] Finally, a third premise is that the latent bacillus can resist the anaerobic environment[7] and that growth can be reactivated years later following contact with some resuscitating factor.[6]

In contrast, the dynamic hypothesis takes into account basic physiological processes in the host—such as cell half-lives or the regeneration of damaged tissues—that would make it extremely difficult for a granuloma to survive for years.[3] It is impossible, for example, for an anaerobic environment to be created in the granulomas, given that ongoing and steady consumption of oxygen would be required (as in the case of bacterial flora in the teeth[8] or in the large intestine[9]). Note, furthermore, that nobody has explained exactly who the prince is that kisses the sleeping beauty awake. In other words, precisely how does a resuscitating factor breach the fortress where the latent bacillus resides? This question is even more puzzling if we bear in mind that the resuscitating factor is produced by actively growing bacilli.[10] The dynamic hypothesis considers, moreover, that *Mycobacterium tuberculosis* is capable of generating LTBI by means of a combination of 3 standard properties: a very slow growth rate, the capacity to produce tissue necrosis, and the possession of a thick, hydrophobic cell wall.[3]

Thus, when *M tuberculosis* enters the alveolar space, it needs to be phagocytosed by alveolar macrophages in order to be able to avoid a hostile environment that is dominated by surfactants and by molecules and enzymes that are capable of slowly destroying its thick cell wall.[11] Once inside the macrophages, a small number of bacilli can multiply and so maintain themselves in the host. Once exponential (log phase) growth and active development begin, the bacillus prevents phagosome-lysosome fusion and causes macrophage death.[12] This cell destruction prejudices the bacillus by confronting it once again with an extracellular environment that, by now, is even more hostile; this is because the released content of the macrophages (and the neutrophils) creates an acid environment containing oxygen radicals, nitrogen radicals, and bactericidal enzymes.[13] In this stressful environment, the bacillus enters a new stationary phase until it is again phagocytosed by other macrophages attracted by the inflammatory process. This cycle is repeated time and

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again, resulting in 2 populations of bacilli: those in the exponential growth phase and those in the stationary phase. The stationary-phase bacilli accumulate among the cell detritus,[3] which is responsible for the appearance of lipid bodies inside the macrophages that phagocytose the detritus, leading to the generation of foamy macrophages.[14]

This process is interrupted once an immune response develops (aimed mainly at the growing bacilli[15]) and the infected macrophages are activated. Bacillus growth is thus halted, and foamy macrophages become increasingly evident. This happens because, when not destroyed by bacillus growth, they have the opportunity to accumulate more and more lipid bodies through phagocytosis of the necrotic tissue and of the bacilli in the tissue. However, these bacilli neither multiply nor die,[16] due to the fact that the macrophages—stimulated by a large quantity of cellular and bacterial detritus—can generate nitric oxide and, with it, a bacteriostatic concentration of nitrogen radicals.[16,17] The macrophages also suppress the action of the specific lymphocytes that should activate them.[18] The process is further affected by 2 other factors: the huge quantity of lipid bodies contained within the macrophages reduces efficiency of phagosome-lysosome fusion,[19] and abnormal antigen presentation prevents activation of the macrophages by specific lymphocytes.[20] One way or another, foamy macrophages begin to accumulate in the alveolar space surrounding the granuloma, and as a result stationary–phase bacilli that have accumulated inside some of the macrophages are drained off or "escape." This then, is the process that underpins the dynamic hypothesis: no longer do we have a prince charming who awakens the sleeping bacillus; what we have, rather, is a street sweeper who seems to indiscriminately collect bacilli along with cell debris.

However, it can be argued that the street sweeper is not so indiscriminate—that the sweeping action is part of a deliberate host strategy that includes a potent immune response focused on the identification and elimination of growth-phase bacilli (as the most dangerous and vulnerable bacilli), leaving the task of draining the least dangerous bacilli (the stationary-phase bacilli, which are more difficult to destroy[21]) to the restorative dynamics of the parenchyma. The bacilli are capable of adapting to this situation—taking advantage of it, in fact, to survive. Thus, once the bacilli reach the alveolar fluid, they are transmitted to the upper airways, to be expelled or else swallowed and then destroyed in the stomach. Concealed in this process is a well-known mechanism which, curiously enough, is little taken account of in the pathophysiology of TB, namely, the generation of aerosols. These are produced in the upper airways for the purpose of moistening and warming inhaled air.[22] Forming part of the aerosols are bacilli contained in the alveolar fluid and originating in cavitated lesions, noncavitated lesions, or small LTBI-generated lesions. These 3 kinds of lesions will clearly result in different concentrations of bacilli in the fluid—the first 2 cases represent different capacities for exogenous infection, whereas in the third case this capacity will be null.[23] However, note that a key concept underpinning the dynamic hypothesis is the fact that the main goal of the aerosols is to return to the alveolar space. It is also interesting to note that slow bacillus growth means that the stationary phase is prolonged,[24] and—given that bacilli in this state are far more resistant to stress than those in the active growth phase[21]—the capacity for inducing new infections will be greater.[21]

LTBI would thus be the result of an endogenous reinfection—that is, it would be a consequence of constant diffusion and reactivation. This explains why the administration of isoniazid for 6 to 9 months is effective. The classic static hypothesis indicates that, after the bacilli are disseminated and before immunity is induced, small granulomas develop in the upper pulmonary lobes where the latent bacilli accumulate. According to this hypothesis, the growth of the latent bacilli can be reactivated many years later, once stimulated by a resuscitating factor and enhanced by an episode of immunodepression.[5,6] However, if this hypothesis were accurate, the current treatment for LTBI would be ineffective given the lengthy period of time in which these bacilli remain in the latent state in situ. In this case, preventing reactivation would require prolonged administration of isoniazid over many years—for life, even, if we took into account other equally questionable classic concepts.[25]

The dynamic hypothesis does not dispute the fact that a high oxygen level makes the upper lobes an excellent site in which the bacilli can grow more intensely to the point where they create cavities.[26,27] However, it does posit that the presence of bacilli in this location is the consequence of constant and recurring endogenous infection and not the result of bacilli persisting in the lung apex for many years (they would have previously been drained off).

The dynamic hypothesis considers that 6 to 9 months´ treatment enables the host to naturally drain stationary-phase bacilli, thereby avoiding the possibility of reactivating bacillus growth and perpetuating bacillus existence.

Chemotherapy has a further beneficial effect. It eliminates accumulated foamy macrophages around the granulomas by preventing the growth of bacilli in the macrophages and reducing the inflammatory response.[28] This, in turn, eliminates a source of local immunodepression. Foamy macrophages are characterized by the generation of nitric oxide, with which they suppress the effector lymphocytes that attempt to activate them.[16,18] However, chemotherapy also leads to a more generalized local immunodepression. Since the bacilli cannot multiply, there is a generalized reduction in the immune response in the entire granuloma[28]; this explains why short-course chemotherapy is ineffective. Given the lack of inflammatory and local immune response, any remaining stationary-phase bacilli can therefore reinitiate growth.

The strategy used with the RUTI vaccine departs from the hypothesis that initial antibiotic treatment is necessary in order to eliminate actively growing bacilli and reduce the number of foamy macrophages. Inoculation with the RUTI vaccine reinduces an immune response to growing bacilli. The induction of a multiantigen cellular and humoral immune response against structural antigens[29-32] ensures that the new macrophages cleaning up the necrotic tissue will be capable of both recognizing stationary-phase bacilli and of being activated for the purpose of destroying the bacilli inside them.[30]

The fact that this strategy has been demonstrated as valid in several experimental TB models in mice, hamsters, and goats[29-32] led us to commence clinical development with the aim of reducing antibiotic treatment to 1 month plus 2 doses of RUTI. The overriding aim is to test the efficacy of our hypothesis on those patients in the greatest need, namely, patients with LTBI and coinfected with human immunodeficiency virus (whose risk of TB reactivation is 20 times greater than that of other individuals[1]). Within 5 years, and thanks to RUTI, we expect to be able to state without a shadow of a doubt that the prospects for treating TB are no less than glowing.

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