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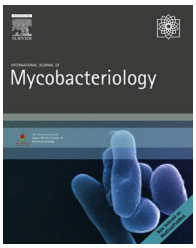

Full Length Article

Reactivation or reinfection in adult tuberculosis: Is that the question? ☆

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ABSTRACT

Looking at the chapter on "natural history" in any tuberculosis (TB) reference book, there is a kind of certainty regarding TB in adults. That is the concept of "post-primary" TB described as the reactivation of dormant bacilli hidden in an old lesion developed during infancy due to a type of local immunosuppression. Intriguingly, this concept involves at least two major uncertainties: how can dormant bacilli remain for such a long period, almost a lifetime, in an old lesion, taking into account granuloma dynamism; and what sort of local immunosuppression is the one that facilitates reactivation? The controversy between reactivation and exogenous reinfection as the cause of active TB started very soon in TB research. Interestingly, this "balance" was disturbed in the 1960s when the "Unitary Concept" became very successful in supporting the reactivation dogma. The "Unitary Concept" was mainly based on the data of tuberculin surveillance during the pre-antibiotic era as well as the data obtained from experimental modelling in animals. At the same time, the "Three-risks model" appeared to explain the relationship between the risk of infection and TB incidence, granting reinfection a key role in adult TB together with primary infection. This role was reinforced by the studies of recurrence based on molecular epidemiology, and a better knowledge of the immune response, granuloma dynamics, and lung physiology. Now it is a matter of taking it into account when designing new prophylactic and therapeutic strategies and also reflecting it in text books to better illustrate to our students.

Introduction—"The Unitary Concept

Reviewing the origin of the dogma of reactivation of an old lesion as the main source of tuberculosis (TB) in adults, it appears that this theory was written to convince the scientific community about the rarity of exogenous reinfection in TB.

The author of this theory, William Stead, introduced the topic citing the publications written by several authors around the 1920s supporting the role of exogenous reinfection, putting it on a par at least with the exacerbation of dormant foci [1]. In particular, the author cited several German specialists (i.e., Assmann, Redeker, and Braeuning) that sup-

☆ "... per això, malgrat la boira, cal caminar." (...therefore, in spite of the fog, you need to walk.) Lluis Llach (1974).

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ported exogenous reinfection as a key factor to developing adult TB (also called as chronic pulmonary TB). They named this concept "The New Theory" and soon after, several American doctors (i.e., Opie, Pinner, Myers, Longe, Medlar, and Terplan) supported it, to the point it was included in the Diagnostic Standards and Classification of the National Tuberculosis Association [2]. The "New Theory" was based on the experience of the wide use of roentgenograms, which started during the First World War to identify TB among the soldiers [3], and was thereafter used mainly for the control of therapeutic pneumothorax. With this technology, they found the "early infiltrates" not detectable by physical examination. These minimal lesions were detected in heavily exposed young adults (nurses or medical students) as they were assumed to have been previously infected in childhood, and the German doctors concluded that those early infiltrates in the upper lobes were the consequence of exogenous reinfection.

However, several Scandinavian specialists posed a strong argument against "The New Theory". According to the tuberculin skin test (TST) surveys among nursing and medical students between 1920 and 1930, there were a considerable number of patients who underwent TST. They then supported the concept that the majority of TB cases in adults, considered to be caused by reinfection, were actually cases of progression of recent primary infections [4].

Does TB incidence depend only on tuberculin status?

Stead considered that the main argument to determine the role of reinfection in adult TB incidence was to compare the incidence of TB between heavily infected persons (close contacts), considering their TST status, and non-exposed persons (control group) in order to relativize the factor of reinfection. He used data from the pre-antibiotic era [5–7]. Interestingly, he found that previous TST-positive (+) persons had a significantly lower incidence of TB in both groups than TST-negative (–) persons. In particular, the ratio between close contacts and controls in TST+ was approximately 2×, whereas that in TST− was approximately 5× (Table 1). Surprisingly, the author considered the higher TB incidence in close contacts of the TST+ group irrelevant, arguing that the contact group was mainly integrated by nursing and medical students. According to Stead, this group developed more TB because they were subjected to "longer working hours and greater bodily fatigue" than the TST+ control group. Therefore, he considered this higher ratio of re-exposure (exogenous reinfection) irrelevant to the TB incidence in TST+ persons and concluded that the most important risk in TST+ persons was reactivation.

Table 1 – Incidence (rate/1000 person-years) of Tuberculosis in Different TST groups [5–7].

	Close contact	Control
TST+	7.6*	4.3*
TST–	49.4**	10.8**

Note. TST = tuberculin skin test.
* $p < .05$.
** $p < .01$.

What is the upper lobe scenario about?

This idea concludes that TB in adults is mainly the consequence of an old lesion in the apex, induced after the hematogenous dissemination that takes place following primary infection. This is in contrast to primary lesions, which usually occur in the middle or lower lobes. Stead used the presence of lesions in the upper lobes in 20% of autopsies of patients with successfully controlled primary lesions [1] as an argument to support this; the other argument was that such lesions tend to have more active bacilli and to be more prone to reactivation than the primary lesions. Following the criteria of the Scandinavian authors [4], the author also favored the possibility that typical adult lesions could occur after a primary infection in the upper lobe, as has recently been demonstrated using molecular epidemiology tools [8].

In this regard, it is important to bear in mind that the upper lobes represent 20% of the lung volume [9,10], so the presence of such lesions is exactly what should be expected.

Stead also supported the concept that the most common site for metastatic implantations is the pulmonary apex because the very high oxygen tension provides a favorable milieu for the bacilli. High oxygen pressure is precisely a consequence of low blood perfusion. Therefore, lesion localization should be less important at this site. It has been clearly demonstrated that TB lesions progress more in the upper lobes [11,12]. The fact is that, in the upper lobes, there is a small breathing amplitude which favors an increased local bacilli–alveolar macrophages ratio leading to an excessive inflammatory response; this in turn favors the recruitment of neutrophils and the induction of exudative lesions. These lesions are the ones that are able to grow quicker and avoid encapsulation by the interlobar septae. Thus, active disease can be induced [13] by local dissemination of new daughter lesions that will finally coalesce, also aided by exogenous reinfection [14]. Equally, the higher tension borne by these septae, as a consequence of the force of gravity, makes these structures less reactive against the presence of local lesions. An interesting and very "graphic" observation was described in miliary TB cases where the lung nodules are larger in the upper lobes than in the lower [15–17].

Lymphatic and hematogenous dissemination as a source of pulmonary and extrapulmonary lesions

After a primary infection, there is a delay in the immune response, and thus, the bacillary bulk that reaches the draining lymph nodes is higher than that in the presence of a previous immune response. In addition, the lymph node is not homogenous at all; in fact, there is a proximal site where macrophages are infected and a real infection is developed, and there is a distal site where dendritic cells are affected, generating the specific lymphocytes. Fortunately, these lymphocytes do not go directly to the infected site of the lymph node; they reach the cava vein, the right heart, and the lung again where they are attracted to the infected sites and

further activate the infected macrophages. Once there is a control of these lesions, and there is an "excess" of lymphocytes, they reach the infective site of the lymph nodes. Thanks to this mechanism, the bacillary load is controlled earlier in the lung than in the organ that generates the protection, the lymph node. Meanwhile, the infection of the lymph node may overwhelm its capacity, and some of the bacilli may also reach the right heart and generate new lesions in the lungs [18].

The presence of extrapulmonary lesions (other than lung lymph nodes) requires a direct shunt toward the arterial capillary. This is the only way to reach the left atrium, the left ventricle, and the systemic vessels. It requires a large lesion and the induction of an important neovascularization process. As happens with tumors, there is also a lymphatic network surrounding the neovascularization that drains the bacilli to the lymph node again; however, the new blood capillaries are more permeable and fragile than the regular ones, increasing the chances of bacilli reaching extrapulmonary sites. In fact, it has been demonstrated recently that *Mycobacterium tuberculosis* (Mtb) is a great inducer of neovascularization [19]. On the other hand, dissemination in the lungs also benefits the development of bigger lesions. It has been very well demonstrated in the zebrafish model that disseminating bacilli tend to reach the sites which are already infected both because of the increased perfusion due to the inflammatory response and also because of the quality of the capillary network that increases the frequency of particle accumulation [20].

In conclusion, dissemination is more likely to occur in persons with a delayed immune response, such as children or immunodepressed persons, where it has the particularity of affecting the lymph nodes, contrary to what happens in immunocompetent persons. This has been recently demonstrated using molecular epidemiology and comparing atypical with typical radiological parameters [8]. Even so, it is still widely accepted that these patrons are linked to primary and post-primary (i.e., after a reactivation of an old lesion) forms of TB, respectively.

"Dynamic hypothesis versus resuscitation from old lesions

Dormant bacilli from TB lesions are drained towards the upper bronchi with the alveolar fluid and from there toward the pharynx and the stomach where they are destroyed. The clearest evidence for this is the use of gastrointestinal lavage in children to detect bacilli, even when they suffer from noncavitary pulmonary TB lesions [21]). This has been one of the arguments used to defend the "dynamic hypothesis" (i.e., the constant endogenous reinfection that maintains the latent infection) [22].

Mtb is not the only bacterium that is able to resist and persist under stress conditions. In fact, this occurs across the whole spectrum of bacteria [23], and therefore, it is not uncommon. What is peculiar is that chemotherapy (isoniazid [INH]), which is only active against growing bacilli, would have any activity against dormant bacilli, the ones responsible for maintaining the latent infection [24]. The only explanation here again comes from the "dynamic hypothesis" based on the constant drainage of bacilli. Consequently, as has been proven experimentally, there is a chance of reinfecting the parenchyma by the production of infective aerosols from the infected alveolar fluid. Considering that INH shows its maximum activity during the first 15 days of treatment, due to its capacity to kill actively growing bacilli [25], the only explanation for such a prolonged period of INH administration would be to avoid the endogenous reinfection process by keeping bactericidal levels constant to kill the growing bacilli that may induce secondary lesions.

Stead did not acknowledge this discrete drainage from "controlled" lesions; he only considered the local drainage of infective material coming from large, apparent lesions. As a consequence, he could not imagine that in "dormant lesions" like the ones of the upper lobes, there is a constant drainage of the dormant bacilli; therefore, the risk of reactivation is reduced over time. Furthermore, these lesions tend to calcify, thereby drastically reducing their bacillary load [26].

Influence of experimental modeling

Stead also based his theory on data from experimental animal models. In this case, size is very important. Mice, guinea pigs, or rabbits have a very small lung surface area. One of the consequences is that the detection of new lesions by the immune response is far easier in them than in big mammals, like humans, where the surface area is equivalent to a tennis court [27] and where the distances between the different lesions can be huge. This makes the constant establishment of new lesions more likely, especially considering that protection against TB requires at least a conserved Th1 cellular response, as the AIDS epidemic has dramatically shown. Contrary to what would happen with antibody-mediated protective immunity, lesions must be found by the specific lymphocytes, and this requires the induction of a local inflammatory response to attract them. As the local inflammatory response requires some time in the case of Mtb infection, the bacilli have the chance to constantly reinfect "virgin" areas. Hence, TB lesions can be found in various stages of progression.

Lessons learned from contact tracing

Another piece of evidence comes from the main risk of acquiring active TB: to be in close contact with a patient. This means at least >6 h a day for a period that depends on the diagnostic delay, around 60–90 days [28]. Does this mean that induction of the infection is rare or on the contrary, does this mean that during this period, the contact is infected on multiple occasions and consequently is more likely to induce active TB? Likewise, what happens in countries with a high incidence, the Republic of South Africa, for instance? Does it mean that people with active TB have been infected just once? Or that they are constantly being reinfected and, therefore, have a higher chance of developing active TB? The paper showing children who developed sensitive TB, although they were in close contact with their parents suffering from multidrug-resistant TB, is just an illustration of this complex-

ity, also linked to the relative virulence of the strain [29]. In fact, Stead also explains a case like that in his paper [1]. Ignoring this factor has led to a curious phenomenon, for instance, in the design of new vaccines: they are designed against only one single challenge [30]. In this regard, other authors have started to work on the concept of multiple consecutive infections and are trying to understand, for instance, the inefficacy of bacillus Calmette–Guérin vaccine (BCG) in countries with a high risk of infection [31]. It is also important to note that infection confers some protection, similar to what is obtained by BCG vaccination, at least in terms of control of the bacillary load, as has been demonstrated by experimental modeling [32,33]. This is an interesting point to consider in the contact tracing strategy. To look for TST might have a limited value for the prediction of active TB in groups with a high prevalence of TST+. Again, in this regard, TST has only some predictive value in the converters, as we will discuss in the next chapter.

Reinfection has a place when trying to understand the whole picture

At the same time when Stead was preparing his paper, a little "revolution" started at the other side of the Atlantic, at the Royal Netherlands Tuberculosis Association (KNVC), where the first attempts to understand the whole TB epidemiology picture started in 1965. In particular, it was in the context of "The International Tuberculosis Surveillance Research Unit (TSRU)" supported by KNVC and the abundant epidemiological data from the Netherlands and with Dr. Karel Styblo as the first Principal Investigator [34]. Among the several findings produced by the TSRU were the epidemiological indexes: (a) the annual risk of infection; (b) the ratio between the annual risk of infection and the incidence rate of infectious (smear positive) cases; and (c) the risk of progression from infection to disease and the issue of endogenous reactivation (or exacerbation) versus exogenous origin of the development of TB.

It was against this background that, using the proposal of Dr. Johannes Holm at a TSRU meeting in 1969 and the first exploratory analysis performed by Dr. K. Styblo, Sutherland et al. [37], from the British Medical Research Council (MRC) published several papers related to a pioneer model to fit the annual risk of infection with TB disease incidence using the epidemiological data from the Netherlands [26,35,36]. Thus, he established the "Three-risks model" for TB disease, stating that (1) those with a recent primary infection had a characteristic risk of developing progressive primary tuberculosis, (2) those with a distant (i.e., not recent) primary infection and a recent reinfection had a characteristic risk of developing exogenous tuberculosis, and (3) those with a distant primary infection, but no recent reinfection had a characteristic risk of developing endogenous tuberculosis.

In particular, Sutherland established the border between recent and distant at 5 years, taking into account his own data as investigator of the MRC Tuberculosis Vaccine trial that enrolled 35,000 unvaccinated adolescents. In this study, they identified a group of 2,170 TST converters, of whom 113 (5.2%) developed TB. In particular, the cumulative incidence was of 58% (1st year), 80% (2nd year), and almost 100% (5th year) with no incidence after 8 years [37] (Fig. 1).

This data is in agreement with the duration of the INH treatment as chemoprophylaxis. It offers an efficacy of 90% after 9–12 months of treatment [38]. It can be interpreted that during this time, 90% of the dormant bacilli have been drained from the lesions, and INH has avoided the possibility of secondary lesions being induced and causing active TB. In this regard, it can be considered that INH treatment accelerates the drainage ratio after the first killing of growing bacilli (up to a 90% of the bacillary load), thus considerably reducing this period compared with the natural draining process. In this case, taking into account the data of the MRC [37], approximately 3 years are needed to drain the same percentage of dormant bacilli (Fig. 1).

Although the "Three-risks model" has been refined by other authors, none of them have considered the incidence of "residual TB," those patients that have suffered TB and have not been properly treated with chemotherapy. These patients have large lesions with no definitive fibrosis and calcification and with a high percentage of viable dormant

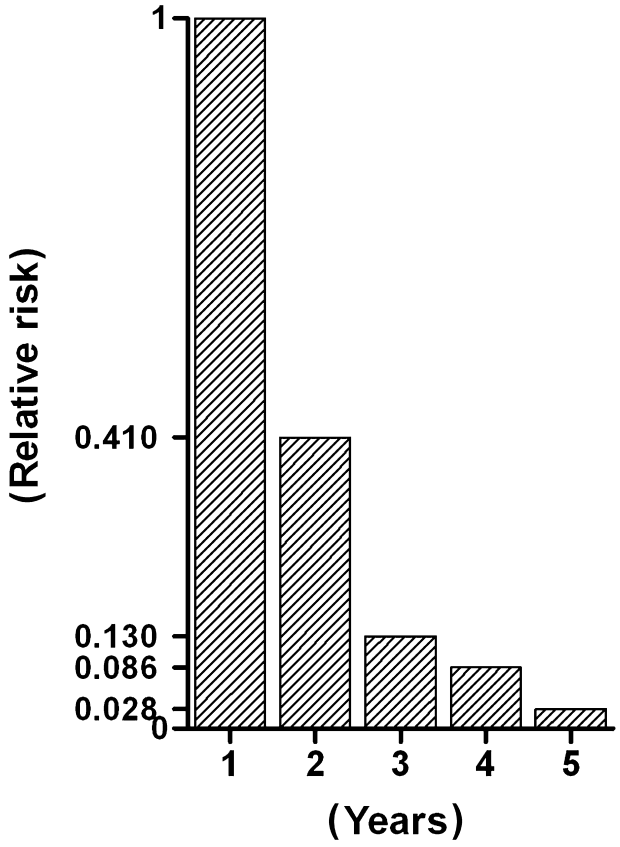

Fig. 1 – Origin of the 5-year period for developing tuberculosis after infection. Incidence of tuberculosis in unvaccinated TST convertors in the MRC Tuberculosis Vaccination Trial [37]. Out of 35,000 unvaccinated adolescents, there were 2170 TST convertors, of which 113 developed tuberculosis. *Note.* TST = tuberculin skin test; MRC = Medical Research Council. Adapted from: Vynnycky E, Fine PE. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. Epidemiol Infect [Internet]. 1997 Oct [cited 2016 Sep 8];119(2):183–201.

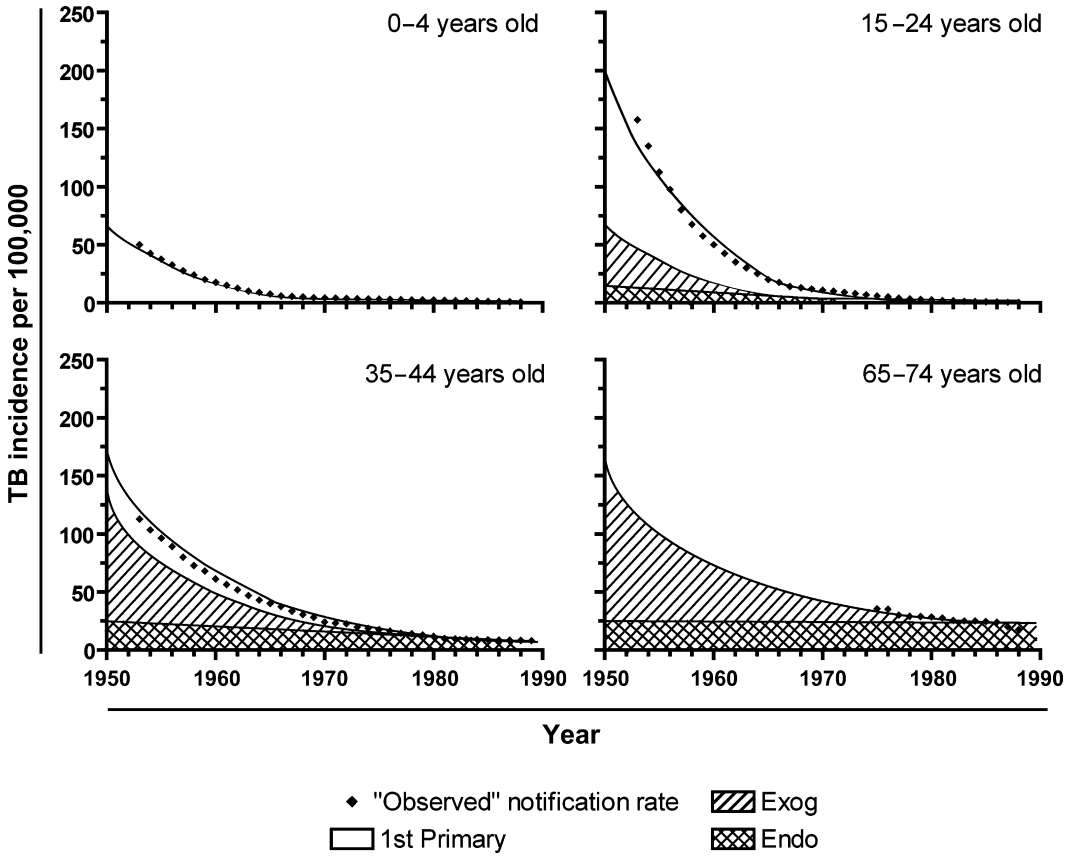

Fig. 2 – Relationship between Risk of Infection and Incidence of Tuberculosis. This is a refinement of the "Three-risks model" proposed by Vynnykcy and Fine [39] to include the age variable. In this model, the incidence of reinfection is paramount in order to fit the real data from tuberculosis incidence in England and Wales. Adapted from: Vynnycky E, Fine PE. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. Epidemiol Infect [Internet]. 1997 Oct [cited 2016 Sep 8];119(2):183–201.

bacilli, susceptible to be drained and to cause TB long after suffering their first episode. This is probably the reason for an excess of the reactivation percentage in these models.

It is worth noting that Vynnycky and Fine included age dependency in the model [39] (Fig. 2). They found that for most age groups, the risk of developing the first primary episode after initial infection exceeded that of developing endogenous and exogenous disease. Previous infection was found to impart 16% and 41% protection against disease subsequent to reinfection among adolescents and adults, respectively, but little protection against reinfection.

Reinfection is, thus, an important event with largely unknown consequences; for example, it can be speculated that multiple exposures may even precipitate progression to disease. Thus, the influence of incidence on the reinfection rate appears to be a fundamental issue [40] (Fig. 3).

Study of recurrence: relapse versus reinfection

Adequately treated patients are still at a high risk of developing recurrent pulmonary disease (defined as an episode of TB following the cure of a previous episode). Recent estimates for the recurrence rate of TB across different regions point to an

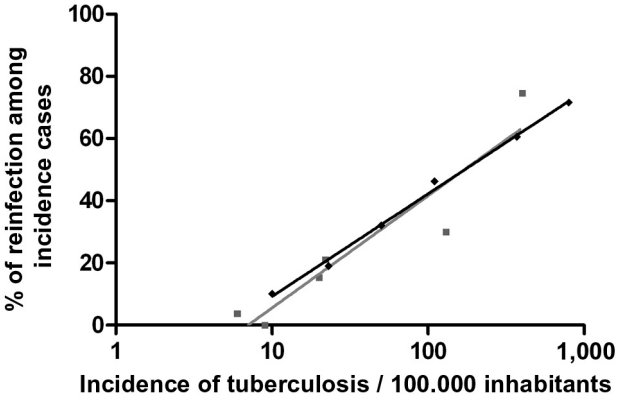

Fig. 3 – Reinfection Increases with an Increase in Tuberculosis Incidence. Data from Uys et al. [40] relating the risk of reinfection with tuberculosis incidence, adjusting the "Three risks model" with the reinfection data obtained by Wang et al. [47]. Adapted from: Uys PW, van Helden PD, Hargrove JW. Tuberculosis reinfection rate as a proportion of total infection rate correlates with the logarithm of the incidence rate: a mathematical model. J R Soc Interface [Internet]. 2009 Jan 6 [cited 2016 Sep 8];6(30):11–5.

average of 2290 cases/100,000 person-years at 12 months after treatment completion. In high-incidence regions, the average TB recurrence rate can reach 7,850/100,000 person-years [41].

The issue of whether or not a first episode of TB imparts some measure of immunity to further TB infections has not been unequivocally established, but it may have an impact [42,43]. On the other hand, a potential confounding effect is that a patient experiencing a second episode of TB may simply be demonstrating that he/she has a higher susceptibility, either innate or due to environmental conditions [44,45]. It is also possible that an episode of TB actually renders the patient more susceptible to infection or likely to become ill. All these issues are surveyed in Verver et al. [42].

The study of recurrence has provided new evidence on the role of reinfection as DNA fingerprinting can be used to evaluate the homology between the strain that caused the first episode and the recurrent one. However, this concept can hide an important percentage of reinfections by strains that are very prevalent in the community and cannot be distinguished from the one obtained from the first episode.

In spite of this limitation, it was soon appreciated that exogenous reinfection may be the origin of a majority (up to 90%) of disease cases in regions with a high risk of infection [40,46,47].

Even so, exogenous reinfection tends to be related to regions with a high incidence; therefore, it is not being considered in those with a low incidence. This has led to the use of network models of TB transmission where the population is not homogeneous, and thus, taking into account that individuals are more likely to be in contact with those with whom they share a home, a school or a workplace than with those with whom they have no particular contact [48]. This approach has demonstrated that in large communities with low TB incidence, nonrandom mixing of the population means that reinfection may play a larger role in disease dynamics than previously recognized. This result does not depend on the explicit inclusion of subpopulations with compromised immunity or otherwise distinctive disease risk. Prolonged or intense contact, such as the interactions occurring within closed settings like households, workplaces, and hospitals, is generally considered necessary for transmission of Mtb.

It is well known that some groups have much higher risk of acquiring infection and developing disease than the majority of the population: (1) incarcerated prisoners (20 times higher in Brazil) [49]; (2) persons living with HIV (8 times higher in a South African community) [50]; and (3) geographical hotspots within urban settings (3 times higher in people living in the poorest areas in Rio de Janeiro) [51], among others.

It has been shown that in a high-incidence setting near Cape Town, South Africa, the rate of reinfection TB disease after the cure of a previous TB disease episode is about four times greater than the rate of first-time TB disease. It is not known whether this elevated rate is caused by a high reinfection rate due to, for instance, living circumstances, or a high rate of progression to disease specific to the patients, or both. In order to address this question Uys et al. [52] analyzed an extensive data set from clinics attended by patients with TB in a high-incidence setting near Cape Town, South Africa, and found that, in fact, the (average) rate of reinfection (as opposed to the rate of reinfection disease) after the cure of a previous TB disease episode is initially about 0.85 per annum. This rate diminishes rapidly over time, and after about 10 years, this rate is similar to the rate of infection in the general population. In addition, the rate of progression to disease after reinfection is initially high but declines in subsequent years down to the figure typical for the general population. These findings suggest that the first few months after the cure of a TB disease episode form a critical period for controlling reinfection disease in a hyperendemic setting and that monitoring such cured patients could preempt a reinfection progressing to active disease.

Conclusion

The support to reactivation as the main source of TB is adults have a series of weak points. Having analyzed these, this review concludes that exogenous reinfection together with primary infection appear to be the major sources of adult TB, contrary to what is usually cited in TB reference books.

According to the data shown, active TB is developed during the first 2 years after infection or even after recurrence being almost impossible for it to occur after 5 years. This is explained by the constant drainage of the dormant bacilli from the lesions and the progressive destruction of the persisting ones with calcification. This process can be accelerated with INH chemoprophylaxis, with the initial killing of growing bacilli and avoiding the induction of further secondary lesions, reducing the probability of developing active TB after 9–12 months' treatment up to 10%; the natural process requires a period of 3 years.

Reinfection is possible because the type of immune response required, cell-mediated immunity, needs a certain local inflammatory response to locate lesions. This fact is easy to perceive with the presence of lesions in different stages of progression in the same individual.

The data on DNA fingerprinting in recurrence also points towards the greater importance of the reinfection process, considering the presence of different fingerprints between the strains of the initial disease and the subsequent ones. Even so, this process is unable to precisely identify the most prevalent strains, which must be the ones with a higher incidence in the initial process and naturally in the subsequent ones.

Finally, even if it may be true that reactivation is possible, there is another factor, which has previously not been considered and is usually not recorded, that is, the prevalence of spontaneously healed TB (without chemotherapy), because of the large area affected, which probably behaves as residual or silent TB and which may eventually recrudesce at advanced ages. This is precisely observed in countries with low incidence.

Overall, the impression is that reinfection should be considered as the major source of adult TB in high-incidence countries, and together with primary disease, in low-incidence countries. This concept should aid the development of better strategies for TB control.

Conflicts of interest

The author is founder and CEO/CSO of Manremyc, the "Spin-off" of the Institut Germans Trias i Pujol (IGTP) that is developing the use of *M. manresensis* as a food supplement to reduce the risk of TB development.

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