

# DNA fingerprinting of *Mycobacterium tuberculosis* from patients with and without AIDS in Rio de Janeiro

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## Abstract

Isolates of *Mycobacterium tuberculosis* derived from patients with AIDS from a single hospital in Rio de Janeiro were typed using a standardized RFLP technique detecting *IS6110* polymorphism. Nineteen isolates were obtained from 15 different patients. Eleven distinct *IS6110* patterns were found, with 4 banding patterns shared by 2 patients. The clustering value of 53% was much higher in comparison with clustering of *M. tuberculosis* strains from TB patients without clinical signs for HIV infection from randomly selected health centers. We present these results as preliminary data on *M. tuberculosis* strain polymorphism in Brazil and on the higher risk for recent transmission amongst patients with AIDS.

It has been demonstrated that infection with HIV is a risk factor for the development of tuberculosis (TB) and this can result either from reactivating latent TB or from a newly acquired infection (1). Individual strains of *Mycobacterium tuberculosis* (Mtb) can be identified by molecular typing methods, and transmission of a strain throughout a community can thus be monitored (2). Increased risk for recent transmission amongst individuals with HIV has been confirmed by fingerprinting studies in outbreaks (3), in urban settings such as New York (4) and San Francisco (5), sometimes involving multidrug-resistant strains (6). The prevalence of TB in Rio de Janeiro is 140 per 100,000 inhabitants and the epidemiological

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situation of Brazil is different from that of African countries because TB has been endemic for centuries and, in contrast to developed countries, in Brazil, the TB epidemic never disappeared. The prevalence of PPD positivity is high and for decades now, BCG vaccination has been applied to most infants.

In contrast to TB, the HIV epidemic in Brazil has a demographic pattern similar to that observed in developed countries. Using a standardized protocol based on the detection of restriction fragment length polymorphism generated by the variation in number and position of the insertion sequence *IS6110* in the bacterial genome (7), we have typed isolates of Mtb from TB patients with and without AIDS from Rio de Janeiro, Brazil.

Deoxyribonucleic acids extracted from cultures of *M. tuberculosis* were digested with *PvuII* and internal molecular weight markers (consisting of a mixture of  $\phi$ X-*HaeIII*,  $\lambda$ -*HindIII* and *PvuII*-digested supercoiled DNA) were added to each sample. The DNA patterns were then generated and normalized by consecutive hybridization with part of *IS6110* and with the internal markers labeled with the ECL direct labeling system (Amersham, UK), analyzed and stored in a computer using the GelCompar software (Applied Maths, Kortrijk, Belgium; 8).

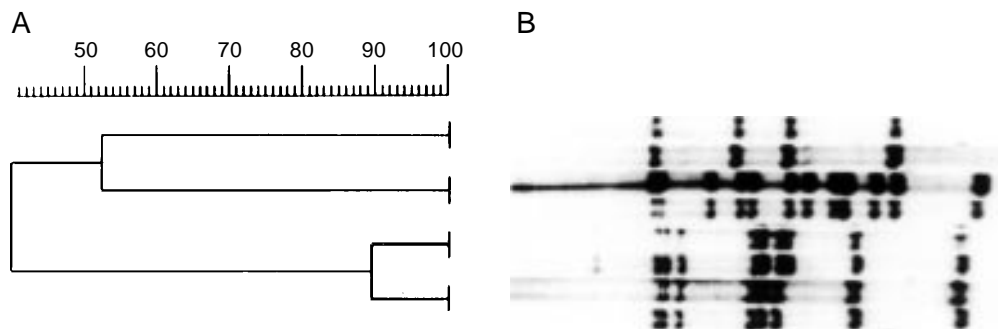
During the period from 1993 to 1994, 57 cultures of Mtb were thus collected and analyzed; of these cultures, 38 were obtained from TB patients without clinical signs for HIV infection seen at randomly selected health centers, while 19 cultures were obtained from 15 different AIDS patients seen at the Evandro Chagas Hospital (Oswaldo Cruz Institute, RJ). Although HIV serology was not performed on TB patients from the health centers in this study, at that time, HIV positivity among TB patients from Rio de Janeiro was 11% (9). Ninety percent of the Mtb isolates had 6 to 15 copies of *IS6110*; only 4 strains, including 2 from patients with AIDS, had less than 6 copies, thus permitting a reliable analysis of strain similarity.

Analysis of the DNA patterns from patients with AIDS showed that two strains had the same 12-copy pattern; two more strains shared a 4-copy pattern and 4 strains had very similar patterns, with two having an identical 8-copy pattern and two more being

identical to the former except for two extra bands (Figure 1). Since outbreak studies have revealed that RFLP patterns with one or two extra bands are not unusual in Mtb clones (10), we inferred that the latter group could have been infected by the same or a closely related strain. Also, the four pairs of isolates that were obtained from different clinical sites of four patients with AIDS were identical (data not shown). Fifty-three percent of the Mtb strains isolated from AIDS patients frequenting the Evandro Chagas Hospital belonged to a cluster. This high level of clustering was not found in the strains isolated from the rest of the TB patients: 2 groups of 2 patients with identical strains were found, representing a clustering of only 11%. Infection with identical strains among the AIDS patients could not be explained by analysis of data such as family relations, address or coincidence of residence at the hospital. However, sporadic unrecorded visits to the hospital do occur and it is known that contact in settings where HIV-infected people socialize poses a risk for transmission of TB (11).

Recent findings have pointed out that transmission of TB may not require "close and prolonged" contact as previously believed, and trivial contact in the community may be sufficient. The AIDS-TB patients studied here had previous contact with the hospital system, at least as outpatients, and although we are not exactly dealing with an outbreak situation, we suppose that these clustered infections were acquired nosoco-

Figure 1 - Computer-generated dendrogram (A) and normalized *IS6110* hybridization patterns (B) of strains of *Mycobacterium tuberculosis* isolated from eight patients with AIDS.



mially. This observation is very important because the standard isolation procedures for AIDS in patients with TB may not be sufficient to prevent nosocomial transmission of TB among these highly susceptible individuals. On the other hand, common exposure within the community may have been sufficient to infect this particular sub-population. Of a total of 53 strains analyzed here, 12 (22.0%) were clustered. When comparing with epidemiological data obtained with molecular markers from low prevalence countries such as the US, Switzerland and the Netherlands, as well as from high prevalence African countries (12), the level of clustering in the strains isolated from TB patients in this study is low and clustering amongst HIV-infected patients surprisingly high.

Two studies comparing strains of *M. tuberculosis* in HIV-seropositive and -seronegative patients with TB, one performed in Tanzania (13), and the other in Italy and Pakistan (14), are contradictory. Clustering in Tanzania was independent of the patient's HIV status while the latter study demonstrated a significantly higher clustering in HIV-positive individuals. This could partly be explained by the use of different criteria for the definition of HIV status. Indeed, Small

et al. (5) already demonstrated that the risk of belonging to a cluster increases with increasing immunosuppression. One of the possible reasons for the low clustering in general observed here could be that, unlike patients with AIDS, most patients were diagnosed in ambulatory services located in different neighborhoods of Rio, while in most of the other studies patient sampling occurred in one or in a small number of settings. Although AIDS and non-AIDS patients represent different neighborhoods of Rio, only patients with AIDS were treated at the same hospital. Also, the number of samples analyzed in this preliminary study is relatively small.

We are now starting a large prospective study to screen TB patients representing most of the city's neighborhoods. In conclusion, this study points to a large variety of TB isolates in Rio de Janeiro and demonstrates a high level of transmission of TB among AIDS patients attending a particular hospital.

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