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The IS*6110* Restriction Fragment Length Polymorphism in Particular Multidrug-Resistant *Mycobacterium tuberculosis* Strains May Evolve Too Fast for Reliable Use in Outbreak Investigation

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To study possible nosocomial transmission of multidrug-resistant (MDR) *Mycobacterium tuberculosis*, strain types and other information on 24, mostly human immunodeficiency virus-positive patients, were collected. Isolates from 11 patients had identical IS*6110* restriction fragment length polymorphism (RFLP) patterns as well as spoligotype patterns and resistance profiles. Noticeably, nine other isolates from related cases also exhibited identical spoligotypes but slightly different RFLP patterns. These results indicate that for some MDR strains, the evolutionary clock of IS*6110* RFLP may run too fast for reliable interpretation of strain typing results over a period of a few years.

The high tuberculosis (TB) morbidity and mortality due to multidrug-resistant (MDR) TB have caused major concern regarding the clinical management and prevention of dissemination of the disease (10, 21). In several countries, hospital outbreaks due to MDR TB were reported in association with human immunodeficiency virus (HIV)-positive patients (6, 15, 16). The short intervals of only 4 to 16 weeks for HIV-positive patients from diagnosis to death and the high transmission rates from patients to health care providers (4) have posed an important public health problem.

From 1989 on, the number of HIV-positive patients admitted for medical treatment at the Dr. Cetrángolo Hospital (CH), located in the northern suburbs of Buenos Aires, Argentina, began to increase (13). About 29% of the HIV-positive patients between 1992 and 1997 had one or more TB episodes. In this period, 19% of the TB cases were caused by MDR *Mycobacterium tuberculosis* strains. The number of HIV-negative or noninvestigated patients with MDR TB remained steady and amounted to about 2% of the total number of TB cases recorded in the hospital. Both HIV-positive and HIV-negative patients, with or without TB, received medical treatment as in- or outpatients. However, all of them shared the same hospital facilities, and most of the time, no effective containment was implemented to prevent transmission of TB (13).

Restriction fragment length polymorphism (RFLP) typing of *M. tuberculosis* isolates has proven to be a useful tool to investigate nosocomial outbreaks (4, 12, 15, 16). Spoligotyping is a new method for typing *M. tuberculosis* complex isolates. This method is based on the polymorphic nature of short sequences which are interspersed among the conserved direct repeats in the genomic direct repeat region (8, 9, 11). In this study, the spoligotyping method, with a lower level of discrimination than that of IS*6110* RFLP typing (5), was used as an additional tool to validate the IS*6110* RFLP typing results. In order to examine the transmission of TB in our hospital, both methodologies were applied.

A total of 74 *M. tuberculosis* isolates were obtained from 58 patients. Thirty-six of these patients were coinfected with HIV. The isolates were identified by AccuProbe tests for *M. tuberculosis* complex (GenProbe, San Diego, Calif.) and by standard biochemical procedures (3). The susceptibility to antituberculostatics was determined by the proportion method (2) on Löwenstein-Jensen medium.

The 18 MDR TB patients among the HIV-negative patients had undergone several episodes of anti-TB treatment while receiving medical treatment in the hospital during an average period of 3 years (range, 2 to 5 years). In contrast, all HIV-positive patients infected with MDR TB had a single episode of the disease, and they had an average survival period of 5.8 months (range, 4 to 18 months) from the onset of the disease.

Seventy-four *M. tuberculosis* strains, isolated from 58 patients, were analyzed by RFLP typing and spoligotyping (11, 17, 18, 20). A group of 24 MDR TB patients, formed by 20 clustered patients and 4 nonclustered patients, was selected for further investigation. During alternate periods from 1992 to 1997, all of these patients were hospitalized or received medical treatment as outpatients, and they sometimes shared the same rooms. Figure 1 shows the RFLP and the spoligotyping patterns as well as the drug resistance profiles of the MDR isolates retrieved from the 20 patients, epidemiologically linked on the basis of the strain typing results. In this figure, the data on hospitalization period and HIV status of the patients also are indicated.

The MDR strains from HIV-positive patients 1 to 11 (group 1) exhibited identical RFLP patterns (type II) and spoligotype patterns (type B), as well as the same resistance profiles. Since the patients concerned had overlapping dates of admission to and/or release from the hospital and they did not receive anti-TB treatment previously, it was highly likely that a person-

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A

Patient number	Health care period	HIV status	Drug susc.	RFLP pattern	RFLP type	Spoligo pattern	Spoligo type
1	01/92 to 02/93	+	HSR		II		B
2	04/92 to 03/93	+	HSR		II		B
3	05/92 to 03/93	+	HSR		II		B
4	10/92 to 04/93	+	HSR		II		B
5	11/92 to 05/93	+	HSR		II		B
6	01/93 to 05/93 05/93 to 07/93** 07/93 to 10/93	+	HSR		II		B
7	04/93 to 03/94	+	HSR		II		B
8	07/93 to 03/94	+	HSR		II		B
9	08/93 to 06/94	+	HSR		II		B
10	09/93 to 06/94	+	HSR		II		B
11	02/94 to 11/95	+	HSR		II		B

B

Patient number	Health care period	HIV status	Drug susc.	RFLP pattern	RFLP type	Spoligo pattern	Spoligo type
12	01/92 to 12/92** 01/93 to 11/93 01/96 to 12/96* 12/97 to date	-	HSR HSRZ HSREZ HSREZ		VIIIa		C
13	1994 to 1997	-	HSR		VIIIb		C
14	1993 to 1994*	+	HSR		VIII		ND
15	1993 to 1994*	+	HSR		VIII		ND
16	09/94 to 02/95	+	HSRZ		VIIIc		C
17	01/94 to 01/95 04/95 to 10/95* 11/95 to 04/96	+	HSREZ		VIIId		C
18	02/95 to 04/96	+	HSREZ		VIII		C
19	12/95 to 07/96	+	HSREZ		VIII		C
20	01/96 to 08/96	+	HSREZ		VIII		C

FIG. 1. Patient information, drug resistance profiles, and DNA fingerprints of *M. tuberculosis* isolates related to the two described TB outbreaks. (A) First outbreak; (B) second outbreak. The column "Drug susc." indicates resistance to the drugs abbreviated as follows: H, isoniazid; S, streptomycin; R, rifampin; E, ethambutol; Z, pyrazinamide. ND, not determined. Dates are given as month/year. *, period of patient's attendance at hospital H1; **, period of patient's attendance at hospital H2.

to-person transmission of MDR TB occurred among these patients.

The second group comprised nine MDR-TB strains isolated from patients 12 to 20. Although five highly similar RFLP patterns (type VIII) were found in this group of nine strains, all had identical spoligotype patterns (type C). The spoligotype patterns of the two described outbreak strains were not found in the database comprising 285 spoligotype patterns of *M. tuberculosis* isolates from Argentina. The five RFLP patterns distinguished among the nine isolates related to the second outbreak were pattern VIII (five instances) and the patterns VIIIa to VIIId (once each). RFLP pattern VIII was found in

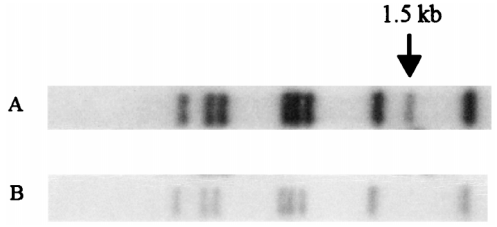

FIG. 2. IS*6110* RFLP patterns of two *M. tuberculosis* strains isolated from an HIV-negative patient (patient 23) at an interval of 1 year. The RFLP pattern of the first isolate, shown in lane A, contains one additional band at the 1.5-kb position. Both strains exhibited the unique spoligotype F.

five MDR TB isolates recovered from five HIV-positive patients (patients 14, 15, and 18 to 20). Patient 20 was a health care worker at the CH. The diagnosis of patients 14 and 15 occurred during 1993 to 1994 in a different hospital in Buenos Aires (H1). The RFLP pattern VIIIa was found in an isolate from an HIV-negative person (patient 12) who had a disease progression of 6 years, 1991 to 1997, and received medical assistance in several hospitals including CH and H1 (1992 to present). RFLP pattern type VIIIb (patient 13) was exhibited by two isolates from one HIV-negative patient. These isolates were obtained at an interval of 1 year. All nine of these strains were resistant to isoniazid, streptomycin, and rifampin, and some of the strains were resistant also to pyrazinamide (patients 12 and 16 to 20) and ethambutol (patients 12 and 17 to 20).

The third group of isolates comprised nonclustered patients 21 to 24. The RFLP patterns V, XIII, and XIV and the spoligotypes D, G, and E were obtained from different MDR TB isolates from three HIV-negative patients (patients 21, 22, and 24, respectively [data not shown]). The patients had a disease progression of 3 years; they remained positive by smear examination during this period although they were receiving appropriate therapy. From the remaining HIV-negative patient of the third group, patient 23, we obtained two isolates, at an interval of 1 year (Fig. 2). The RFLP patterns of these isolates differed in a single band of 1.5 kb, present in the first isolate and absent in the second. Both strains had the same spoligotype, F.

All isolates from the first group of patients (patients 1 to 11) had identical RFLP and spoligotype patterns as well as resistance profiles. In contrast, the isolates from the second group of patients (patients 12 to 20) had identical spoligotype patterns and highly similar, but not identical, RFLP patterns. The second outbreak strain provoked an outbreak in hospital H1 which also extended to other hospitals (15). Since we are quite sure that all of the isolates from the second group of patients are derived from a common ancestor within a period of 4 years, it is tempting to speculate on the instability of IS*6110* RFLP in these strains with regard to bacterial factors and host-dependent influences. Also, the serial isolates recovered from MDR TB patient 23 showed a change in the RFLP pattern. Many reports have proved the stability of IS*6110* RFLP during in vivo and in vitro (7, 19) incubation of *M. tuberculosis* strains. In contrast, this study indicates that particular strains, such as those causing the second outbreak, may have a higher mutation frequency. In a recent study by Yeh et al. (22), it was found that serial isolates from 14 (29%) of 49 patients showed changes in their DNA genotypes between isolates (12 in IS*6110* RFLPs and 2 in polymorphic GC-rich sequence RFLPs). However, the changed IS*6110* RFLPs were not related to the bacterial drug susceptibility or to the patient's HIV serum status or adherence to therapy. In contrast, in this study the instability of IS*6110* RFLP found in the second outbreak strain may be somehow related to the selective pressure of a combination of drugs, as these drug-resistant patients were difficult to treat. On the other hand, the patient population in the first outbreak did not differ significantly from the patient group in the second microepidemic; however, we did not observe any alteration in the IS*6110* RFLP of this strain. Therefore, the factor influencing the transposition frequency may be strain dependent. For instance, IS*6110* elements in the second outbreak strain may be inserted in particular genomic promoter regions, resulting in a higher transposition pressure.

Spoligotyping, in addition to IS*6110* RFLP, can be useful in determining more distant relationships among isolates. This has been proven for the first time in the disclosure of the Beijing genotype family of *M. tuberculosis* strains (20). One representative of this type of *M. tuberculosis* strain, the W variant, has been transmitted in New York hospitals since the early 1990s (14). In the study by Moss et al. (14), 8 of 128 (6.5%) W-type MDR TB strains isolated in New York City hospitals in 1992 also exhibited IS*6110* RFLP patterns slightly different from those of the predominant type. Within a relatively short period (a few years), this W-type strain developed several lineages with slightly different, but recognizable, IS*6110* RFLPs (1). In our current study, the relative instability of IS*6110* RFLP was found in one of two MDR outbreak strains; however, not fewer than four of nine of the IS*6110* RFLP patterns showed a minor and different alteration. Therefore, the transposition rate may be strongly related to the *M. tuberculosis* genotype represented.

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