Epidemiological Study of Tuberculosis in Palermo, Italy: IS6110 Fingerprinting of Mycobacterium tuberculosis Strains Isolated in the Years 1994–1998

A. Nastasi, C. Mammina

Summary
In some industrialized countries reemergence of tuberculosis has been recorded. Most cases are thought to be caused by reactivation of infections that had been acquired many years before, but in some geographical areas up to 40% of diagnosed infections have been estimated to be newly acquired, based on the results of molecular epidemiological methods. Restriction fragment length polymorphism of the insertion sequence IS6110 of Mycobacterium tuberculosis has been widely used to evaluate epidemiological patterns of transmission in various communities.

We have used IS6110 fingerprinting to analyze 101 strains which were isolated between June 1994 and June 1998 from 95 inhabitants of the province of Palermo, Italy, an area with an endemic rate for tuberculosis ranging between 5.1 and 8.0 per 100,000 persons in the last 5 years.

89 different patterns have been obtained, 87.4% of the patients were infected by presumably unrelated isolates. Six microepidemics were also recognized. These data suggest that reactivation largely exceeds recent infection.

Key Words
Mycobacterium tuberculosis · Epidemiology · Italy · Genotyping · Transmission

Introduction
Tuberculosis remains a major public health problem causing morbidity and mortality worldwide [1]. Although approximately 95% of cases occur in developing countries, tuberculosis has recently reemerged even in some industrialized countries [2–5], where most cases are thought to be due to reactivation of infections that had been contracted many years before. However, the relative contribution of recent transmission to the overall incidence of disease needs to be reliably established. Indeed, understanding transmission routes of Mycobacterium tuberculosis is a critical step in the development of a rational approach to disease control.

The presence of repetitive genetic elements in M. tuberculosis has permitted the identification of individual strains by DNA fingerprinting [6, 7]. M. tuberculosis strains in particular have been classified by using the insertion sequence [IS] IS6110 as a genetic marker [7–13]. Each fingerprint is a measure of both the number of insertions and their relative sites of insertion within the genome. By using this typing methodology, a number of studies have elucidated the epidemiology of M. tuberculosis and have provided information concerning sources and routes of transmission, the degree of recent transmission versus reactivation, and the identification of patterns which may be linked to geographical source, resistance patterns or virulence properties [13–16].

The aim of this study was to evaluate the genetic diversity of M. tuberculosis strains isolated from patients living in the province of Palermo, Italy, during a 4-year period. In this province, the incidence rate for tuberculosis ranged 5.1 in 1993 to 5.6 per 100,000 inhabitants in 1997 with a maximum of 8.0 per 100,000 in 1996. For comparison, in Italy the incidence of tuberculosis in 1995 was 9.12 per 100,000 [17].

M. tuberculosis strains isolated from 95 patients between June 1994 and June 1998 were typed by IS6110- restriction fragment length polymorphism (RFLP) to identify interstrain relationships and patterns of transmission of this pathogen within a defined geographical area.

Patients and Methods
Patients
101 strains of M. tuberculosis were recovered from 95 patients, who where hospitalized for tuberculosis in the years 1994–1998. The patients represent 35% of the patients with tuberculosis who were reported (positive and negative culture) to the Territorial Tuberculosis Registry of the province of Palermo during the period beginning in January 1994 and ending in June 1998. The following...
data were collected for all patients: age, geographical origin, HIV serological status, clinical extension of tuberculosis.

**Bacterial Strains**

The strains analyzed in this study were obtained from the microbiology laboratories of five hospitals in Palermo: Policlinico, Ospedale “Cervello”, Ospedale “Villa Sofia”, Ospedale “Guadagna”, a care center for infectious diseases, and Ospedale dei Bambini, a pediatric treatment center. *M. tuberculosis* strains were isolated by culture on Löwenstein-Jensen or MGIT (Becton-Dickinson) medium. Colonies were identified by using standard biochemical tests [18] and by application of the polymerase chain reaction (PCR) – RFLP analysis procedure described by Telenti et al. [19]. Isolates were maintained on Löwenstein-Jensen slants at + 4°C until DNA extraction. The strains were isolated from bronchial aspirate (47), sputum (23), bronchoalveolar lavage fluid (8), urine (6), lymph node (5), pleural fluid (4), gastric aspirate (4), synovial fluid (2), cerebrospinal fluid (1), and ascites (1).

For three patients, multiple isolates were available. For one patient, two isolates were recovered from pleural fluid and bronchial aspirate – isolates nos. 58 and 59 – within a few days; from another, three patient strains – isolates nos. 13, 19 and 77 – were recovered from sputum specimens on three separate occasions two months and two years apart, respectively. Four strains of a multidrug-resistant *M. tuberculosis* – isolates nos. 16, 31, 36 and 60 – were recovered from sputum specimens of a 48-year-old patient two months apart during 1994.

**DNA Fingerprinting and RFLP Analysis**

Extraction of DNA from isolates of *M. tuberculosis* and Southern blotting with labeled IS6110 DNA were performed using the previously described methodologies [7]. The DNA probe was labeled with an enhanced chemiluminescence gene detection system (Amersham). In brief, *M. tuberculosis* DNA was extracted, digested with PvuII, electrophoresed and hybridized with a fragment of the insertion sequence IS6110, measuring 245 base pairs (bp) and generated by PCR. Each Southern blot included DNA from *M. tuberculosis* Mt14323 as an external standard [7]. The resulting autoradiographs were compared by the Gel Manager software (Biosystematica) and RFLPs were clustered by the unweighted pair grouping method using averages (UPGMA). The accuracy of the procedure was evaluated by comparing the IS6110 patterns of strain Mt14323, which was present in a single lane on each autoradiograph. All lanes that were found to have similar patterns by computer analysis were visually compared and classified. A cluster was defined as a group of two or more isolates from different patients, whose RFLP fingerprints were clustered by the unweighted pair grouping method using averages (UPGMA). Isolates with unique fingerprints were classified as nonclustered.

**Results**

**Patients**

The 95 patients with tuberculosis analyzed in this study included seven children and 88 adults; the ages ranged from 2 to 12 years for children and from 16 to 83 years for adults (median age = 48 years). HIV serological status was positive for five and negative for 90. Five adult patients were