Molecular Epidemiology of *Mycobacterium tuberculosis* Infection in Israel

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Restriction fragment length polymorphism analysis with IS6110 and DR-r probes was used to study 69 *Mycobacterium tuberculosis* isolates obtained from Israeli patients and new immigrants from the former Soviet Union and Ethiopia. DNA fingerprinting identified unique patterns for almost all isolates, indicating that most patients were infected with a unique strain imported from their country of origin and that their latent infection was reactivated in Israel.

In recent years, the incidence of tuberculosis has increased worldwide (13). The incidence of tuberculosis also increased in Israel due to immigration from high-incidence countries (such as the former Soviet Union and Ethiopia) (3). For the last decade, the incidence of tuberculosis among Israelis born in Israel or living in the country for many years is 4 cases per 100,000 people, whereas the tuberculosis rates of recent immigrants from the former Soviet Union (38 to 172/100,000) and Ethiopia (500 to 3,000/100,000) are much higher (3). From 1990 to 1996, Israel absorbed more than 750,000 immigrants, one-seventh of the whole population. The proportion of drug-resistant *Mycobacterium tuberculosis* strains in Israel was greatly influenced by these waves of immigration (14). Typing of *M. tuberculosis* strains has improved the understanding of tuberculosis transmission (1, 4, 6, 7, 10–12, 17, 21). Restriction fragment length polymorphism (RFLP) can differentiate strains of *M. tuberculosis*, particularly when insertion sequence IS6110 probes are used (2, 4, 6, 12, 15–18). This is a standardized method (16) commonly used for analyzing the molecular epidemiology of tuberculosis. We present here the first RFLP analysis of strains obtained in Israel from Ethiopian, Russian, and local Israeli patients.

Sixty-nine *M. tuberculosis* isolates were chosen (55 from the Israeli mycobacterium reference laboratory, A. Felix Public Health Laboratories, Tel Aviv, and 14 from the mycobacterial laboratory of Hadassah Hospital, Jerusalem, Israel) to include isolates of Ethiopian, former Soviet Union, and Israeli origin, as well as different antibiotic susceptibility patterns. Isolates were cultured from patients with pulmonary tuberculosis between 1992 and 1996 and identified as *M. tuberculosis* complex with the Accu Probe kit (Gen-Probe, San Diego, Calif.). Seventeen isolates were excluded: nine with identical RFLP patterns shown to be a laboratory contamination and eight originating from the same patients or family. Twenty percent of the isolates were from patients born in Israel or long-term residents, 39% were from former Soviet Union immigrants, and 32% were from Ethiopian immigrants. There was no information on the country of origin for 9% of the isolates. Fifty-five percent of the isolates were susceptible to all antimycobacterial drugs tested; 39% were resistant to at least one antimycobacterial drug, and there was no information on drug susceptibility for 6% of the isolates. Isolates were coded, so that the patients’ country of origin and the antimicrobial susceptibility patterns of the isolates were revealed only after molecular typing was performed.

RFLP analysis was done as described by van Embden et al. (16). The probes used were the 245-bp PCR product of the right IS6110 arm (19), and the 36-bp DR-r oligonucleotide that detects direct repeats (8). The IS6110 DNA probe was prepared by PCR using INS-1 and INS-2 primers (19). The DR-r oligonucleotide (5′-GTCGTCAGACCCAAAACCCGGAGG GGACGGAAAAAC-3′) was synthesized (DNA synthesizer 394; Applied Biosystems Inc.). Probes were labeled with digoxigenin (Roche Molecular Biochemicals, Roche Diagnostics GmbH) by random priming (IS6110 probe) or by 3′-end labeling (DR-r). Isolates were cultivated on Lowenstein-Jensen slants, and chromosomal DNA was extracted using 50 μl of lysozyme (10 mg/ml) and 75 μl of sodium dodecyl sulfate-proteinase K and cetyltrimethylammonium bromide-NaCl mixtures (16). DNAs were digested with 20 U of PvuII, which cleaves the IS6110 sequence once. Restriction fragments were separated by 0.8% agarose gel electrophoresis (19), transferred to nylon membranes (Roche Molecular Biochemicals), hybridized to the probes described above, and detected by chemiluminescence (DIG system; Roche Molecular Biochemicals). DNAs from *M. tuberculosis* H37Rv and BCG 1173P2 Pasteur strains were included as controls. The fingerprinting patterns were compared by visual examination. Clustering was defined as the occurrence of two or more isolates from different patients that showed an identical pattern for at least six IS6110 bands. When there were less than 6 IS6110 bands, clustering was defined as the occurrence of similar RFLP patterns using both IS6110 and DR-r as probes.

The RFLP patterns of the 25 drug-susceptible isolates from patients living in different localities of Israel differed from each other (Fig. 1).

Fourteen *M. tuberculosis* isolates (10 susceptible to all anti-
mycobacterial drugs and 4 resistant) from patients living in Jerusalem but originating from countries representing the diversity found in Israel exhibited different IS\textsubscript{6110} RFLP patterns (Fig. 2).

Thirteen drug-resistant \textit{M. tuberculosis} isolates (11 from new immigrants from the former Soviet Union) were examined. Eight of these were multidrug resistant (MDR). Only the RFLP patterns of isolates 4 and 6 and isolates 8 and 10 were similar (Fig. 3), emphasizing the low rate of clustering among \textit{M. tuberculosis} strains in Israel (4 of 52 [7.7%]). Isolates 8 and 10 were MDR with identical susceptibility patterns, whereas isolates 4 (MDR) and 6 (streptomycin resistant) had different susceptibility patterns. No obvious contact could be found between these cases.

The IS\textsubscript{6110} copy number of the isolates ranged from 1 to 14 (mean, 7 copies). No correlation was found between the IS\textsubscript{6110} copy number and the origin of the patient or antibiotic susceptibility. Three isolates had a single copy of IS\textsubscript{6110}. The size of the band for isolate 12 was similar to that of BCG, which always has a single copy of IS\textsubscript{6110} (Fig. 2). In a situation where it is impossible to differentiate between \textit{M. tuberculosis} with a single IS\textsubscript{6110} copy and BCG, RFLP with an unrelated probe is recommended. RFLP using a DR-r probe was performed and demonstrated that this isolate was identical to 1173 BCG-P2 strain (data not shown).

Israel has a unique epidemiological situation, where immigrants from many countries with varied incidence of tuberculosis concentrate in a relatively small area. RFLP analysis of 52 isolates of \textit{M. tuberculosis} showed a considerable diversity, suggesting that most patients examined were infected with unique strains. RFLP revealed a 92% genetic diversity, a rate higher than that observed in other developed countries with populations of various geographical origins (1, 7). Our results indicate that each immigrant imported the mycobacteria from his or her country of origin and the disease reactivated in Israel, soon after arrival. This RFLP study does not indicate any trend of cross infection of tuberculosis between new immigrants themselves during their stay at immigration centers or between new immigrants and local Israelis. However, the selection of the isolates was performed retrospectively and the number of strains analyzed was limited. A larger prospective study over a longer period of time is required to determine whether this epidemiological trend continues. The polymorphism among the isolates from Ethiopian immigrants was unexpectedly high, as they came from a country with a high prevalence of tuberculosis where the polymorphism is usually low (2, 5, 9, 17). This finding is even more surprising, because many of these immigrants lived for a prolonged period in crowded conditions before coming to Israel. The low rate of clustering indicates that tuberculosis among the study population results mainly from reactivation of latent infection.

Our experience and that of others (20) suggest that \textit{M. tu-
tuberculosis strains have a low penetration-diffusion pattern in the human population.

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