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Restriction Fragment Length Polymorphism of *Mycobacterium tuberculosis* Strains Isolated from Greenland during 1992: Evidence of Tuberculosis Transmission between Greenland and Denmark

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In order to describe the transmission of tuberculosis (TB) at the clonal level in a defined geographic region during a certain period of time, all isolates of *Mycobacterium tuberculosis* collected during 1992 from Greenland were subjected to analyses of DNA restriction fragment length polymorphism (RFLP). The RFLP patterns obtained by probing the genomic DNA with the repetitive insertion segment IS*6110* revealed a high degree of similarity among the isolates, indicating a relatively high transmission rate and a close relationship between the individual *M. tuberculosis* clones. This was further confirmed by reprobing the Southern blots with two more-stable genetic markers, IS*1081* and the DR sequence. The RFLP patterns were compared with those of 245 *M. tuberculosis* strains collected from Denmark during the same period (representing 91% of all new, bacteriologically verified cases of TB in Denmark in 1992). One of the three prevalent IS*6110*-defined clusters was traced to a group of immigrants from Greenland living in a small, defined geographical region in Denmark and to a group of Danish citizens either with known contact with these immigrants or, in other cases, with a record of previous travel or working activities in Greenland. The study showed that the present technique is extremely helpful in monitoring the spread of TB and thereby also contributing to improved disease control.

Tuberculosis (TB) still remains one of the major infectious diseases causing morbidity and mortality throughout the world (20, 27). Although 95% of all new TB cases and deaths occur in developing countries (26), TB, considered a disease of poverty and of the past, has returned even in industrialized countries, in part because of the AIDS pandemic (4, 22). Multidrug-resistant tuberculosis has emerged as a major problem among human immunodeficiency virus (HIV)-infected persons in New York and in Florida (4). Because infection with HIV is associated with increased susceptibility to TB or at least with accelerated progression and mortality of the disease, it is feared that the incidence of TB will increase even further as the HIV epidemic continues to develop. There is therefore an urgent need for new tools to assist in TB control. In this context, epidemiological investigations are of great importance. A thorough understanding of the transmission and pathogenesis of TB is fundamental for the development of a rational approach to disease control. The identification of the infectious source and the tracing of individuals in contact with the infected persons are important aspects of limiting the dissemination of TB. The discovery of strain-specific DNA fingerprint patterns on the basis of the occurrence of repetitive DNA elements in the chromosome of *Mycobacterium tuberculosis* has provided epidemiologically very useful tools for monitoring the spread of individual strains (17, 19, 21). The use of this novel technique in conjunction with conventional microbiological and clinical investigations is providing increased insight into the current epidemiology of TB.

As described previously (3, 7, 10, 16–18, 23, 28, 31, 33, 35), five different repetitive DNA elements have been discovered in *M. tuberculosis* complex strains. The insertion sequence IS*6110*, belonging to the IS*3* family of insertion elements of enterobacteria, has been widely used as a probe for strain differentiation (3, 6, 11, 14, 17, 23). The DNA fingerprint patterns generated with this probe after digestion of genomic DNA with the endonuclease *Pvu*II are relatively simple and amenable to computerized analyses and comparison. Moreover, the degree of the restriction fragment length polymorphism (RFLP) generated appears well suited for epidemiologic investigations. So far, the DNA fingerprinting technique for *M. tuberculosis* isolates with the IS*6110* probe has mainly been used to confirm suspected cases of transmission (2, 4, 5, 8, 9, 12, 13, 15) and to detect laboratory contamination (25). Recently, two population-based epidemiological studies of TB by conventional and molecular methods were reported (1, 24). In this study, all new isolates of *M. tuberculosis* obtained from Greenland during the year 1992 were investigated. The TB transmission between Denmark and Greenland, two geographically separated and epidemiologically quite different parts of one kingdom, was also investigated by study of isolates obtained from immigrants from Greenland living in Denmark as well as isolates derived from Danish TB patients in the same period of time. The RFLP patterns obtained with IS*6110* as a probe were compared with those obtained with two other genetic markers, IS*1081* and the DR region.

MATERIALS AND METHODS

Bacterial strains. The isolates of *M. tuberculosis* were collected as a part of a cross-sectional study performed during 1992 at the Mycobacteria Department at Statens Seruminstitut in Copenhagen, Denmark. This laboratory serves as a central diagnostic service laboratory for all of Denmark including the

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Faroe Islands and Greenland. A total of 272 strains, representing 92% of all new, bacteriologically verified cases of TB in Denmark, the Faroe Islands, and Greenland in 1992, were examined. Eight percent of all the new cases were not examined because the bacterial cultures were not available for DNA extraction. Of these analyzed isolates, 49 are described in this report either because they originated from a sample submitted from Greenland (n = 27), because they were obtained from immigrants from Greenland living in Denmark (n = 9), or because their RFLP patterns could be aligned to a prevalent pattern found in the samples from Greenland (n = 13). The isolates were identified as *M. tuberculosis* on the basis of standard microbiological tests. All isolates were fully susceptible to isoniazid, streptomycin, rifampin, and ethambutol.

DNA probes. Three different DNA probes were used in the present study. They were (i) a 245-bp probe directed against the right arm of IS*6110* (32), (ii) a 236-bp probe directed against IS*1081*, which is a 1,346-bp sequence related to the IS*256* family of insertion elements originally found in *Staphylococcus aureus* (7, 32), and (iii) a probe directed against the so-called DR sequence, which is a directly repeated sequence of 36 bp clustered in one region of the genome of *M. tuberculosis* complex species and interspersed by nonrepetitive sequences of 36 to 41 bp (16, 32). All three probes were prepared by PCR and purified by agarose electrophoresis as described previously (16, 17, 34). They were all nonradioactively labelled by use of an enhanced chemiluminescence kit (Amersham International plc, Little Chalfont, United Kingdom), as instructed by the manufacturer.

RFLP analyses. DNA fingerprinting was performed essentially as described previously (30). The technique includes the following steps: growth of *M. tuberculosis* strains on Löwenstein-Jensen slopes at 37°C for 3 weeks; extraction of genomic DNA from the bacteria; digestion of the DNA with the restriction endonuclease *Pvu*II; separation of the digested DNA by electrophoresis in 0.8% agarose gel in a buffer containing 90 mM Tris base, 90 mM boric acid, and 2 mM EDTA overnight (voltage, 0.8 V/cm); transfer of the separated DNA from the gel to a positively charged nylon membrane (Hybond N+; Amersham) in a vacuum transfer device (Milliblot V system; Millipore Corp., Bedford, Mass.); probing for the repetitive elements with labelled DNA probes; and detection of bound probes by the enhanced chemiluminescence direct nucleic acid detection system (Amersham).

To facilitate the determination of the size of each IS*6110*-hybridizing fragment in computer-assisted analyses for comparison of fingerprints of *M. tuberculosis* strains, we used, as recommended previously (30), a mixture of *Hin*dIII-digested lambda DNA and *Hae*III-digested ϕX174 DNA as an external marker and a mixture of *Pvu*II-digested supercoiled DNA ladder and *Hae*III-digested ϕX174 DNA as an internal marker. The internal marker was added to the wells together with the cleaved *M. tuberculosis* DNA and visualized by reprobing the blots with enhanced chemiluminescence kit-labelled molecular size marker standards. In addition to the two molecular size marker standards, a lane of *Pvu*II-digested chromosomal DNA of an *M. tuberculosis* reference strain, Mt.14323 (strain collection, Laboratory of Bacteriology and Antimicrobial Agents, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands), was included in each gel as a standard.

Analyses of RFLP patterns. The pictures obtained from both hybridization with the IS*6110* probe and rehybridization with the internal marker probe were scanned by a scanner interfaced with a personal computer in which the program Bioimage Whole Band Analyzer, version 3 (Millipore Corporation, Ann Arbor, Mich.), was installed. On the basis of the molecular sizes of the hybridizing fragments and the number of IS*6110* copies of each isolate, the similarity values among all the fingerprint patterns were calculated with a deviation value of 4%, and the relationship between all the isolates was defined mathematically. The RFLP patterns obtained by using the IS*1081* probe and the DR probe were compared visually.

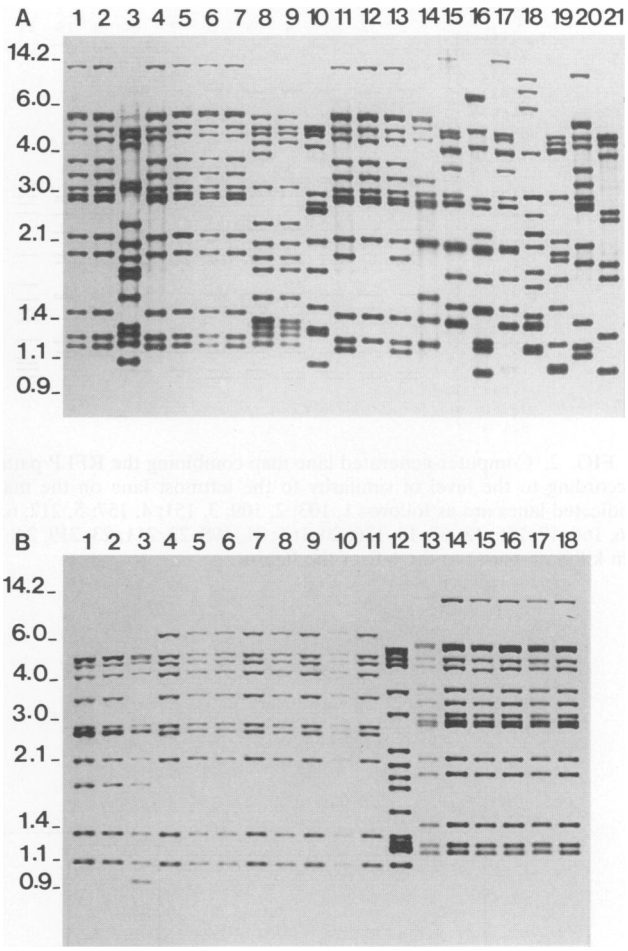

FIG. 1. IS*6110* fingerprints of *M. tuberculosis* strains. Lanes 11, 13, 16, 18, 20, and 21 in panel A and lanes 13, 15, and 17 in panel B represent strains isolated from immigrants from Greenland living in Denmark. Lanes 14, 16, and 18 in panel B represent strains derived from Danish patients. The remaining lanes contain samples submitted from Greenland. The isolate identification numbers of the individual isolates shown in the figure are as follows. (A) Lane 1, 103; lane 2, 109; lane 3, 126; lane 4, 151; lane 5, 157; lane 6, 217; lane 7, 222; lane 8, 160; lane 9, 228; lane 10, 67; lane 11, 144; lane 12, 159; lane 13, 227; lane 14, 297; lane 15, 137; lane 16, 156; lane 17, 261; lane 18, 238; lane 19, 177; lane 20, 292; lane 21, 299. (B) Lane 1, 80; lane 2, 161; lane 3, 166; lane 4, 176; lane 5, 198; lane 6, 211; lane 7, 219; lane 8, 229; lane 9, 232; lane 10, 245; lane 11, 240; lane 12, 111; lane 13, 120; lane 14, 4; lane 15, 5; lane 16, 65; lane 17, 53; lane 18, 70. The IS*6110* fingerprint pattern of the reference strain was deleted from this figure. Sizes of standard DNA fragments are indicated to the left of each panel in kilobase pairs.

Patient information. The filing of the data used in this study was approved by the Danish Ministry of Health (kt.j.nr. 93/0763-9) as required by Danish legislation. The ethical aspects of this study, i.e., the cases in which patients were contacted directly and questioned about possible contacts with

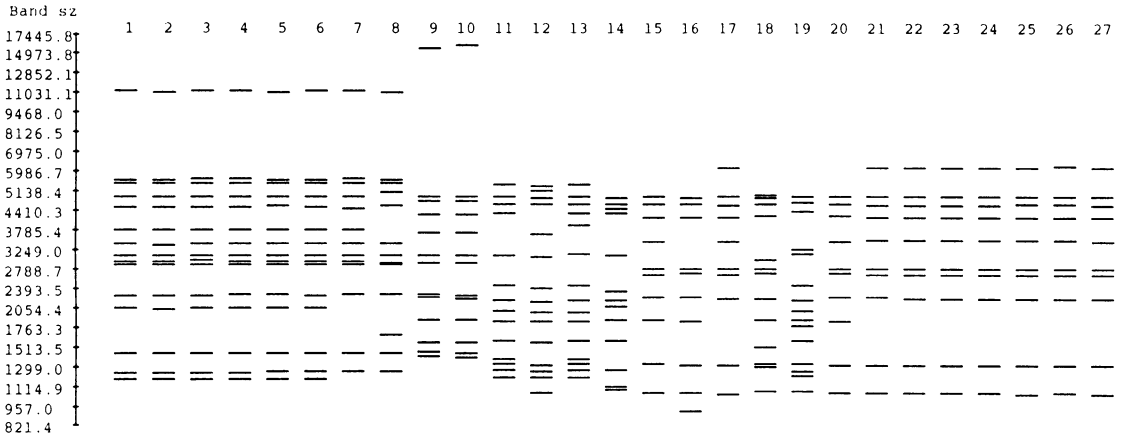

FIG. 2. Computer-generated lane map combining the RFLP patterns of all 27 isolates from Greenland. The lanes are arranged in a hierarchy according to the level of similarity to the leftmost lane on the map (strain number 103). Strain identification numbers corresponding to the indicated lanes are as follows: 1, 103; 2, 109; 3, 151; 4, 157; 5, 217; 6, 222; 7, 159; 8, 297; 9, 261; 10, 137; 11, 228; 12, 111; 13, 160; 14, 177; 15, 80; 16, 166; 17, 176; 18, 67; 19, 126; 20, 161; 21, 198; 22, 211; 23, 219; 24, 229; 25, 232; 26, 245; 27, 240. Sizes of standard DNA fragments are indicated (in kilobase pairs) to the left of the figure.

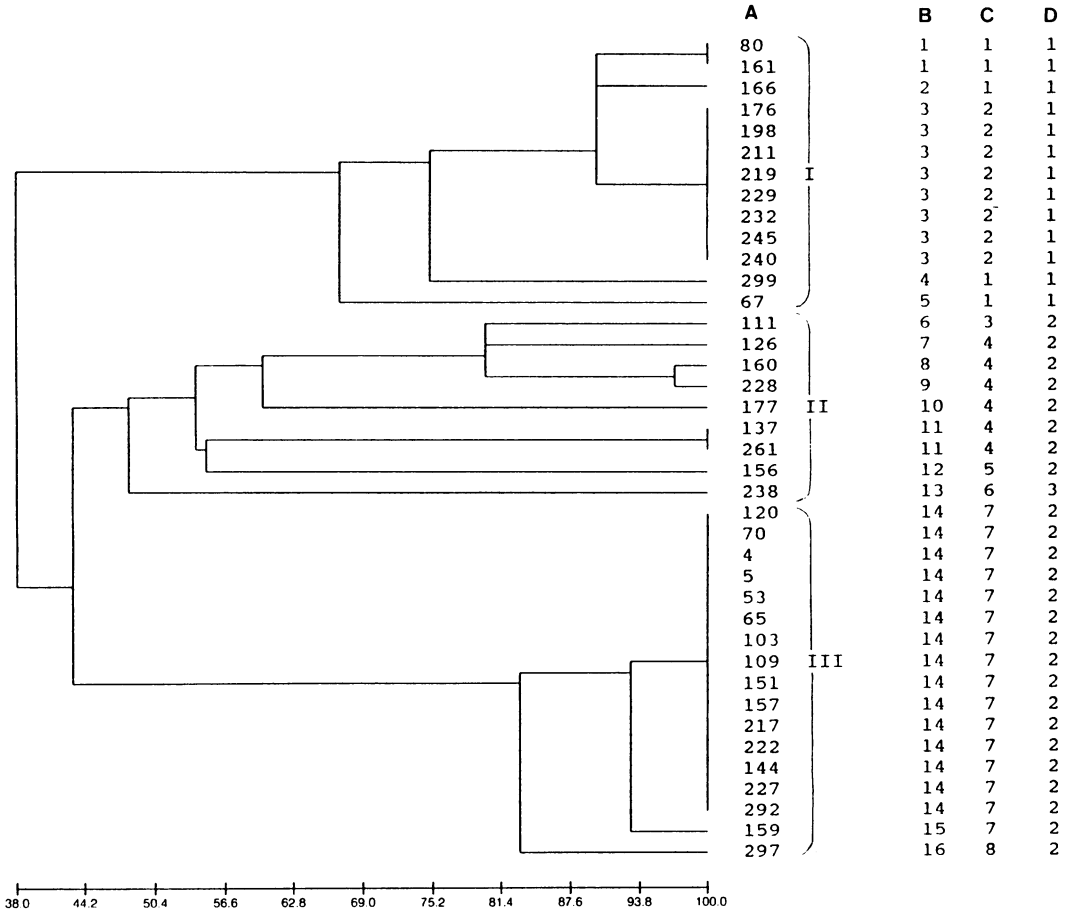

FIG. 3. Dendrogram based on computer-assisted comparison of DNA fingerprints obtained by IS*6110* probes compared with DNA fingerprinting patterns obtained by IS*1081* and DR. Included in the figure are all 39 fingerprints from Fig. 1. The levels of similarity among the patterns are given as percentages, as indicated below the dendrogram. Brackets I, II, and III indicate clusters defined by the computer-assisted analysis of the IS*6110*-defined patterns. (A) Isolate identification numbers; (B) IS*6110*-defined pattern; (C) DR-defined pattern; (D) IS*1081*-defined pattern. Isolates 53, 227, 144, 292, 120, 5, 156, 238, and 299 were obtained from immigrants from Greenland living in Denmark. Isolates 4, 65, and 70 were obtained from Danish patients. The remainder are the isolates submitted from Greenland.

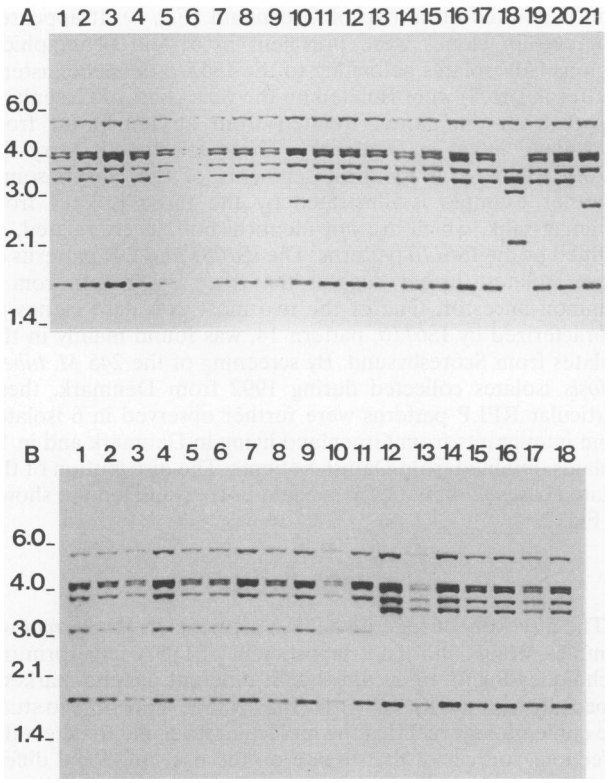

FIG. 4. IS*1081* DNA fingerprints obtained from reprobing the blots shown in Fig. 1. Panel and lane designations are as defined in the Fig. 1 legend. Sizes of standard DNA fragments are indicated to the left of each panel in kilobase pairs.

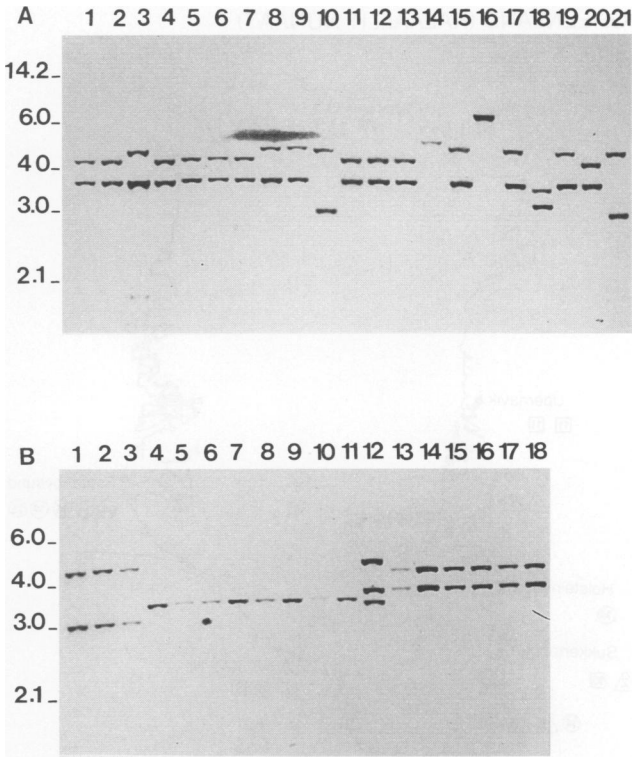

FIG. 5. DR DNA fingerprints obtained from reprobing the blots shown in Fig. 1. Panel and lane designations are as defined in the Fig. 1 legend. Sizes of standard DNA fragments are indicated to the left of each panel in kilobase pairs.

other TB patients and travel activities, were evaluated and approved by the ethical committee of Copenhagen [J.nr. (KF) 01-503/93].

RESULTS

With the purpose of characterizing TB transmission at the clonal level in a defined and relatively isolated region with a high TB incidence, we analyzed *M. tuberculosis* isolates from all new cases of bacteriologically verified TB diagnosed in Greenland in the year 1992 ($n = 27$) by use of a DNA fingerprinting technique. By the same method, we analyzed 91% of all new cases of bacteriologically verified TB in Denmark in 1992 ($n = 245$), including all samples from immigrants from Greenland living in Denmark ($n = 9$). The RFLP patterns of the isolates obtained from Greenland were compared with the RFLP patterns of the isolates collected in Denmark. Six immigrants from Greenland and 13 Danish patients were observed to be infected with a clone either identical to or very similar to one of the most prevalent clones in Greenland.

Characteristics of IS*6110* RFLP patterns. The IS*6110* DNA fingerprints of 39 of the 49 *M. tuberculosis* isolates are shown in Fig. 1. The fingerprints of the other 10 isolates obtained from the Danish patients are not shown, because these isolates were fingerprinted in several separate experiments. An overview of the RFLP patterns of all the *M. tuberculosis* isolates from Greenland is illustrated with a computer-derived lane map shown in Fig. 2. According to a computer-assisted comparison of the DNA fingerprints, the RFLP patterns fell into only three clusters, as shown by the dendrogram in Fig. 3. The isolates of two of the clusters (clusters I and III) exhibited a very high degree of similarity. The number of IS*6110* copies per genome ranged from 9 to 15. Ten out of 27 isolates from Greenland carried 15 copies of IS*6110*. Two prevalent patterns, containing 10 and 15 copies of IS*6110*, were found in eight and six isolates, respectively, which account for 52% of the total number of samples from Greenland. In contrast to previously reported studies (6, 32, 33) and to ongoing studies on strains isolated in Denmark and Tanzania (34a), we did not find any isolate carrying only one copy of the IS*6110* element. The lowest number of IS*6110* copies per isolate was nine.

Comparison of RFLP patterns associated with two other genetic markers. Only two transposable elements have so far been found in the *M. tuberculosis* complex: the above-mentioned IS*6110* and IS*1081* (32). IS*1081* has previously been demonstrated to be much more stably integrated in the chromosome than IS*6110* (32). In fact, the RFLP patterns obtained with IS*1081* as a probe did not allow strain differentiation to nearly the same degree as those obtained with IS*6110*. We wanted to determine whether the IS*1081* defined clusters would follow the IS*6110* clustering. The RFLP patterns obtained with IS*1081* as a probe are shown in Fig. 4. The number of IS*1081* copies per chromosome was six for all of the 39 isolates analyzed. Only three patterns were observed among the 39 isolates. As summarized in the table in Fig. 3, a clear correlation between the clusters defined by IS*1081* and by IS*6110* was observed. All the isolates included in cluster I defined by IS*6110* typing showed identical IS*1081* banding patterns, and the isolates included in cluster II and cluster III showed another IS*1081* banding pattern, with the exception of one isolate in cluster II (number 238), which gave rise to

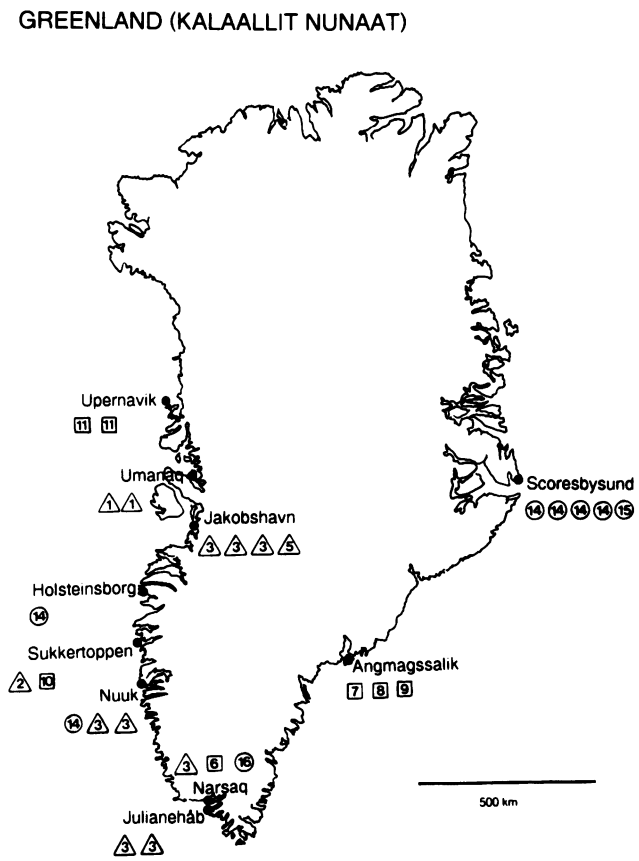

FIG. 6. Map of Greenland on which the distribution of different IS*6110*-defined *M. tuberculosis* clones is marked. Open triangles, cluster I; open squares, cluster II; open circles, cluster III. The numbers inside these symbols refer to the IS*6110*-defined pattern of each isolate.

pattern 3. This isolate is, however, the most distantly related of the isolates in that cluster, as determined by IS*6110* probing.

The DR sequences of *M. tuberculosis* had previously been described as useful markers for strain differentiation when applied on *Alu*I-cleaved DNA and had proved to be especially useful in characterization of strains carrying only one copy of IS*6110* (32). In an attempt to assess the applicability of a less polymorphic marker to characterize the relationship between isolates carrying high numbers of IS*6110* copies per chromosome, the blots were also typed with the DR probe and compared with the other genetic markers. Reprobing the blots with the DR probe revealed eight different patterns among the 39 isolates (Fig. 5). The fingerprints obtained from DR reprobing were more polymorphic than those obtained by the IS*1081* probing but less polymorphic than those demonstrated by the IS*6110* probe. The DR typing supports the clusters defined by IS*6110* (Fig. 3). The isolates showing identical IS*6110* banding patterns exhibited identical DR banding patterns, e.g., the isolates included in pattern 3 and in pattern 14 defined by IS*6110*. Even patterns 14 and 15 in cluster III, which differed in IS*6110* banding patterns by one band, gave rise to the same DR picture. A clear relation between the IS*6110* typing and the IS*1081* and DR typing was observed.

Geographical distribution of *M. tuberculosis* clones and transmission of TB between Greenland and Denmark. The distribution of IS*6110*-defined *M. tuberculosis* clones in Greenland is marked on a map of Greenland (Fig. 6). It appeared that certain clones were prevalent in certain geographical regions. All isolates belonging to the IS*6110*-defined cluster I (patterns 1 to 5) were isolated on the west coast of Greenland (apart from one strain isolated from an immigrant from Greenland living in Denmark). These isolates all carry the IS*1081* element at identical segments of the chromosome. Another example is illustrated by the three isolates from Angmagssalik, which are not identical but closely related, as defined by the IS*6110* patterns. The IS*1081* and DR patterns of these isolates further suggest that they originated from a common ancestor. One of the two most prevalent clones as characterized by IS*6110*, pattern 14, was found mainly in the isolates from Scoresbysund. By screening of the 245 *M. tuberculosis* isolates collected during 1992 from Denmark, these particular RFLP patterns were further observed in 6 isolates from immigrants from Greenland living in Denmark and in 13 isolates obtained from Danish patients. The distribution of the related cases and the possible chain of transmission are shown in Fig. 7.

DISCUSSION

The discovery of repetitive DNA elements in *M. tuberculosis* complex strains and the establishment of DNA fingerprinting techniques for *M. tuberculosis* with different genetic markers, especially with the IS*6110* probe, have made it possible to study the epidemiology of TB at the molecular level and to detect the infectious sources of the disease on the basis of clonal differentiation of *M. tuberculosis* isolates. A better understanding of the dissemination of the bacteria in a defined population will hopefully improve the detection of new cases and the control of disease transmission. The present study was intended to provide an overview of the molecular epidemiology of TB in Greenland during a 1-year period, 1992. Greenland is a home-ruled region of Denmark with a population of approximately 50,000. Only coastal regions of the 2,200,000-km^2 country are inhabited. The largest city is the capital, Nuuk, with a population of 12,000 citizens. There are many cultural and socioeconomic differences between Denmark and Greenland, and although the peoples of the two countries in principle have access to the same kind of health system the incidence of TB is much higher in Greenland than in Denmark (>50 cases per 100,000 people in Greenland and <10 cases per 100,000 people in Denmark, according to the National Surveillance System, Department of Epidemiology, Statens Seruminstitut, Copenhagen, Denmark). The IS*6110*-associated RFLP patterns observed in the isolates from Greenland were characterized by a large number of IS*6110* copies and a high degree of similarity, suggesting a close relationship between the *M. tuberculosis* clones circulating in Greenland. This was further demonstrated by probing of the samples with the other known transposable element of *M. tuberculosis*, the IS*1081* element, which apparently is mobilized with a much lower frequency than IS*6110*. The patterns obtained with this probe were very uniform but grouped the isolates in agreement with the IS*6110*-defined clusters. Similarly, the use of the so-called DR region as a probe grouped the isolates in accordance with the IS*6110*-defined clusters. Both the DR probe and the IS*1081* probe had less discriminatory power in strain differentiation than the IS*6110* probe. However, the use of these three markers in combination appears promising in defining the relationship between various clones during the process of evolution.

In general, *M. tuberculosis* isolates with identical DNA fingerprints, especially when they consist of multiple IS*6110*-

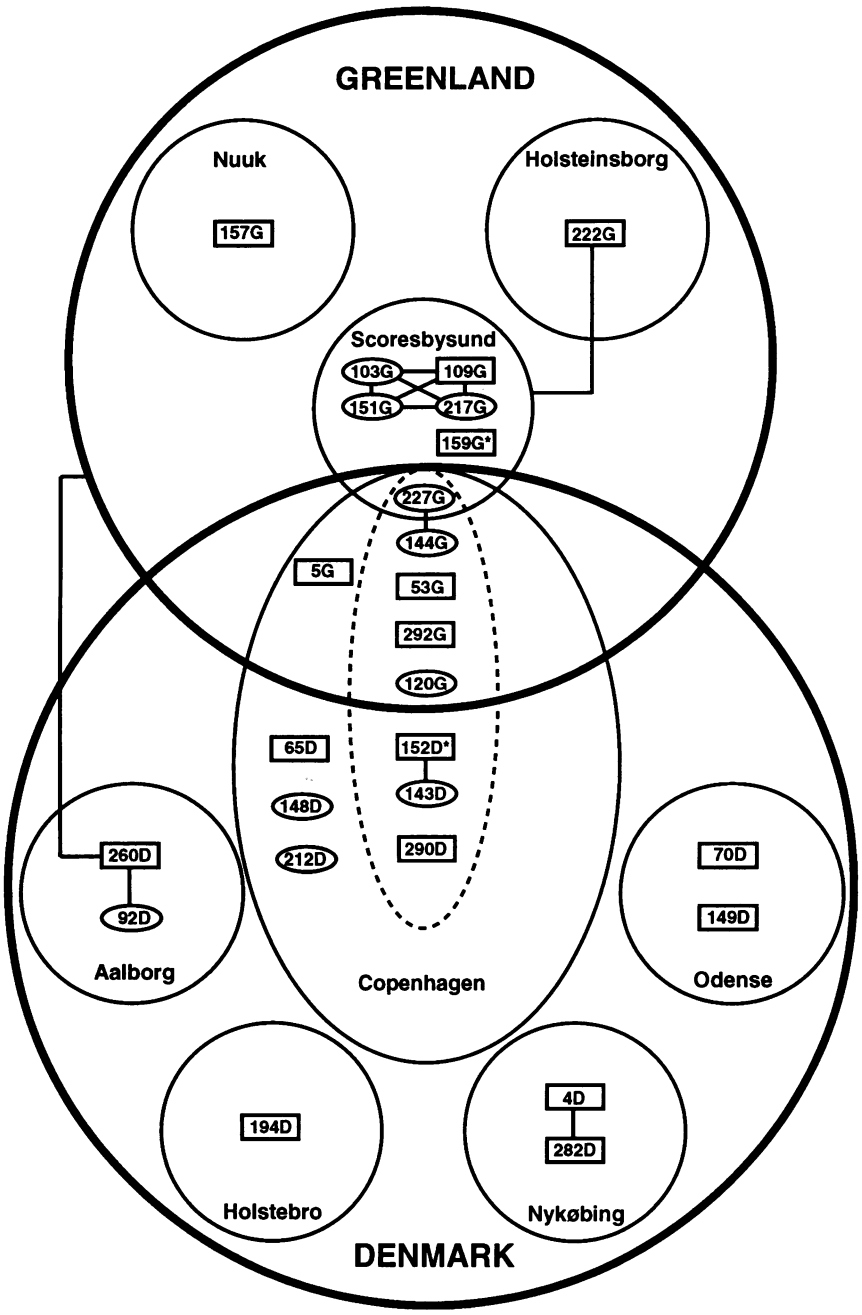

FIG. 7. Transmission of an *M. tuberculosis* clone belonging to the IS*6110*-defined cluster III (patterns 14 and 15) between Greenland and Denmark. Each strain is indicated by its strain identification number inside a rectangle when isolated from a male patient and within an oval when isolated from a female patient. Patients originating from Greenland are indicated by a G, and Danish patients are indicated by a D. Known contacts are indicated by solid lines, e.g., married couples, persons living at the same address, close neighbors, friends, etc. The dashed line encloses patients living in or with close contact to a certain small region of Copenhagen with a high percentage of inhabitants with social and health problems. Many immigrants from Greenland live in or frequently visit this neighborhood. Patient 227G lived in Scoresbysund before moving to Copenhagen in late 1991; patient 222G worked as a sailor and had close contact with the inhabitants of Scoresbysund; patient 260D worked in Greenland for 4 months before contracting TB; patient 53G visited Greenland in 1990.

hybridizing bands, are epidemiologically related (6, 19, 29, 33). This is further confirmed by our observation that certain clones of *M. tuberculosis* were prevalent in certain geographical areas, where the inhabitants appeared to have relatively frequent contacts with each other. The degree of DNA polymorphism in the isolates from Greenland tended to correlate with the degree of geographical isolation of a region. In Scoresbysund, a fairly isolated municipality of 400 inhabitants on the east coast of Greenland, only one cluster of closely related isolates was observed, whereas in Nuuk, the capital city of Greenland, and in cities on the southwest coast, more diversity was noted. This observation is in good agreement with previous reports

(15) in which it was suggested that geographical separation of a pool of infection may lead to the evolution of distinct bacterial clones. Another factor contributing to the degree of DNA polymorphism of *M. tuberculosis* isolates in a population is the relative contribution of newly acquired TB versus reactivated infection. The finding that two prevalent fingerprint patterns constituted 52% of the isolates from Greenland and that only very limited polymorphism was observed supported the notion that the rate of transmission of TB in Greenland was high in 1992. Because Greenland is a relatively isolated geographic region, it is expected that, on the basis of the present survey, a continued study of all new isolates in the following years will provide data describing in detail the TB distributional trends in that particular part of the world and will also contribute insight into the evolution of *M. tuberculosis.*

Another result from the present study is the observation that one of the two most prevalent clones (pattern 14) found in Greenland was also isolated from 13 Danish patients and from 6 immigrants from Greenland living in Denmark. The clinical information available for these patients confirmed in several instances the epidemiological relationship of these cases. It was found that many of the patients appeared, as indicated in Fig. 7, to have had some level of relationship or contact, such as couples living together, families, neighbors, or friends. In some cases, the direct source of infection could not be detected, and in all cases, the possibility of another common source of infection certainly exists. A certain region of Copenhagen appears to have been a central arena for the transmission of this particular clone of *M. tuberculosis*. This part of Copenhagen attracts many immigrants from Greenland and is known to house a high proportion of persons with severe social and health problems. One of the immigrants inhabiting this area originated from Scoresbysund, the center for this particular *M. tuberculosis* clone. One of the patients infected with this clone was believed to have infected three other persons diagnosed in 1993, i.e., after this study was completed. Mycobacterial DNA was available from one of these patients, and it was demonstrated that it was not identical to the clone of pattern 14 (data not shown). In other words, the source of infection was falsely believed to be known. The present technique will, in combination with a good health care program, be extremely useful in monitoring and hopefully controlling the spread of the disease. The results from a cross-sectional study of *M. tuberculosis* strains obtained through 1992 from the rest of the Danish population are currently under investigation.

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REFERENCES

1. **Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Drucker, and B. R. Bloom.** 1994. Transmission of tuberculosis in New York City, an analysis by DNA fingerprinting and conventional epidemiologic methods. N. Engl. J. Med. **330:** 1710–1716.
2. **Beck-Sagué, C., S. W. Dooley, M. D. Hutton, J. Otten, A. Breeden, J. T. Crawford, A. E. Pitchenik, C. Woodley, G. Cauthen, and W. R. Jarvis.** 1992. Hospital outbreak of multidrug resistant *Mycobacterium tuberculosis* infections. JAMA **268:**1280–1286.
3. **Cave, M. D., K. D. Eisenach, P. F. McDermott, J. H. Bates, and J. T. Crawford.** 1991. IS*6110*: conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. Mol. Cell. Probes **5:**73–80.
4. **Centers for Disease Control.** 1991. Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons, Florida and New York, 1988–1991. Morbid. Mortal. Weekly Rep. **40:**585–591.
5. **Centers for Disease Control.** 1992. Transmission of multidrug resistant tuberculosis among immunocompromised persons in a correctional system, New York 1991. Morbid. Mortal. Weekly Rep. **41:**507–509.
6. **Chevrel-Dellagi, D., A. Abderrahman, R. Haltiti, H. Koubaji, B. Gicquel, and K. Dellagi.** 1993. Large-scale DNA fingerprinting of *Mycobacterium tuberculosis* strains as a tool for epidemiological studies of tuberculosis. J. Clin. Microbiol. **31:**2446–2450.
7. **Collins, D. M., S. K. Erasmuson, and D. M. Stephens.** 1991. Identification of insertion sequence, IS*1081*, in *Mycobacterium bovis*. FEMS Microbiol. Lett. **83:**11–16.
8. **Daley, C. L., P. M. Small, G. F. Schecter, G. K. Schoolnik, R. A. McAdam, W. R. Jacobs, Jr., and P. C. Hopewell.** 1992. An outbreak of tuberculosis with the human immunodeficiency virus: an analysis using restriction fragment length polymorphisms. N. Engl. J. Med. **326:**231–235.
9. **Dooley, S. W., M. E. Villarrino, M. Lawrence, L. Salinas, S. Amil, J. V. Rullan, W. R. Jarvis, A. B. Bloch, and G. M. Cauthen.** 1992. Nosocomial transmission of tuberculosis in a hospital unit for HIV-infected patients. JAMA **267:**2632–2634.
10. **Doran, T. J., A. L. M. Hodgson, J. K. Davies, and A. J. Radford.** 1992. Characterization of a novel repetitive DNA sequence from *Mycobacterium bovis*. FEMS Microbiol. Lett. **96:**179–186.
11. **Dwyer, B., K. Jackson, K. Raios, A. Sievers, E. Wilshire, and B. Ross.** 1993. DNA restriction fragment analysis to define an extended cluster of tuberculosis in homeless men and their associates. J. Infect. Dis. **167:**490–494.
12. **Edlin, B. R., J. I. Tokars, M. H. Grieco, J. T. Crawford, J. Williams, E. M. Sordillo, K. R. Ong, J. O. Kilburn, S. W. Dooley, K. G. Castro, W. R. Jarvis, and S. D. Holmberg.** 1992. An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **326:**1514–1521.
13. **Fischl, M. A., R. B. Uttamchandani, G. L. Daikos, R. B. Poblete, J. N. Moreno, R. R. Reyes, A. M. Boota, L. M. Thompson, T. J. Cleary, and S. Lai.** 1992. An outbreak of tuberculosis caused by multiple-drug resistant tubercle bacilli among patients with HIV infection. Ann. Intern. Med. **117:**177–183.
14. **Genewein, A., A. Telenti, C. Bernasconi, C. Mordasini, S. Weiss, A.-M. Maurer, H. L. Rieder, K. Schopfer, and T. Bodmer.** 1993. Molecular approach to identifying route of transmission of tuberculosis in the community. Lancet **342:**841–844.
15. **Godfrey-Fausset, P., P. R. Mortimer, P. A. Jenkins, and N. G. Stoker.** 1992. Evidence of transmission of tuberculosis by DNA fingerprinting. Br. Med. J. **305:**221–223.
16. **Hermans, P. W. M., D. van Soolingen, E. M. Bik, P. E. W. de Haas, J. W. Dale, and J. D. A. van Embden.** 1991. The insertion element IS*987* from *Mycobacterium bovis* BCG is located in a hot-spot integration region for insertion elements in *Mycobacterium tuberculosis* complex strains. Infect. Immun. **59:**2695–2705.
17. **Hermans, P. W. M., D. van Soolingen, J. W. Dale, A. R. J. Schuitema, R. A. McAdam, D. Catty, and J. D. A. van Embden.** 1990. Insertion element IS*986* from *Mycobacterium tuberculosis*: a useful tool for diagnosis and epidemiology of tuberculosis. J. Clin. Microbiol. **28:**2051–2058.
18. **Hermans, P. W. M., D. van Soolingen, and J. D. A. van Embden.** 1992. Characterization of a major polymorphic tandem repeat in *Mycobacterium tuberculosis* and its potential use in the epidemiol-

ogy of *Mycobacterium kansasii* and *Mycobacterium gordonae*. J. Bacteriol. **174:**4157–4165.

19. **Mazurek, G. H., M. D. Cave, K. D. Eisenach, R. J. Wallace, Jr., J. H. Bates, and J. T. Crawford.** 1991. Chromosomal DNA fingerprint patterns produced with IS*6110* as strain-specific markers for epidemiologic study of tuberculosis. J. Clin. Microbiol. **29:** 2030–2033.
20. **Murray, C. J. L., K. Styblo, and A. Rouillon.** 1990. Tuberculosis in developing countries: burden, intervention and cost. Bull. Int. Union Tuberc. Lung Dis. **65:**6–24.
21. **Otal, I., C. Martín, V. Vincent-Lévy-Frebault, D. Thierry, and B. Gicquel.** 1991. Restriction fragment length polymorphism analysis using IS*6110* as an epidemiological marker in tuberculosis. J. Clin. Microbiol. **29:**1252–1254.
22. **Raviglione, M. C., P. Sudre, H. L. Rieder, S. Spinaci, and A. Kochi.** 1993. Secular trends of tuberculosis in Western Europe. Bull. W. H. O. **71:**297–306.
23. **Ross, B. C., K. Raios, K. Jackson, and B. Dwyer.** 1992. Molecular cloning of a highly repeated DNA element from *Mycobacterium tuberculosis* and its use as an epidemiological tool. J. Clin. Microbiol. **30:**942–946.
24. **Small, P. M., P. C. Hopewell, S. P. Singh, A. Paz, J. Parsonnet, D. C. Ruston, G. F. Schecter, C. L. Daley, and G. K. Schoolnik.** 1994. The epidemiology of tuberculosis in San Francisco, a population-based study using conventional and molecular methods. N. Engl. J. Med. **330:**1703–1709.
25. **Small, P. M., N. B. McClenny, S. P. Singh, G. K. Schoolnik, L. S. Tomkins, and P. A. Mickelsen.** 1993. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. J. Clin. Microbiol. **31:**1677–1682.
26. **Snider, D. E.** 1993. Tuberculosis: the world situation. History of the disease and efforts to combat it, p. 13–31. *In* J. D. H. Porter and K. P. W. J. McAdam (ed.), Tuberculosis: back to the future. London School of Hygiene and Tropical Medicine Third Annual Public Health Forum. John Wiley and Sons Ltd., Chichester, England.
27. **Sudre, P., H. G. ten Dam, and A. Kochi.** 1992. Tuberculosis: a global overview of the situation today. Bull. W. H. O. **70:**149–159.
28. **Thierry, D., M. D. Cave, K. D. Eisenach, J. T. Crawford, J. H. Bates, B. Gicquel, and J. L. Guesdon.** 1990. IS*6110*, an IS-like element of *M. tuberculosis* complex. Nucleic Acids Res. **18:**188.
29. **Thierry, D., O. Matsiota-Bernard, E. Pitsouni, C. Costopoulos, and J.-L. Guesdon.** 1993. Use of the insertion element IS*6110* for DNA fingerprinting of *Mycobacterium tuberculosis* isolates presenting various profiles of drug susceptibility. FEMS Immunol. Med. Microbiol. **6:**287–298.
30. **van Embden, J. D. A., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, T. M. Shinnick, and P. M. Small.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
31. **van Embden, J. D. A., D. van Soolingen, P. M. Small, and P. W. M. Hermans.** 1992 Genetic markers for the epidemiology of tuberculosis. Res. Microbiol. **143:**385–391.
32. **van Soolingen, D., P. E. W. de Haas, P. W. M. Hermans, P. M. A. Groenen, and J. D. A. van Embden.** 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **31:**1987–1995.
33. **van Soolingen, D., P. W. M. Hermans, P. E. W. de Haas, D. R. Soll, and J. D. A. van Embden.** 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J. Clin. Microbiol. **29:**2578–2586.
34. **van Soolingen, D., P. W. M. Hermans, P. E. W. de Haas, and J. D. A. van Embden.** 1992. Insertion element IS*1081*-associated restriction fragment length polymorphism in *Mycobacterium tuberculosis* complex species: a reliable tool for recognizing *Mycobacterium bovis* BCG. J. Clin. Microbiol. **30:**1772–1777.

34a. **Yang, Z. H., et al.** Unpublished data.

35. **Zainuddin, Z. F., and J. W. Dale.** 1989. Polymorphic repetitive DNA sequences in *Mycobacterium tuberculosis* detected with a gene probe from a *Mycobacterium fortuitum* plasmid. J. Gen. Microbiol. **135:**2347–2355.