Abstract  
[Background and objective] The incidence and annual risk of infection of tuberculosis (TB) have decreased rapidly in Japan because of the development of anti-TB medicines and nutritional and hygienic improvements after World War II. The incidence of tuberculosis is currently high among elderly people, reflecting the fact that the prevalence rate of TB infection had been extremely high during their youth. This would suggest that most current cases of TB in the elderly are reactivation of infections acquired in their youth. TB reactivation in various organs have both common and unique aspects. We evaluated the frequency of endogenous reactivation of TB in various organs by examining the TB incidence rate over a 30-year period (1975–2005) in Japan. [Methods] The incidence rate of TB in each organ was obtained for each 10-year birth cohort, using reports of newly registered TB patients in Japan in 1975, 1985, 1995, and 2005. Specifically, the incidence rates of pulmonary TB, lymph node TB, bone-joint TB, kidney TB, and meninges TB were analyzed. [Results] Chronological changes in TB incidence rates in each organ were characterized by a rapidly declining phase followed by a stationary phase in every organ except pulmonary TB. Incidence rates among the already infected population in the stationary phase were 3.0 (lymph node TB), 1.2 (bone-joint TB), 0.5 (kidney TB), and 0.3 (meninges TB) per 100,000 cases, respectively. [Conclusions] Once infected with TB, the incidence rate of TB in these organs does not decrease below the above-mentioned values.

Key words: Tuberculosis, Incidence rate, Reactivation, Extra-pulmonary TB

Introduction

The interval from infection with mycobacteria to the onset of tuberculosis (TB) has been studied before the advent and spread of antibiotic therapy. For example, a study on the trends in the incidence rate of TB after infection was conducted by Chiba\(^1\) by following 1,192 railway workers (15- to 29-year-old males) for 30 years who had had a positive conversion in the tuberculin reaction. The incidence rate was reported to be 16% within 1 year, 1% after 10 years, 0.3% after 20 years, and 0.1% after 30 years.\(^2\) Taken together with the reports by Ferebee\(^3\) and Hart and Sutherland\(^4\) who studied chronological changes in the incidence rate of the disease among contacts of TB patients, a pattern was observed in which occurrences were concentrated in the first 1–2 years after positive conversion and became sporadic over the longer term as illustrated in Fig. 1A where the logarithm of the incidence rate was plotted on the y-axis.\(^5\)\(^–\)\(^8\) However, long-term trends in the incidence rate were unclear due to the limited numbers of cases. For example, the incidence rate after 30 years was 0.1% in Chiba’s report\(^1^;\) this would indicate that there was only one incident, considering that there were originally 1,192 cases. It was unclear whether this particular case was actually reactivation from infection 30 years prior or re-infection. Despite these shortcomings, the incidence rate decreased almost linearly on the logarithmic graph (i.e., exponentially) for 10 years or longer after infection. The decline rate is approximately \(-0.11\)/year (Fig. 1A) on the logarithmic scale.

Different from pulmonary TB, chronological changes in the incidence rate of extra-pulmonary TB after the initial infection have rarely been reported. Wallgren\(^9\) reported that the incidence of bone-joint TB was high in the first one year and then decreased gradually, and was very low after 4 years. His data, illustrated on a logarithmic scale in Fig. 1B, demonstrated a linear decrease (slope \(-0.38\)/year) in the incidence of bone-joint TB.

What would be the ultimate incidence rate more than 10 years after the initial infection, which was not described in the above reports? Will the incidence rate continue to decrease exponentially with time, or will other features appear? It is

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DOCTOR’S DELAY IN ENDOBRONCHIAL TUBERCULOSIS

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Abstract  [Objective] The aim of this study was to investigate the current status of doctor’s delay in diagnosing endobronchial tuberculosis (EBTB) and to elucidate the risk factors contributing to the delay. [Methods] Retrospective clinicopathological analysis. [Patients] Sixty-two patients with EBTB were admitted at our hospital between 1999 and 2010. Their backgrounds, symptoms, diagnoses at initial consultation, delay in diagnosis, and clinical examination results were analyzed. [Results] Of the 62 patients, 59 had acid-fast, bacilli-positive sputum smear test results at admission. Among the 40 patients with total diagnostic delay of more than 2 months, only 11 experienced long patient’s delay exceeding 2 months. However, 22 patients experienced long doctor's delay of more than 2 months (28% vs. 55%, respectively, p<0.05), suggesting that doctor's delay contributes more to total delay than patient's delay. Fever was less frequent in patients with long doctor's delays than in those without (0% vs. 18%, respectively), at the initial consultation. In addition, radiographs showed that patients with long doctor's delays more frequently presented with shadows in the lower lung field (50% vs. 23%, p<0.05), and most of these patients had noncavitary shadows on admission. All 7 patients diagnosed with bronchial asthma at the initial consultation had long doctor's delays. [Conclusion] These findings demonstrate that long doctor's delays in diagnosing EBTB remain an issue. The clinical features of EBTB with long doctor's delays were confirmed to be quite different from those of pulmonary tuberculosis.

Key words: Endobronchial tuberculosis, Doctor's delay, Radiographic findings, Bronchial asthma

Introduction

Tuberculosis is one of the most prevalent infectious diseases in Japan. In 2010, there were 23,261 newly diagnosed tuberculosis patients and the incidence of tuberculosis was 18.2 per 100,000 population, which makes Japan a middle-incidence country for tuberculosis14). However, amid the trend towards a long term, slow decline in the incidence rate of tuberculosis, the total diagnostic delay of tuberculosis remains an ongoing problem in clinical practice21). The total delay in diagnosis is called total delay, which is a combination of the patient’s and doctor's delays. Patient's delay is defined as the interval between the onset of symptoms and the initial consultation, whereas doctor's delay is defined as the interval between the initial consultation and diagnosis of tuberculosis15). The length of doctor's delay in pulmonary tuberculosis (PTB) diagnosis has been decreasing because of recent advances in examination methods22) such as polymerase chain reactions4) and Quanti FERON-TB5).

Unfortunately, total delay, especially doctor’s delay, is known to be high in the diagnosis of endobronchial tuberculosis (EBTB) associated with PTB39)–40). This is because EBTB is difficult to distinguish from common diseases such as cold, bronchitis, or bronchial asthma on the basis of symptoms, considering the following features39)–42): (i) EBTB is common in women and young individuals; (ii) it is frequently associated with severe cough as the chief complaint and is often accompanied by wheezing; and (iii) on radiography, the X-ray shadows often appear as minimal shadows without cavities as a result of comorbid PTB. EBTB patients have a high rate of sputum smear positivity for acid-fast bacilli (AFB)39)–42), and the risk of contact infection is high. Therefore, decreasing the length of doctor’s delay is essential, not only for the doctors themselves, but also for maintaining public health, in general.

The objective of this study was to investigate the current status of doctor's delay in EBTB. We also aimed to elucidate the risk factors that can prolong doctor's delay in EBTB patients.

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GENETIC RESEARCH ABOUT
MYCOBACTERIUM AVIUM COMPLEX

Kenji OGAWA

Abstract  We conducted four genetic studies on the Mycobacterium avium complex (MAC). (1) M. avium genotyping: A total of 70 clinical isolates from patients with pulmonary MAC infections were typed by MATR-VNTR, IS1245-RFLP, and MIRU-VNTR analyses to compare discriminatory powers of these typing methods. To allow a comparison of discriminatory powers, the Hunter-Gaston discriminatory index (HGDJ) was calculated, giving a HGDJ of 0.960 for IS1245-RFLP, 0.949 for MIRU-VNTR, and 0.990 for MATR-VNTR, demonstrating that MATR-VNTR analysis is the best of the three genotyping methods. (2) Genetic characteristics of M. avium: Japanese clinical isolates of M. avium were subjected to insertion sequence (IS) analyses. First, an analysis of 81 isolates by heat shock protein 65 identified all isolates as belonging to the subspecies of M. avium subsp. hominis suis. Another analysis by IS901 identified about 70% of the isolates as IS901-carriers. IS901 had been thought to be carried by the subspecies that infect birds: M. avium subsp. avium and M. avium subsp. silvaticum. Studies have reported that most human isolates in the U.S. and Europe carry no IS901. The prevalence of IS901-carriers among Japanese clinical isolates of M. avium is thus a significant characteristic. A further analysis of the IS901 showed that compared with M. avium subsp. avium, the clinical isolates shared 60 point mutations of nucleotide sequence. This novel insertion sequence was designated “ISMav6”. (3) The CAM resistance gene in MAC: This study assessed the correlation between CAM-susceptibility and mutation of the gene involved in drug resistance (A DNA sequence analysis identified mutations at positions 2058 and 2059 in domain V of 23S rRNA). Furthermore, a system was developed to rapidly detect the presence/absence of CAM resistance by ARMS-PCR, a procedure used to detect gene mutations. The utility of this new system was also evaluated. A total of 253 clinical isolates were tested for drug susceptibility, with 227 isolates identified as sensitive and 26 as resistant. Sequence analyses showed that all 28 strains randomly selected for testing from the sensitive strains were wild type, whereas 24 of the 26 resistant strains were mutant type. The rest of the 2 strains were subsequently confirmed to be mutant type after they were isolated from contaminations with sensitive strains. These results showed an association between drug susceptibility and drug-resistant gene mutation. In addition, ARMS-PCR provided a sensitivity of 84.6% (22/26) and a specificity of 100% (28/28) for the detection of gene mutations. The lower sensitive was probably attributable to the fact that one of the 4 strains was a combination of wild type and mutant type. These results indicated that compared with drug-susceptibility tests, ARMS-PCR provides earlier results on the presence/absence of drug resistance and has the capability of rapid detection even when the specimen contains a mixture of sensitive and resistant strains. (4) Development of a VNTR analysis for M. intracellulare: Bioinformatics analyses were used to develop a VNTR analysis for M. intracellulare and to evaluate the utility of the VNTR analysis. First, the Tandem Repeat Finder (TRF) software was used to conduct a search of TR loci on the genomic data of M. intracellulare ATCC 13950 published in December 2007, resulting in the identification of 16 TR loci, which were used in VNTR analyses of 74 isolates from pulmonary MAC infections. The HGDJ was 0.988, suggesting an excellent discriminatory power. Furthermore, a stability evaluation of the VNTR loci was conducted in isolates from patients with long-term bacilli discharge. The VNTR loci were stable without changes for up to 4 years in 14 such patients. These results indicated that this method is useful in M. intracellulare genotyping and in determining whether the cause of recurrence in recurrent patients is endogenous from the remnant bacilli or exogenous from another infection of different bacilli, given that the VNTR loci have been confirmed to be stable.
Key words: MAC, VNTR, HGDI, IS901, ISMav6, Clarithromycin-resistant gene, ARMS-PCR method, MLVA method

Introduction

Let me begin with the reasons I started genetic research on the *Mycobacterium avium* complex (MAC). The number of pulmonary MAC patients started to increase from around 1992. Some of the cases progressed to respiratory failure, leading to death while the patients were still in their 50s. Although pulmonary MAC was regarded as sequelae of tuberculosis at the time, most of the patients were middle and old age women who had no apparent pre-existing disease. The disease stood out in my mind as an intractable infection with no decisive therapeutic agents available. I decided to study treatment protocols with what were available then. My study on the clinical efficacy of multidrug therapies was published in this journal in 2005. In the mean time, I felt that there was a limit to what the present-state treatments can achieve. At that time, other research groups were conducting studies on host factors and human genetics, but none of the studies provided clinically applicable results. Although no precise epidemiological data are available, the number of pulmonary MAC cases in Japan seems to have increased drastically over the last 20 years, giving rise to the speculation that there may be some human infection-related mycobacterial factors present in the MAC that inhabits Japan. Thereupon I began to study the correlation between mycobacterial genotypes and clinical condition by applying variable numbers of tandem repeats (VNTR) typing, which had been used for mycobacterial identification in tuberculosis, on clinical isolates of *M. avium* and comparing VNTR typing with the then standard protocol, insertion sequence 1245-restriction fragment length polymorphism (IS1245-RFLP) typing.

*Mycobacterium avium* Gene Typing Methods

The IS1245-RFLP typing reported by Guerrero et al. in 1995 has been used as the standard protocol for *Mycobacterium avium* typing. However, some strains have no IS1245 sequence, and this method is unstable as the reproducibility or even the resolution declines as the number of copies increases, as with *M. tuberculosis*. It is therefore unsuitable for multi-center studies, which require comparative analyses of data.

Meanwhile, in Japan, Nishimori et al. in 2003 published a VNTR typing method based on the *Mycobacterium avium* tandem repeat (MATR) loci, which has been used in veterinary medicine. Moriyama et al. and Kasumi et al. applied MATR on human clinical isolates and achieved excellent results. In 2007, Thibault et al. published the *Mycobacterium* interspersed repetitive units (MIRU)-VNTR typing method and reported its utility. Our group compared 3 methods of typing to investigate which is useful in future studies. VNTRs are gene loci that consist of repeat units of 10- to 100-bp minisatellite nucleotide sequence interspersed on chromosomes. The advantage of this method lies in the facts that it uses mainly the simple and rapid PCR technique; that it facilitates the analyses of slow-growth mycobacterium as only a small quantity of culture is needed; and that it provides digital data that make it easy to compare data among study sites. Furthermore, compared with MIRU-VNTR, for which 8 VNTR loci have been identified by an exhaustive search with the Tandem Repeat Finder Program, MATR-VNTR has 16 loci identified by a homology search of tandem repeats that have been observed in *M. tuberculosis*. Although the principle of VNTR typing remains the same, these methods differ substantially in the search methods for VNTR loci. A study was conducted with 70 clinical isolates of *M. avium* to evaluate 4 patterns of analysis: these 3 methods and a combination of IS1245-RFLP plus MATR-VNTR. The Hunter-Gaston discriminatory index (HGDI) was used to compare discriminatory powers. The results shown in Table 1 indicated that MRTR-VNTR (HGDI = 0.990) was the best of the 3 typing methods. The results further showed that its discriminatory power was maximized further when paired with IS1245-RFLP, giving an HGDI of 0.999. The utility of MRTR-VNTR alone is sufficient for the present needs.

**Present Status of the Clinical Application of MATR-VNTR Typing**

Pulmonary *M. avium* infections are air-borne infections, while disseminated *M. avium* infections associated with HIV are primarily enteric infections. In porcines, infections are transmitted by the enteric route, just as in HIV patients. VNTR

<table>
<thead>
<tr>
<th>Typing method</th>
<th>IS1245-RFLP</th>
<th>MATR-VNTR</th>
<th>MIRU-VNTR</th>
<th>IS1245-RFLP plus MATR-VNTR</th>
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<tbody>
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<td>56</td>
<td>27</td>
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<td>22</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>No. of unique isolates</td>
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<td>48</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>HGDI</td>
<td>0.960</td>
<td>0.990</td>
<td>0.949</td>
<td>0.999</td>
</tr>
</tbody>
</table>

HGDI: Hunter-Gaston discriminatory index
DISEASE PROGRESSION OF MYCOBACTERIUM AVIUM PULMONARY INFECTION AND THE MYCOBACTERIAL VARIABLE NUMBER TANDEM REPEAT (VNTR) TYPING

Toshiaki KIKUCHI

Abstract: Nontuberculous mycobacteriosis may progress to fatal chronic respiratory infections. Some cases remain stable over a relatively long period of time. With no well established progression predictors yet available, we conducted a retrospective analysis of the association between mycobacterial variable numbers of tandem repeat (VNTR) and clinical progression in 37 patients who were seen at the Department of Respiratory Medicine, Tohoku University Hospital between 2005 and 2006 and from whose respiratory tract specimens M. avium was isolated and cultured. The disease type in the 15 patients who began an antimicrobial therapy within 1 year after a bacteriological diagnosis was defined as progressive, and that in the 9 patients who began an antimicrobial therapy 2 years or longer after diagnosis was defined as stable. A cluster analysis of the mycobacterial VNTR genotypes showed concentrations of the progressive-type isolates and the stable-type isolates in different clusters. Furthermore, the study demonstrated that multiple logistic regression analysis can be used to construct a model for estimating, with statistical significance, progression of nontuberculous mycobacteriosis based on the mycobacterial VNTR genotype. These results indicated that whether a nontuberculous mycobacteriosis is progressive can be estimated by the VNTR genotyping of the nontuberculous mycobacterium.

Key words: Nontuberculous mycobacteriosis, Treatment standard, Minisatellite repeat, Computational biology, Cluster analysis

Introduction

Nontuberculous mycobacterioses (NTM) are granulomatous infections caused by Mycobacteria other than the tuberculosis complex and Mycobacterium leprae. With no established chemotherapies having reliable curative potential, NTM may progress to fatal chronic respiratory infections. The disease progression of NTM, however, is variable. Some cases progress relatively rapidly, and yet some cases remain stable for decades. The reasons for the variable disease progression are unknown, and no NTM progression predictors have been established. Treatment guidelines for NTM, therefore, suggest that making the diagnosis does not, per se, necessitate the initiation of therapy, and that it is difficult to distinguish between patients who require immediate therapy and those in whom such a decision can be withheld.

Given the above context, this study aims to estimate the probability of disease progression of NTM and to provide a guideline on treatment suitability by a retrospective analysis of the association between mycobacterial genotypes and the clinical progression in NTM patients with Mycobacterium avium, the most common pathogen associated with NTM.

Patient Population and Methods

The patient population comprised all 37 patients from whose specimens M. avium was cultured by the Department of Respiratory Medicine, Tohoku University Hospital between January 2005 and December 2006. Of these cases, 26 were pulmonary NTM diagnosed on clinical progression and chest imaging, and the other 11 were believed to be transient infections that were non-pulmonary NTM (Table). Of the 26 patients with pulmonary NTM, one died of complication of lung cancer and could not be followed up; another one had hypersensitivity pneumonitis; 15 began an antimicrobial therapy within 10 months of M. avium detection, and whose disease type was defined as progressive; and 9 began an antimicrobial therapy 2 years or longer after M. avium detection, and whose disease type was defined as stable.

The genotype of M. avium was determined by the variable numbers of tandem repeats (VNTR) genotyping method (Fig. 1) based on the number of repeat units in the tandemly repetitive sequence in 16 loci selected from the minisatellite regions

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