Original Article

ANALYSIS OF USEFULNESS OF A WHOLE BLOOD INTERFERON GAMMA ASSAY (QuantiFERON®TB-2G) FOR DIAGNOSING ACTIVE TUBERCULOSIS IN IMMUNOCOMPROMISED PATIENTS

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Abstract [Purpose] Recently, there have been many reports that QuantiFERON® TB-2G (QFT-2G) is useful for diagnosing active tuberculosis. However, it remains controversial whether QFT-2G is useful for diagnosing active tuberculosis in immunocompromised patients as well as immunocompetent ones. Therefore, we analyzed whether QFT-2G sensitivity is decreased in immunocompromised patients compared with that in immunocompetent patients and what factors affect the QFT-2G sensitivity.

[Subjects and methods] The subjects consisted of 159 patients (105 males, 54 females; age 64.0 years [14−91]) who were diagnosed with active tuberculosis and underwent the QFT-2G test in Nishi Kobe Medical Center between July, 2006 and December, 2008. We analyzed these patients with regard to age, sex, white blood cell count in peripheral blood (WBC), lymphocyte count in peripheral blood (Lym), serum total protein, serum albumin, and QFT-2G sensitivity, and compared the findings between immunocompetent and immunocompromised patients. Immunocompromised patients consisted of those with diabetes mellitus, malignant disease, chronic renal failure, systemic steroid administration and AIDS. To test significance of differences, we used Mann-Whitney test for categorical variables, and t test for continuous variables.

[Results] One hundred fifty one patients had pulmonary tuberculosis (including 8 with bronchial tuberculosis), 11 tuberculous pleurisy, 2 miliary tuberculosis, 2 intestinal tuberculosis, 1 tuberculous lymphadenitis, 1 tuberculosis of the hip joint, and 1 tuberculosis of the vertebra (there was some overlap among cases). In the entire patient group, positive QFT-2G results were detected in 125 (78.6%). In the immunocompetent and immunocompromised patients, positive results were seen in 82 (78.8%) and 43 (78.2%), respectively; these proportions were not significantly different ($p=1.00$). In all patients, Lym was significantly lower in patients with intermediate, negative or indeterminate QFT-2G results than in QFT-2G-positive patients ($p<0.001$).

[Conclusion] In our analysis, QFT-2G sensitivity did not significantly differ between immunocompetent and immunocompromised patients. Therefore, it is considered that QFT-2G is useful for diagnosing active tuberculosis in immunocompromised as well as immunocompetent patients.

Key words: QuantiFERON® TB-2G, Active tuberculosis, Immunocompromised patients, Peripheral blood lymphocyte

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Original Article

LIVER DYSFUNCTION DURING TREATMENT OF LATENT TUBERCULOSIS INFECTION

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Abstract [Purpose] The indications for treatment for latent tuberculosis infection were revised in 2007 to reflect that any subject with a higher risk of tuberculosis regardless of age should be treated. We worried about the incidence of liver dysfunction due to isoniazid (INH) in patients older than 30 yrs. of age. We evaluated the frequency of liver dysfunction due to INH according to age and discussed the possibility of its prevention.

[Methods] We reviewed the clinical records of 99 patients younger than 29 yrs. and 229 patients older than 30 yrs. who were treated for latent tuberculosis infection from August 2007 to December 2008 at our clinic. The liver function tests (AST and ALT) were performed before the treatment, one and a half months after the start of the treatment, and almost every month during the treatment. We defined liver dysfunction as an AST and/or ALT greater than 100 IU/L.

[Results] Seven out of the 99 younger patients (7.1%) and 42 out of the 229 (18.3%) older patients developed liver dysfunction. The difference between the two age groups was statistically significant according to the chi-square test ($p < 0.01$). After the occurrence of liver dysfunction, 35 out of 49 patients (71%) completed the treatment by maintaining the same or a decreased dose of INH, while the medication was discontinued in 9 patients who were then followed up by chest X-ray examination. Two of these 49 patients discontinued the medication by themselves.

[Conclusions] The frequency of liver damage due to INH was higher in persons older than 30 yrs. In this group, 3 persons developed severe liver damage with ALT and/or AST higher than 1,000 IU/L. Early detection is required to avoid serious damage. Thus, we decided to perform liver function tests more often, i.e., at 2 weeks after the onset of treatment and every month thereafter.

Key words: Latent tuberculosis infection, INH, Liver dysfunction

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A CASE HAVING CHYLIFORM PLEURAL EFFUSION CAUSED BY FORMER TUBERCULOUS PLEURISY

Kazumi NISHIO, Kana HARADA, Yasushi NAKANO, Shinji AIDA, and Ken OKABAYASHI

Abstract A 49-year-old male who had been treated for pulmonary tuberculosis and tuberculous pleurisy in 2007 was referred to our hospital with the complaint of dyspnea on exertion in Nov. 2009. Chest X-ray showed increased pleural effusion compared with that remaining after the previous treatment of pleurisy in 2008. A chest CT revealed that fluid collection was surrounded by thickened pleura. Thoracocentesis was performed, and yellow milky liquid was obtained. The pleural effusion contained few cells. The triglyceride concentration was 83 mg/d*l*, and the cholesterol level was very high at 628 mg/d*l*. Based on these findings we diagnosed this case as chyliform pleural effusion. Both smear of acid-fast bacilli and PCR-TB test of the pleural effusion were positive, but culture was negative for mycobacterium, suggesting that this chyliform pleural effusion was produced by the former episode of tuberculous pleurisy, not by the recent reactivation of tuberculous pleurisy. The ADA concentration in the pleural effusion was high at 91.7 IU/*l*. No increase in the amount of pleural effusion was observed after thoracocentesis without any anti-tuberculosis therapy.

Key words: Chyliform pleural effusion, Pseudochylothorax, Cholesterol, Tuberculous pleurisy, ADA

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GENETIC RESEARCH ABOUT *MYCOBACTERIUM AVIUM* COMPLEX

Kenji OGAWA

Abstract A variable-number tandem-repeat (VNTR) typing method using the *Mycobacterium avium* tandem repeat (MATR) loci (MATR-VNTR) is employed in Japan for epidemiological studies using clinical isolates of *M. avium*. In this study, the usefulness of this MATR-VNTR typing method was compared with that of the IS*1245*-restriction fragment length polymorphism (IS*1245*-RFLP) typing method and a mycobacterial interspersed repetitive-unit (MIRU)-VNTR typing method reported previously (Boschiroli, C. Hubbans, P. Overduin, K. Stevenson, M.C. Gutierrez, P. Supply, and F. Biet, Clin Microbiol.) Seventy clinical isolates identified as *M. avium* from human immunodeficiency virus-negative patients with MAC infections were used. MATR-VNTR typing using 15 loci distinguished 56 patterns of different allele profiles, yielding a Hunter-Gaston discriminatory index (HGDI) of 0.990. However, IS*1245*-RFLP and MIRU-VNTR typing yielded HGDIs of 0.960 and 0.949, respectively, indicating that MATR-VNTR has an excellent discriminatory power compared with MIRU-VNTR and IS*1245*-RFLP typing. Moreover, concomitant use of the MATR-VNTR method and IS*1245*-RFLP typing increased the HGDI to 0.999. MATR-VNTR typing is inexpensive and easy to perform and could thus be useful in establishing a digital multifacility database that will greatly contribute to the clarification of the transmission route and pathophysiology of *M. avium* infections.

Mycobacterium avium (n＝81) from patients with pulmonary infections who were HIV-negative and isolates (n＝33) from HIV-positive patients were subjected to genetic analysis by PCR detection of three *M. avium*-specific insertion sequences (IS*901*, IS*1245* and IS*1311*) and nucleotide sequencing of the heat-shock protein 65 (*hsp65*) gene. All clinical isolates were identified as '*M. avium* subspecies *hominissuis*' by sequence analysis of *hsp65*. Compared with clinical isolates of *M. avium* reported elsewhere, IS*1245* was found less frequently in Japanese isolates (96/114 isolates, 84％) and IS*901* was detected more frequently (76/114 isolates, 67％). One isolate was found to lack IS*1311*, which has not been reported previously for '*M. avium* subsp. *hominissuis*.' Nucleotide sequence analysis of the PCR products for IS*901* revealed that all clinical isolates had the same new insertion sequence, designated IS*Mav6*, which had 60 point mutation compared with the nucleotide sequence of the original IS*901*. These results suggest that '*M. avium* subsp. *hominissuis*' with IS*Mav6* is prevalent in Japan. IS*Mav6* may have implications for the virulence of *M. avium* and contribute to an increase of *M. avium* infections in this country.

Clarithromycin (CAM) is the key drug in the various treatment regimens of *Mycobacterium avium* complex (MAC) diseases and the only drug for which drug susceptibility has been shown to correlate with a clinical response in these diseases. A point mutation at position 2058 or 2059 of the 23S rRNA gene has been reported to occur in high-level CAM-resistant isolates. This study examined a correlation between the results from a drug susceptibility test and the mutation of genes involved in drug resistance in MAC. Furthermore, we adapted a rapid detection method using amplification refractory mutation system (ARMS)-PCR to identify a mutation in 23S rRNA gene in MAC isolates. Using MICs of CAM, drug susceptibility was tested for 253 clinically-isolated MAC strains. Of these, 28 CAM-sensitive strains and 26 CAM-resistant strains were analysed by sequence analysis and ARMS-PCR. The drug susceptibility test showed that a bimodal distribution was associated with 227 CAM-sensitive strains and 26 CAM-resistant strains. Sequence analysis revealed that all 28 CAM-sensitive strains tested were wild type, whereas 24 of the 26 CAM-resistant strains were mutant type. The sensitivity of the sequence and ARMS-PCR analyses were 92.3％ and 84.6％, respectively, and specificity of both was 100％. We found a correlation between drug susceptibility testing and the mutation of drug-resistant genes in this study. ARMS-PCR allowed rapid determination of CAM resistance, even when samples consisted of the mixed type of CAM-sensitive and CAM-resistant strains.

MAC is divided into *Mycobacterium avium*, and the less-epidemiologically studied *Mycobacterium intracellulare*. Genetic typing for *M. intracellulare* using variable number of tandem repeats (VNTR) has not yet been developed. The aim of this study was to identify VNTR loci in the genome of *M. intracellulare* and apply them as an epidemiological tool to clinical isolates. Here, we identified 25 VNTR loci on the *M. intracellulare* genome, which 16 showed variations among clinical isolates in the number of tandem repeat motifs. Among the 74 *M. intracellulare* isolates, 49 genotypes were distinguished using the 16 VNTR loci, resulting in a Hunter-Gaston discriminatory index of 0.988. Moreover, all 16 VNTR loci were stable in different sets of isolates recovered within time

intervals ranging from 2 to 1551 days from 14 separate patients. These results indicate that for use as epidemiological markers of *M. intracellulare*, the loci in this VNTR assay highly discriminating and stable over time.

Key words: MAC, VNTR, HGDI, IS*901*, IS*Mav6*, Clarithromycin resistance gene, ARMS-PCR, MLVA

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MANAGEMENT OF ADVERSE EFFECTS WITH ANTITUBERCULOSIS CHEMOTHERAPY

Chairpersons: [1]Kazunari TSUYUGUCHI and [2]Masako WADA

Abstract Tuberculosis has now become a curable disease with chemotherapy. So it is natural that the present issues in tuberculosis management are focused on how to complete standard chemotherapy. In this context, management of adverse effects constitutes an essential part of antituberculosis chemotherapy, as well as directly observed therapy. In this symposium, discussions were held about three major subjects on this issue.

First, hepatotoxicity develops frequently and has sometimes fatal outcome, which makes it the most problematic adverse effect. "Management of hepatotoxicity during antituberculosis chemotherapy" was published by the Japanese Society for Tuberculosis (JST) in 2006. Dr. Shinsho Yoshiba evaluated this recommendation and pointed out that the criteria for discontinuation of drug based on AST, ALT and bilirubin levels is too sensitive and the concept of predicting fulminant hepatic failure (FHF) is lacking. He stressed the importance of monitoring serum prothrombin time for predicting FHF.

Next, allergic drug reaction such as fever or skin rash often causes distress, although rarely fatal. As isoniazid (INH) and rifampicin (RFP) are key drugs for the cure, readministration of these drugs is often attempted by desensitization therapy. "Recommendation about desensitization therapy of antituberculosis drugs" was also published by JST in 1997. Dr. Yoshihiro Kobashi reported high success rates of 79 percent for INH and 75 percent for RFP according to this

recommendation. He also reported correlated factor with the success, such as the longer period from the discontinuation to the desensitization therapy and lower doses of drugs at starting desensitization.

Finally, we sometimes experience transient worsening of radiographical findings and general symptoms during antituberculosis chemotherapy. This is presumed to be due to allergic reaction to dead bacilli without requiring discontiunation of the drug. Differential diagnosis includes drug-induced pneumonia requring discontinuation and true worsening of pulmonary tuberculosis due to drug resistance requiring change in therapy. Dr. Masanori Akira reported that presence of ground-glass attenuation and/or consolidation by HRCT suggests transient worsening or drug-induced pneumonia, whereas presence of centrilobular nodules and/or tree-in bud suggests true worsening.

We believe that these findings from the symposium will add useful information for management of adverse effects and be helpful for implementation of antituberculosis chemotherapy.

1. Hepatotoxicity of antituberculosis drugs: Shinsho YOSHIBA (Sempo Tokyo Takanawa Hospital)

Antituberculosis drugs are sometimes hepatotoxic. Doctors who are responsible for the treatment of patients with tuberculosis should always be aware of their hepatotoxicity, because it seldom leads to fulminant hepatic failure.

The Japanese Society for Tuberculosis proposed criteria based on the levels of AST, ALT and bilirubin for the prevention of such grave hepatic injury in 2006. In recent years attempts have been made to predict fulminant hepatic failure (FHF) before patients develop coma. Yoshiba's formula using prothrombin time, etiology, cholinesterase and bilirubin is widely accepted as useful to predict FHF. Introduction of the formula to this area is recommended.

2. Desensitization therapy for allergic reactions of antituberculous drugs: Yoshihiro KOBASHI, Mikio OKA (Division of Respiratory Diseases, Department of Medicine, Kawasaki Medical School)

We evaluated the usefulness of desensitization therapy for patients showing allergic ractions of INH and RFP according to the guideline proposed by the Japanese Society for Tuberculosis.

Adverse reactions were 22 patients with drug eruption, 22 with drug fever and 6 with drug fever plus eruption. The clinical effect of desensitization therapy was good in 27 out of 36 patients for RFP (75%), and in 19 out of 24 patients for INH (79%). The comparative study between patient group with success desensitization therapy and that with failure desensitization therapy was not a significant difference except for initiation period of desensitization therapy.

3. The imaging features of early transient radiographic progression, true worsening of TB, and drug induced pneumonitis during TB treatment: Masanori AKIRA (Department of Radiology, NHO Kinki-chuo Chest Medical Center)

HRCT findings of the new lesions in the early transient radiographic progression are enlargement or confluence of the original lesions, development of areas of ground-glass attenuation and/or consolidation ipsilateral to the original lesion, and development of areas of ground-glass attenuation and/or consolidation in the subpleural region contralateral to the lesion. These CT findings may suggest a local hypersensitivity reaction to drug or massive dead tubercle bacilli per se. In contrast, CT findings of patients with multiple drug-resistant tuberculosis and true progression are centrilobular nodules, tree-in-bud appearance, nodules, and cavitation. These CT findings may suggest a bronchogenic spread from the original tuberculous lesions.

Key words: Anti-tuberculosis drugs, Adverse effects, Drug-induced hepatotoxicity, Desensitization therapy, Transient worsening on chest film

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The 85th Annual Meeting Mini-Symposium

THE CLINICAL APPLICATION OF QuantiFERON TB-2G: ITS USEFULNESS AND LIMITATIONS

Chairpersons: [1]Shigeki SATO and [2]Hideaki NAGAI

Abstract QuantiFERON TB-2G (QFT) is widely used in clinical settings for the identification of tuberculosis infection because of its high level of utility. It is well known that QFT stimulates peripheral blood lymphocytes *in vitro* by means of *M. tuberculosis*-specific protein, and that infection is identified by measuring the interferon-γ released. Interpretation of QFT results is therefore difficult in immunosuppressed subjects in whom the function of immunocompetent cells, including lymphocytes, is suppressed, making it difficult for them to produce interferon-γ.

There is a high incidence of tuberculosis among hemodialysis patients. It has been conjectured that the use of powerful immunosuppressive agents following kidney transplantation results in a high risk of tuberculosis. How QFT results change immediately following kidney transplantation is an extremely interesting question. In recent years, an increasing number of institutions have been using TNF-α inhibitors to treat rheumatoid arthritis patients. Is QTF useful for identifying whether patients have latent tuberculosis infection before the administration of anti-TNF antibodies? In particular, many rheumatoid arthritis patients may have been given methotrexate or glucocorticoids, which suppress the immune system, prior to the administration of TNF-α inhibitors, possibly making it difficult to interpret the QFT results. We must be aware of this limitation when performing QFT on immunosuppressed patients. It is also important that we understand the clinical parameters influencing QFT results (such as lymphocyte counts). The morbidity rate of tuberculosis is high among healthcare workers, particularly nurses. A number of studies have reported that QFT is useful in hospital infection control for tuberculosis, but the effectiveness of QFT for monitoring the health of healthcare workers is still not fully understood.

In this symposium, we will debate how far QFT can be used and the extent of its usefulness under exceptional circumstances.

1. How do we manage kidney transplant recipients with latent tuberculosis infection?: Norihiko GOTO (Transplant Surgery, Nagoya Daini Red Cross Hospital)

It is unclear whether QuantiFERON®-second generation (QFT-2G) is useful for diagnostic screening and follow up of latent tuberculosis infection (LTBI) in immunosuppressed kidney transplant (KTx) recipients. The QFT-2G assay that included response to mitogen stimulation was performed before and 6 months after KTx. Non responder was 0 (0%) at baseline, 3 (3%) at 6 months. Response to mitogen stimulation was 9.7±5.3 IU/mL at baseline vs. 10.4±5.0 IU/mL at 6 months after KTx ($p=0.29$). QFT-2G is a useful screening test for LTBI and active tuberculosis (TB) even during maintenance of immunosuppression of KTx.

2. QuantiFERON-TB Gold in Japanese rheumatoid arthritis patients for assessing latent tuberculosis infection prior treatment of anti-tumor necrosis factor antibody: Shogo BANNO (Division of Rheumatology and Nephrology, Department of Internal Medicine, Aichi Medical School of Medicine)

To determine the positive rate of LTBI in RA patients using the QFT-2G test, we divided RA patients into two groups: with or without old TB findings by chest CT. With a cutoff level set at 0.35 IU/m*l*, the positive rate of QFT-2G in LTBI was detected only 5.8%, when setting cutoff at 0.1 IU/m*l* (lower cutoff level), 23.1% was detected in LTBI patients. The positive TST results were significantly increased in non-LTBI patients compared than in LTBI patients. The QFT-2G test was not affected by the treatment of MTX, and the incidence of indeterminate result was low. The QFT-2G was useful compared to TST before administration of TNF inhibitors in RA patients, because of superior specificity of QFT-2G.

3. Clinical parameters that influence the sensitivity of T-cell assays: Haruyuki ARIGA (National Hospital Organization Tokyo National Hospital)

The detection of tuberculosis (TB) infection in compromised hosts is essential for TB control, but T cell assay might be influenced by the degree of cell-mediated immunosuppression. The relationship between immunocompetence and specific interferon (IFN)-γ response in whole blood QuantiFERON-TB Gold (QFT) is uncertain. Immune-related clinical indicators associated with the degree of antigen-specific IFN-γ production were analysed using a large immunologically-unselected population with obvious TB infection. The absolute number of blood lymphocyte in TB patients was significantly associated with specific IFN-γ production in a linear regression model. Sensitivity of 2 IFN-γ Release Assays, QFT and ELISPOT, partly depends on peripheral lymphocyte counts. At low lymphocyte count conditions, ELISPOT assay is superior to whole blood QFT for detecting tuberculosis infection.

4. QuantiFERON TB-2G among staffs in the hospitals of Nationao Hospital Organization: Susumu OGURI, Chihiro NISHIO, Kensuke SUMI, Masayoshi MINAGUCHI, Tomomasa TSUBOI, Atuo SATOU, Osamu TOKUNAGA, Takeshi MIYAMOMAE, Takuya KURASAWA (National Hospital Organization Minami-Kyoto National Hospital)

[Purpose] To investigate the infection rate of tuberculosis among staffs working in the hospitals of NHO.

[Method] Questionnaires were sent to the hospitals and the responses were analyzed.

[Result] Among the staffs working in the hospitals with tuberculosis wards, positive rate of QuantiFERON TB-2G was 6.9%, probable positive rate was 5.6%. On the other hand, among the staffs working in the hospitals without tuberculosis wards, positive rate was 4.4%, probable positive rate was 3.9%.

[Conclusion] It is necessary to monitor the infection rate among hospital staffs.

Key words: QuantiFERON-2G (QFT), Tuberculosis, Interferon-γ, Transplant, TNF, Immunosuppression

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NON-TUBERCULOUS MYCOBACTERIOSIS WHAT HAS BEEN COMING OUT

Chairperson: Akira KAJIKI

Abstract Diagnosis of non-tuberculous mycobacteriosis is relatively easy, because of recent technological advances (HRCT, MGIT, PCR, DDH etc). Although many reports of this disease have been published, there are many problems to resolve.

1. Prevalence of non-tuberculous mycobacteriosis: Shigeki SATO (Department of Medical Oncology and Immunology, Nagoya City University Graduate School of Medical Sciences)

Questionnaire surveys to determine the prevalence of non-tuberculous mycobacterial (NTM) disease were carried out in 2001, 2007, and 2009. The NTM disease rate was estimated at 5.9/100,000, confirming that Japan has one of the world's highest NTM disease rates. Examination of the proportions of *M. avium* and *M. intracellulare* disease in Japan by region revealed that the *M. avium/M. intracellulare* disease ratio increased in different regions since past reports. In the 2007 survey, the *M. avium* disease rate had increased over the 2001 level. *M. kansasii* had a high disease rate in the Kinki and Kanto regions. Disease rates tended to be high in regions that have a metropolis. However, the disease rate was low in Aichi Prefecture, so that the presence in a region of a metropolis is probably not of itself a factor causing a high disease rate. The distributions of the bacteria causing NTM thus vary among different countries and regions.

2. Polyclonal infection of *Mycobacterium avium* using variable numbers of tandem repeats (VNTR) analysis: Tomoshige MATSUMOTO (Department of Clinical Research and Development, Center for Infectious Diseases, Osaka Prefectural Hospital Organization, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases)

Mycobacterium avium complex (MAC) is refractory to therapy, containing rifampicin (RFP), ethambutol (EB), and clarithromycin (CAM). It was widely accepted that therapeutic difficulties of pulmonary MAC treatment was caused by highly resistance to antibiotics or repeated re-infection from environment. Variable number of tandem repeats (VNTR) analysis of MAC is available. So, we studied the MAC-VNTR of clinical isolates from 29 patients with pulmonary MAC, refractory to the therapy. Compared the clinical isolates before

with after each therapy, clinical isolates derived from the all except one patient showed the same VNTR patterns, before and after.

According to MAC-VNTR analysis of the clinical isolates we studied, frequency of polyclonal infection was low (1/29). We concluded that the highly resistance to antibiotics or the repeated same VNTR type infection from environment made refractory pulmonary MAC.

3. An approach to identify susceptibility genes in patients with non-HIV-related pulmonary *Mycobaterium avium* complex (MAC) infection: Naoto KEICHO (Department of Respiratory Diseases, Research Institute, National Center for Global Health and Medicine)

Mycobacterium avium complex causes human pulmonary disease. Th1 T cells play a role in protective immunity from mycobacterial infection. Genetic defect of Interferon-gamma/Interleukin-12 axis is known to cause familial non-tuberculous mycobacterial infection. On the other hand, non-mendelian type of genetic abnormalities such as polymorphisms of HLA, CFTR and SLC11A1 (NRAMP1) genes has also been investigated as disease susceptibility genes. Recently our group has reported disease association with MHC-class I related chain-A molecule (MICA), comparing 300 sporadic cases with 300 healthy controls.

4. Genetic feature of *Mycobacterium avium* complex: Taku NAKAGAWA, Kenji OGAWA (Department of Pulmonary Medicine, National Hospital Organization Higashinagoya National Hospital)

The bacterial factors contributing to the pathogenesis of *M. avium* complex infection and diversity of disease progression remain unclear. MATR-VNTR typing is inexpensive and easy to perform and has an excellent discriminatory power compared with MIRU-VNTR and IS*1245*-RFLP typing. MATR-VNTR typing revealed that *M. avium* isolates from HIV-positive patients are analogous to the isolates from pig enterically-transmitted rather than those from HIV-negative patients with pulmonary diseases.

M. avium comprises four subspecies. We performed genetic analysis by using Insertion Sequence (IS) for 114 clinical isolates of *M. avium*. All clinical isolates were identified as *M. avium* subsp. *hominissuis* by sequence analysis of *hsp65*. PCR detection rate of IS*901* was about 70%, while detection rate in Europe and America was 0−8%. Compared with the original IS*901*, 60 point mutations were found in the sequence of the insertion sequence detected from all PCR-positive clinical isolates. This new insertion sequence was designated IS*Mav6*. It became clear that *M. avium* strains in Japan are distinct from strains in Western countries in terms of the prevalence of IS*Mav6*.

We conducted genetic analysis for *M. avium* isolates collected from 11 hospitals all over Japan, but MATR-VNTR typing failed to show that distinct clusters correlate with disease progression or region.

Genetic typing for *M. intracellulare* using VNTR has not yet been developed. We identified VNTR loci in the genome of *M. intracellulare* ATCC1395 and applied them as a molecular epidemiological tool to clinical isolates.

5. Infection source of pulmonary *Mycobactrium avium* complex (MAC) disease: Yukiko NISHIUCHI (Toneyama Institute for Tuberculosis Research Osaka City University Medical School), Ryoji MAEKURA (National Hospital Organization Toneyama National Hospital)

Pulmonary MAC disease is characterized as the polyclonal infection and the recurrence, which suggest the presence of polyclonal niche of MAC in environment surrounding patients. We revealed that MAC was recovered from bathrooms but not from other sites of residences. The bathtub inlet was the niche with polyclonal colonization of MAC in the bathrooms of MAC patients. The identical/related genotypic profiles with isolates from patients were revealed by pulsed field gel electrophoresis. These results implied that the residential bathroom might be one of the infectious sources of pulmonary MAC disease.

Key words: Regional prevalence, Polyclonal infection, Host factor, Bacterial factor, Infectious sources

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TUBERCULOSIS ANNUAL REPORT 2009
—Series 1. Summary of TB Notification Statistics in 2009—

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Abstract Annual reports of tuberculosis (TB) statistics in Japan have been compiled mainly using the output of the database obtained through the nationwide computerized tuberculosis surveillance system which has been operated since 1987. This system has been revised several times, with the latest revision conducted in 2007 when much new information was added. Therefore, a plan was drawn up to provide TB epidemiological statistics in Japan on "Kekkaku" and a series of ten reports was already issued as "TB Annual Report 2008". This is the first report of a new series for "TB Annual Report 2009".

The report can be summarized as follows. The TB notification (incidence) rate fell below 20 per 100,000 in 2007 and continued to decline, reaching 19.0 in 2009. However, 24,170 TB patients were newly notified in 2009. For sputum smear positive pulmonary TB, the patient count was 9,675 with an incidence rate of 7.6 per 100,000 in 2009. Since June 2007, it has been legally compulsory to notify latent TB infections (LTBI) requiring treatment; the number in 2009 was 4,119 cases.

Key words: Tuberculosis, Incidence rate, Trend, Sex-age specific, Monthly report, Latent TB infection, Extra-pulmonary TB

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