——————— **Original Article** ———————

EFFECTS OF PROPHYLAXIS ON QuantiFERON®TB-2G RESPONSES AMONG CHILDREN

[1]Kazue HIGUCHI, [2]Kenji OKADA, [1]Nobuyuki HARADA, and [3]Toru MORI

Abstract [Objective] To study the effect of treatment of latent tuberculosis infection (LTBI) on QuantiFERON®TB-2G (QFT-2G) test results.

[Subjects and methods] QFT-2G was used for a contact investigation in a junior high school and those positive or doubtful positive (TB Antigen-Nil response ≥ 0.1 and < 0.35 IU/ml) were indicated for treatment of LTBI with INH. All subjects who completed treatment of LTBI were re-tested with QFT-2G approximately 1 month after completion of treatment and a subset were again re-tested 8 to 11 months after the completion of treatment. The levels of IFN-γ response in each QFT-2G test were compared.

[Results] Initially, 43 subjects (28 QFT-2G positive and 15 doubtful positive) were indicated treatment of LTBI, and 41 (95％) completed 6-months treatment. These 41 subjects were re-tested with QFT-2G approximately 1 month after the completion of treatment. Among 28 pre-treatment positives, 19 remained positive, 6 became doubtful positive, and 3 reverted to negative. Among 13 pre-treatment doubtful positives, 1 converted to positive, 5 remained doubtful positive, and 7 reverted to negative. The QFT-2G responses after the completion of treatment significantly declined compared with the pre-treatment level (geometric means ; before treatment ESAT-6 : 0.30 IU/ml, CFP-10 : 0.09 IU/ml, after treatment ESAT-6 : 0.18 IU/ml, CFP-10 : 0.05 IU/ml, dependent t-test ; ESAT-6 : p＝0.020, CFP-10 : p＝0.005). At 8 to 11 months after the completion of treatment, 30 randomly selected subjects received the third QFT-2G test. Among 19 positives at the completion of treatment, 14 remained positive, 4 become doubtful positive, and 1 reverted to negative. Among 8 doubtful positives at completion of treatment, 4 converted to positive, 3 remained doubtful positive, and 1 reverted to negative. A further decline of QFT-2G responses was not observed. Three subjects negative at the completion of treatment were re-tested and remained negative at the third test.

[Conclusion] QFT-2G responses significantly declined after the treatment of LTBI, despite the rate of reversion in QFT-2G being low. This low reversion rate suggests QFT-2G would not be useful as a marker to evaluate the success of treatment for LTBI. However, the finding that QFT-2G responses significantly decline after the treatment of LTBI suggests the possibility that this decline could be used as a marker of the susceptibility of the infective *M. tuberculosis* strain to the prophylactic drug used. The outbreak investigation has been carried out for over two years, and none of 229 students who were TST positive, but QFT-2G negative and because of this result not indicated treatment of LTBI, have developed TB, suggesting that QFT-2G reflects TB infection more accurately than the TST, even in school children.

Key words : Tuberculosis outbreak, QuantiFERON®TB-2G, Treatment of LTBI, Contact investigation

[1]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (JATA), [2]National Hospital Organization Fukuoka National Hospital, [3]Leprosy Research Center, National Institute of Infectious Diseases

Correspondence to : Kazue Higuchi, Immunology Division, Research Institute of Tuberculosis, JATA, 3－1－24, Matsuyama, Kiyose-shi, Tokyo 204－8533 Japan.
(E-mail : higuchi@jata.or.jp)

TUBERCULOSIS CONTROL IN SHINJUKU WARD, TOKYO
— Promoting the DOTS Program and Its Outcome—

[1]Sumi KAGURAOKA, [2]Masako OHMORI, [3]Yoshiko TAKAO, [1]Mari YAMADA, [3]Masako MUROI,
[4]Michiko NAGAMINE, [5]Keiji FUKAZAWA, [6]Megumi NAGAI, [7]Masako WADA,
[2]Hitoshi HOSHINO, [2]Takashi YOSHIYAMA, [8]Hideo MAEDA, and [2]Nobukatsu ISHIKAWA

Abstract ［Objectives］ The objectives were to report how to promote tuberculosis (TB) control including DOTS (Directly Observed Treatment, Short-course) programs, and to evaluate the results of TB control programs in Shinjuku Ward (Shinjuku-ku).

［Setting and characteristics］ Inhabitants and TB patients in Shinjuku Ward. Shinjuku Ward is located in the center of metropolitan Tokyo and has typical urban TB problems, such as high incidence rate and TB among foreigners and the homeless. The TB incidence rates in Shinjuku Ward decreased from 83.9 per 100,000 population in 1999 to 42.5 per 100,000 population in 2006, however, the rates were still two times higher than the national average. Therefore, one of the important TB programs in Shinjuku has been to actively detect cases among high-risk groups such as foreigners and the homeless.

［Methods］ We observed the trend of case detection rates by health examination with chest X-ray among different high-risk groups, and compared the treatment outcomes before and after DOTS program execution. We also reviewed the changes of re-treatment rates and drug resistance rates.

［Results］ The case detection rates of TB by health examinations of foreign students at Japanese language schools decreased from 0.49％ in 1996 to 0.13％ in 2006 (p＝0.021). Although the case detection rates decreased, they were still about 26 times higher than those of Japanese students. While, the case detection rates among the homeless remained high with 4.7％, 3.3％, 4.5％ and 3.6％ in 1999−2002, respectively, since 2003, however, they had decreased and no TB cases were detected in 2005−2006. The DOTS program for homeless TB patients has been carried out since 2000 and that for the foreigners since 2003. The rates of defaulting during treatment before DOTS were very high among both homeless patients (21.4％) and foreigners (29.8％) in 1998−1999. However, after the introduction of DOTS program, those rates declined to 10.4％ (p＝0.014) among the homeless and 7.8％ (p＝0.002) among foreigners in 2002−2004. The proportion of newly notified patients with previous TB treatment and those with multi-drug resistant TB (MDR-TB) have also decreased after the introduction of DOTS programs. From 2000−2002 to 2003−2006, the re-treatment rates decreased from 19.4％ to 10.0％ (p＜0.001) and MDR-TB rates decreased from 1.6％ to 0.2％ (p＝0.042), respectively.

［Discussion］ The key points of TB control in Shinjuku Ward are to detect TB cases early especially among the high-risk groups, and to assist all TB patients to complete their treatment. In order to expand this strategy, besides promoting active case findings among high-risk groups, we have developed many types of DOTS programs, considering each patient's lifestyle and cooperating with school teachers at schools, pharmacists at pharmacies, home-care specialists at homes or facilities for the elderly, and so on. Among others, a major premise for the homeless and some other socially disadvantaged patients was to guarantee the provision of medicine and living by introducing social welfare services, before starting DOTS programs. This approach might have helped to reduce the defaulting rate, relapse rate and MDR-TB rate.

Key words : Tuberculosis, Shinjuku, DOTS, Public health nurse, Foreigners, Homeless, Treatment outcome

[1]Nishishinjuku Public Health Centre, Tokyo, [2]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, [3]Shinjuku-ku Public Health Centre, Tokyo, [4]Specific Disease Control Section, Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government, [5]Kita-ku Public Health Center, Tokyo, [6]Toshima-ku Ikebukuro Public Health Centre, Tokyo, [7]Chemothrapy Institute, Kaken-Hospital, [8]Tokyo Metropolitan Institute of Public Health Centre

Correspondence to： Sumi Kaguraoka, Nishishinjuku Public Health Centre, Tokyo, 7−5−8, Nishishinjuku, Shinjuku-ku, Tokyo 160−0023 Japan.
(E-mail： sumi.kaguraoka@city.shinjuku.tokyo.jp)

WHAT IS NEEDED TO PREVENT DEFAULTING FROM TUBERCULOSIS TREATMENT?

[1]Kunihiko ITO, [1,2]Takashi YOSHIYAMA, [1]Yohko NAGATA, [1]Noriko KOBAYASHI, [1]Seiya KATO, and [1]Nobukatsu ISHIKAWA

Abstract [Purpose] To investigate the factors relating to defaulting from tuberculosis treatment in Japan, and clarify what is needed to prevent defaulting.

[Object] Tuberculosis patients who were registered at public health centers (PHCs), and interrupted treatment for more than 2 months without the doctors' direction at the end of December 2005.

[Method] Investigation by questionaire sent by post-mail to all public health centers (608 PHCs) in Japan.

[Result] The valid answers was obtained from 89.0% (541/608) of PHCs. Tuberculosis patients who had interrupted treatment, but could be contacted by PHCs' staff were 137, and for those patients the factors relating to defaulting from treatment were analyzed. The factors were classified into 7 categories (there may be more than one factors in one patients) ; factors related to disbelief and/or prejudice for diagnosis and/or treatment (except factors related to drug adverse effects) were observed in 51.8%, factors related to economical problem in 24.1%, factors related to job or studies in 23.4%, factors related to drug adverse effects in 22.6%, factors related to visiting out-patients departments in 6.6%, psychiatric disease and/or drug abuse in 4.4%, others in 9.5%.

[Conclusion] It is needed to prevent defaulting, first, to improve the quality of tuberculosis medical care and services including good and sufficient explanations on TB and how to cure it to patients, and proper managements for drug adverse effects, and then to expand public economical support for the costs of medicine and travel expenses to medical facilities and to make accessible time and place of the tuberculosis outpatient clinic more convenient and flexible for patients.

Key words : Defaulting, DOT, DOTS, Treatment support, Quality of medical care

[1]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (JATA), [2]Department of Respiratory Medicine, Fukujuji Hospital, JATA

Correspondence to : Kunihiko Ito, Research Institute of Tuberculosis, JATA, 3−1−24, Matsuyama, Kiyose-shi, Tokyo 204−8533 Japan. (E-mail : ito@jata.or.jp)

—————— **Case Report** ——————

USEFULNESS OF THE VARIABLE NUMBERS OF TANDEM REPEATS (VNTR) ANALYSIS FOR COMPLEX INFECTIONS OF *MYCOBACTERIUM AVIUM* AND *MYCOBACTERIUM INTRACELLULARE*

[1]Noriko TSUNEMATSU, [3]Mieko GOTO, [1]Yumiko SAIKI, [2]Michiko BABA,
[4]Tadashi UDAGAWA, and [4]Yuko KAZUMI

Abstract   [Purpose] The bacilli which were isolated from a patient suspected of the mixed infections with *Mycobacterium avium* and *Mycobacterium intracellulare*, were analyzed. The genotypes of *M. avium* in the sedimented fractions of treated sputum and in some colonies isolated from Ogawa medium were compared by the Variable Numbers of Tandem Repeats (VNTR).

[Object and method] Case : A woman, aged 57. Mycobacterial species isolated from some colonies by culture in 2004 and 2006 and from the treated sputum in 2006, were determined by DNA sequencing analysis of the 16S rRNA gene. Also, by using VNTR, the genotype of mycobacteria was analyzed.

[Results] (1) The colony isolated from Ogawa medium in 2004 was monoclonal *M. avium*. (2) By VNTR analyses of specimens in 2006, multiple acid-fast bacteria were found in the sputum sediment and in isolated bacteria from Ogawa medium. (3) By analyses of 16S rRNA DNA sequence, *M. avium* and *M. intracellulare* were found in the colonies isolated from the sputum sediment and the Ogawa medium in 2006. (4) The same VNTR patterns were obtained in *M. avium* in 2004 and 2006 when single colony was analyzed. (5) From the showerhead and culvert of the bathroom in the patient's house, *M. avium* was not detected.

[Discussion] By VNTR analyses, it was considered that the mixed infections of *M. avium* and *M. intracellulare* had been generated during treatment in this case. Therefore, in the case of suspected complex infection, VNTR analysis would be a useful genotyping method in *M. avium* complex infection.

[1]Department of Clinical Laboratory, and [2]Internal Medicine, Tokyo Metropolitan Ohtsuka General Hospital, [3]Department of Infection Control and Prevention, University of Tokyo Hospital Internal Medicine, [4]Pathology Division, Mycobacterium Reference Center, Research Institute of Tuberculosis (RIT), Japan Anti-Tuberculosis Association (JATA)

Correspondence to : Noriko Tsunematsu, Department of Clinical Laboratory, Tokyo Metropolitan Ohtsuka General Hospital, 2−8−1, Minamiohtsuka, Toshima-ku, Tokyo 170−8476 Japan.
(E-mail : noriko-o@ohtsuka-hospital.toshima.tokyo.jp)

第83回総会ミニシンポジウム

I. ワクチン研究の現在と将来

座長　¹小林　和夫　　²菅原　　勇

キーワーズ：改良 BCG，弱毒結核菌，成分ワクチン，DNA ワクチン，感染曝露前（予防）ワクチン，感染曝露後（治療）ワクチン

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2. BCG vaccine trials in South Africa
　　Gregory HUSSEY (South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa)
3. Present and future of TB vaccine development research
　　Peter ANDERSEN (Statens Serum Institute, Copenhagen, Denmark)
4. Comments and directions in research and development of TB vaccines
　　Jerald C. SADOFF (Aeras Global TB Vaccine Foundation, Bethesda, Maryland, USA)

　全世界で約20億人（全人口の3分の1）が結核菌（*Mycobacterium tuberculosis*）に既感染，すなわち，無症候性潜伏感染し，毎年920万人が結核を発病，170万人（後天性免疫不全症候群合併23万人を含む）が死亡している（http://www.who.int/tb/en/）。今後10年間に，少なくとも8000万人が結核を発病，2000万人が死亡することが推定されている。

　日本（2006年）では年間2.6万人（罹患率人口10万対：20.6）が結核を発病し，2.3千人（死亡率：1.8）が死亡し，日本においても結核対策は重要な課題である。Robert Koch が1882年に「結核菌」を発見，爾来，120年余が経過した現在でも，国内外を問わず，結核は人類に甚大な健康被害を提供し続けている。

　結核対策における世界的課題として，①薬剤耐性結核菌の出現や蔓延および②HIV−結核菌の重複感染がきわめて重要である。これらの課題を克服する科学的戦略は「安全で有効な結核ワクチン」である。現行結核ワクチンである bacillus Calmette-Guérin（BCG）は乳幼児結核に有効であるが，潜在性結核菌感染を基盤とした多くの成人肺結核や内因性再燃結核に対する BCG 接種の有効性は疑問視されている。

　世界保健機関（WHO）は2015年までに現行 BCG を凌駕する新規結核ワクチンの開発を目指している。新規結核ワクチンの開発戦略は「予防・治療：感染曝露前（予防的）や感染曝露後（治療的）ワクチン」，「ワクチン製剤：改良型 BCG，弱毒結核菌，成分ワクチンや DNA など遺伝子ワクチン」，「接種方法：Prime や Prime-boost ワクチン」などの視点から進捗しており，前臨床試験，さらに，第1相など臨床試験で評価され，有望なワクチン候補が開発されつつある。

　第83回日本結核病学会総会（石川信克会長）において，ミニシンポジウム「ワクチン研究の現在と将来」を企画し，世界の第一線で活躍されている気鋭の結核ワクチン研究者が結核ワクチン開発の現況や将来展望を発表した。ミニシンポジウム「ワクチン研究の現在と将来」が会員諸氏に有用な情報を提供，そして，研究室から臨床に迅速・効率的に"橋渡し（Translation）"し，究極的に人類に甚大な健康被害を提供し続けている結核の制圧に寄与することを祈念している。

¹国立感染症研究所免疫部，²結核予防会結核研究所抗酸菌レファレンスセンター

連絡先：小林和夫，国立感染症研究所免疫部，〒162−8640 東京都新宿区戸山1−23−1（E-mail : kobayak@nih.go.jp）

1. 新しい結核 DNA ワクチン

国立病院機構近畿中央胸部疾患センター臨床研究センター　岡田　全司

1998年, アメリカ合衆国疾病対策予防センター（Centers for Disease Control and Prevention：CDC）および Advisory Council for the Elimination of Tuberculosis（ACET）は新世代の結核ワクチン開発の必要性を発表した。しかしながら, BCG ワクチンに代わる結核ワクチンは欧米でも臨床応用には至っていない。結核ワクチンは, DNA ワクチン, リコンビナント BCG ワクチン, サブユニットワクチンに大別される。DNA ワクチンは予防ワクチン効果の切れ味ではほかより優れていることが多く, 安定性・経済的にも優れている。われわれは BCG ワクチンをはるかに凌駕する 1 万倍強力な結核予防ワクチン効果を示す新しい DNA ワクチン（HVJ–エンベロープ/HSP 65＋IL-12 DNA ワクチン）を開発した。

〔マウス〕の結核感染系では BCG ワクチンをはるかに凌駕する新しい結核ワクチンはきわめて少ない。われわれはプライム・ブースター法を用い, HSP 65 DNA＋IL-12 DNA（HVJ–エンベロープベクター）のワクチンは BCG ワクチンよりも 1 万倍強力な結核予防ワクチンであることを世界に先駆けて明らかにした。このワクチンは, 結核菌由来の HSP 65 蛋白抗原特異的な, CD8 陽性キラー T 細胞および interferon：IFN-gamma 産生 T 細胞の分化も増強した。肺の結核病理像の改善効果も示した。さらに生体内において, CD8 陽性 T 細胞と CD4 陽性 T 細胞の両者がこの結核予防ワクチンに必要であることを明らかにした。

〔治療ワクチン〕さらに, このワクチンは治療結核ワクチン効果も示した。すなわち結核菌をあらかじめ投与したマウスにおいて HVJ–エンベロープ/HSP 65 DNA＋IL-12 DNA ワクチンを 3 回治療投与すると, コントロール群に比較して有意差をもって肺・肝・脾の結核菌数の減少を認めた。多剤耐性結核菌や超薬剤耐性結核（XDR-TB）に対しても治療ワクチン効果を示した。欧米では治療ワクチンは未開発である。モルモット（結核菌吸入感染系）の系でもこのワクチンは BCG より有効であった。〔新しいヒト生体内抗結核免疫解析モデル SCID-PBL/hu〕を用いてもワクチン効果を示した。

さらに, 〔ヒト結核感染モデルに最も近いカニクイザル〕（Nature Med. 1996）を用い, HSP 65 DNA＋IL-12 DNA ワクチンの強力な有効性を得た。カニクイザルに 3 回ワクチン接種後 4 週間後にヒト結核菌を経気道投与し, 1 年以上経過観察した。リンパ球増殖反応・サイトカイン（IFN-gamma, IL-2等）産生の増強および胸部 X線所見・血沈, 体重の改善効果が認められた。さらに, 生存率改善・延命効果も認められた。DNA ワクチン投与群は50％の生存率であり, コントロール群は生存率 0％であった。さらに, サルの系でプライム–ブースター法を用いて, より強力なワクチン開発を行った。その結果, BCG ワクチン・プライム–DNA ワクチン・ブースター法を用いた群は100％の生存率を示した。一方, BCG ワクチン単独群は33％の生存率であった。成人に対して切れ味の鋭い強力な新しい結核ワクチンが切望されているが, BCG ワクチンは乳幼児でほぼ全員に実施されていることより HSP65 DNA＋IL-12 DNA ワクチンが強力な成人ワクチンとなることが示唆された。WHO STOP TB VACCINE Meeting でこのワクチンはきわめて高い評価を受けた。さらに, このワクチンを鼻粘膜または気道内ワクチンとして投与を試みつつある。さらに, カニクイザルの系で治療ワクチン効果およびプライムとブースターの期間を長期間とって, プライム–ブースター法を研究中である。

We have developed a novel tuberculosis (TB) vaccine ; a combination of the DNA vaccines expressing mycobacterial heat shock protein 65 (HSP 65) and interleukin 12 (IL-12) delivered by the hemagglutinating virus of Japan (HVJ)-envelope and -liposome (HSP 65＋IL-12/HVJ). This vaccine provided remarkable protective efficacy in mouse and guinea pig models compared to the BCG vaccine on the basis of C.F.U of number of TB, survival, an induction of the CD8 positive CTL activity and improvement of the histopathological tuberculosis lesions. This vaccine provided therapeutic efficacy against multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) (prolongation of survival time and the decrease in the number of TB in the lung) as well as protective efficacy in murine models. Furthermore, we extended our studies to a cynomolgus monkey model, which is currently the best animal model of human tuberculosis. This novel vaccine provided a higher level of the protective efficacy than BCG based upon the assessment of mortality, the ESR, body weight, chest X-ray findings and immune responses (IFN-γ, IL-2, IL-6 produc-

tion, and lymphocyte proliferation of cynomolgus monkey). All monkeys in the control group (saline) died within 8 months, while 50％ of monkeys in the HSP 65＋IL-12/HVJ group survived more than 14 months post-infection (the termination period of the experiment). Furthermore, the combination of HSP 65＋IL-12/HVJ and BCG by the priming-booster method showed a synergistic effect in the TB-infected cynomolgus monkey (100％ survival). In contrast, 33％ of monkeys from BCG Tokyo alone group were alive (33％ survival). Furthermore, this vaccine exerted therapeutic efficacy in the TB-infected monkeys. These data indicate that our novel DNA vaccine might be useful against *Mycobacterium tuberculosis* for human clinical trials.

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2. BCG vaccine trials in South Africa

South African Tuberculosis Vaccine Initiative, University of Cape Town　　Gregory HUSSEY

The South African Tuberculosis Vaccine Initiative, located within the University of Cape Town, has been involved in a number of BCG vaccine trials over the last few years and in this presentation I will highlight results from some of our studies.

A randomized trial comparing the efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis disease in infants and young children

Intradermal BCG vaccine is currently recommended by the World Health Organization (WHO). Prior to this study, no randomized trial comparing the relative incidence of tuberculosis following intradermal as opposed to percutaneous BCG vaccination had been conducted. 11680 South African newborns were randomized to receive Tokyo 172 BCG vaccine via either the percutaneous (n＝5775) or the intradermal (n＝5905) route within 24 hours of birth and then followed up for 2 years to document and investigate adverse events and suspected tuberculosis (TB) disease. The cumulative incidence of tuberculosis over two years of follow up was 6.13％ [95.5％CI : 5.52−6.79％] in the intradermal group and 6.49％ [5.86−7.18％] in the percutaneous group. No significant differences were found between the routes in the cumulative incidence of adverse events. Our results suggest that the WHO should consider revising its policy of preferential intradermal vaccination to allow national immunization programs to choose percutaneous vaccination if that is more practical.

Determining BCG-induced immune correlates of protection against childhood tuberculosis disease

This study aims to determine what we can measure in the blood of a BCG-vaccinated baby to tell us whether that infant has either been protected, or not protected, against future tuberculosis disease. Defining these ″immune correlates″ is critical for studies of new tuberculosis vaccines. 5675 infants, routinely vaccinated with BCG at birth were enrolled. Blood was collected, processed and cryopreserved at 10 weeks of age, and the infants were followed for at least 2 years. 45 infants developed culture-positive lung tuberculosis over this period (i.e., not protected by BCG). 91 infants did not develop tuberculosis disease despite exposure to adults with tuberculosis in the households (i.e., protected by BCG). We are now in the process of retrieving blood products stored at 10 weeks of age, to compare BCG-induced immunity in the 2 groups. Our comprehensive approach to analysis includes: determining cytokine levels in plasma, evaluating cytokine expression and the memory phenotype of specific T cells, determining specific T cell proliferative and cytokine-producing capacity, assessing the pattern of mRNA expression, and determining whether BCG-induced antibody production patterns may correlate with protection. Results will be presented.

RESEARCH AND DEVELOPMENT OF VACCINES AGAINST TUBERCULOSIS

Chairpersons : [1]Kazuo KOBAYASHI and [2]Isamu SUGAWARA

Speakers :

1. Novel DNA vaccines against tuberculosis : Masaji OKADA (Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center)
2. BCG vaccine trials in South Africa : Gregory HUSSEY (South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa)
3. Present and future of TB vaccine development research : Peter ANDERSEN (Statens Serum Institute, Copenhagen, Denmark)
4. Comments and directions in research and development of TB vaccines : Jerald C. SADOFF (Aeras Global TB Vaccine Foundation, Bethesda, Maryland, USA)

Mycobacterium tuberculosis is one of the most successful bacterial parasites of humans, infecting over one-third of the population of the world as latent infection without clinical manifestations. Over 9.2 million new cases and nearly 1.7 million deaths by tuberculosis (TB) occur annually (http://www.who.int/tb/en/). TB poses a significant health threat to the world population. Global tuberculosis control is facing major challenges today. In general, much effort is still required to make quality care accessible without barriers of gender, age, type of disease, social setting, and ability to pay. Coinfection with *M. tuberculosis* and human immunodeficiency virus (TB/HIV), and multidrug-resistant (MDR) and extensively drug-resistant (XDR)-TB in all regions, make control activities more complex and demanding. Treating and preventing TB is challenging, even in developed countries where there is a modern health care system and infrastructure. Current treatment regimens last six to nine months, and erratic or inconsistent treatment breeds MDR (490,000 new cases/year) and even XDR-TB (40,000 new cases/year), which means that this pandemic could become even more difficult to control throughout the world. TB is a leading cause of death among people who are also infected with HIV, according to the World Health Organization. One-third of the 33.2 million people living with HIV also suffer from TB. The coinfection causes 230,000 deaths annually worldwide. Without proper treatment, approximately 90 percent of people living with HIV die within two to three months of contracting TB (http://www.stoptb.org/wg/tb_hiv/default.asp). The goal of this symposium is to understand the current situation of research and development of novel TB vaccines and the future perspective.

To win the fight against TB, a comprehensive approach is needed that includes new and more effective vaccines as well as improved diagnostics and treatment. The bacillus Calmette-Guérin (BCG) vaccine, created in 1921, is the only existing vaccine against TB. Unfortunately, it is only partially effective. It provides some protection against severe forms of pediatric TB, namely disseminated and meningeal tuberculosis occurring in the first year of life, but is unreliable against adult pulmonary TB, which accounts for most of the disease burden worldwide. Although BCG is the most widely administered vaccine in the world, there have never been as many cases of TB on the planet. There is therefore an urgent need for a modern, safe and effective vaccine that would prevent all forms of TB, including the drug-resistant strains, in all age groups and among people with human immunodeficiency virus (HIV).

Strategies for the research and development (R&D) are included 1) pre-exposure (prophylactic) and 2) post-exposure (therapeutic) vaccines. Based on the preparation, there are 4 types, such as 1) improved BCG, 2) attenuated *M. tuberculosis*, 3) subunit/component vaccines, and 4) DNA vaccines. Speakers have presented and discussed "R&D of novel vaccines against TB" better than current BCG.

To control TB and overcome the issues, such as drug-resistant TB and HIV-TB coinfection, we hope the presentation in the Mini-symposium promotes a more adventurous approach to develop a novel, effective and safe TB vaccine.

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[1]Department of Immunology, National Institute of Infectious Diseases, [2]Mycobacterial Reference Center, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

Correspondence to : Kazuo Kobayashi, Department of Immunology, National Institute of Infectious Diseases, 1−23−1 Toyama, Shinjuku-ku, Tokyo 162−8640 Japan.
(E mail : kobayak@nih.go.jp)

——————— **The 83rd Annual Meeting Mini-symposium** ———————

EVOLUTION OF IGRA RESEARCHES

Chairpersons: [1]Haruyuki ARIGA and [2]Nobuyuki HARADA

Abstract: The progress of genomic analysis in mycobacterium including *M. tuberculosis* (Mtb) allowed us to find Mtb-specific antigens, ESAT-6 and CFP-10, which induce strong interferon-gamma (IFN-γ) from sensitized T cells. Shortly after discovery of these antigens, diagnostic tests for tuberculosis (TB) infection were developed using these antigens. Since ESAT-6 and CFP-10 are absent from all BCG substrains and most of non-tuberculous mycobacterium, these diagnostic tests are not confounded with BCG vaccination and infection of most of non-tuberculosis mycobacterium. These diagnostic tests are called as Interferon-Gamma Release Assays (IGRAs), and currently there are two commercially available tests. One of them, QuantiFERON®-TB Gold (it is called QuantiFERON® TB-2G in Japan, QFT-2G) based on ELISA method has been approved in Japan, and the other is T-SPOT®. *TB* which is based on ELISPOT method and has not been approved in Japan yet. As in general T-SPOT®. *TB* has been shown to be more sensitive than QFT-2G, approval of T-SPOT®. *TB* in Japan would be expected.

However, there are many questions to be solved in IGRAs, since we have just started to use these tests. A paper which integrated these questions was published last year, and it would be helpful[1].

In this mini-symposium, Dr. Peter Andersen reported the progress of development of diagnostic tests for tuberculosis infection, the possibility to distinguish between active TB and latent TB infection (LTBI) which the current IGRAs do not, and the prognostic use of IGRAs (The Japanese content was reported by Chairpersons). Dr. Ariga reported the application of QFT-2G for specimens other than blood. He also reported the interesting data on which T cells responded in QFT-2G. Dr. Higuchi comprehensively reported data on several questions in the QFT-2G test.

Currently the use of IGRAs is expanding rapidly. Under this circumstance, it would be very important to properly understand the characteristics of IGRAs. We hope that this mini-symposium may help for understanding these issues.

1. Interferon-Gamma Release Assays (IGRA) and antigens for detection of latent infection and prediction of disease: Peter ANDERSEN (Department of Infectious Disease Immunology and the SSI Centre for Vaccine Research, Statens Serum Institut, Denmark)

One of the most important challenges in global tuberculosis control is the diagnosis and treatment of latent tuberculosis infection. The currently used method for detection of latent tuberculosis infection, the tuberculin skin test, has low specificity. The identification of antigens specific for *Myco-bacterium tuberculosis* to replace purified protein derivative has therefore been a major international research priority. We have performed a rigorous assessment of the diagnostic potential of antigens that are lacking from the *M. bovis* bacille Calmette-Guérin vaccine strains, as well as from most non-tuberculous mycobacteria. We have identified three antigens with a major diagnostic potential: ESAT-6, CFP-10 and TB 7.7. These antigens are currently used in IGRA tests such as the QuantiFERON® that measure the production of interferon-γ from sensitized T lymphocytes, thereby signalling ongoing infection. In the EU, US and Japan, where these tests have entered the market, the value of this approach in contact tracing has rapidly become apparent. I will suggest that such tests can be modified to identify the individuals among the latently-infected, at most risk of developing active contagious TB. Targeted treatment of this part of the population offers the possibility of preventing TB before it becomes infectious, which would greatly contribute to the eventual control of this global epidemic.

2. Immune responses specific for *M. tuberculosis* antigen—peripheral blood and sites of inflammation: Haruyuki ARIGA (National Hospital Organization Tokyo National Hospital)

To develop a more accurate method for diagnosing active tuberculous pleuritis, as well as peritonitis, meningitis and pericarditis of tuberculous origin, we established an antigen-specific interferon-γ (IFN-γ) release assay using cavity fluid specimens. Study subjects were 30 patients with bacteriologically confirmed active tuberculous serositis and 49 patients with definitive nontuberculous etiology. Culture was performed for 18 h with fluid mononuclear cells in the supernatant of the effusion together with saline or *Mycobacterium tuberculosis*-specific antigenic peptides, early secretory antigenic target 6 and culture filtrate protein 10. IFN-γ concentrations in the culture supernatants were measured by ELISA. In patients with active tuberculous serositis, antigen-specific IFN-γ responses of cavity fluid samples were significantly higher than those of nontuberculous effusion samples. Area under the receiver operating characteristic curve was significantly greater for cavity fluid IFN-γ response than for cavity fluid adenosine deaminase and whole-blood IFN-γ release assay. The cavity fluid IFN-γ release assay could be a non-invasive method for accurately and promptly diagnosing tuberculous serositis in patients in whom active tuberculosis in the cavity space is clinically suspected but for which no bacteriological evidence can be obtained.

3. Several questions in IGRAs: Kazue HIGUCHI (Research

Institute of Tuberculosis, Japan Anti-Tuberculosis Association)

Although it has been recommended to use QFT-2G for contact investigations in the revised guideline for contact investigation last year, there are several questions in QFT-2G. In this mini-symposium, data on several questions in the QFT-2G test were presented. These included the application of QFT-2G to vulnerable individuals in immune system such as infants and HIV-positives, the effects of chemotherapy on the QFT-2G test, the prognosis of development of active TB by QFT-2G, the next generation of QFT-2G, quality assurance of the QFT-2G test, and some problems of the current QFT-2G test. It should be important to research these questions and improve IGRAs based on the basic immunology.

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[1]National Hospital Organization Tokyo National Hospital, [2]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

Correspondence to : Nobuyuki Harada, Immunology Division, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, 3－1－24, Matsuyama, Kiyose-shi, Tokyo 204－8533 Japan. (E-mail : harada@jata.or.jp)