The NTM Host Research ConsortiumInternational workshop 2024

Disseminated NTM disease

“Toward future international collaborative research for NTM”

February 24 2024

Time schedule : Japan Time

14:00 Video message: **Hiroto Yasuura,** *Japan Science and Technology Agency*
Welcome address: **Yoko Yamaguchi**, *Japan Science and Technology Agency*

14:05 opening remarks
Naoki Hasegawa, *Keio University, Japan*

Part1 Chair : **Ho Namkoong**, *Keio University, Japan*

14:10「Genome-wide meta-analysis identifies novel loci for nontuberculous mycobacterial pulmonary disease and a potential causal relationship with body mass index」
Kyungtaek Park, *Institute of Health and Environment, Seoul National University, South Korea*

14:30「The Philippines NTRL and the TB Laboratory Network」
Ramon P. Basilio, *Research Institute for Tropical Medicine, Department of Health, Philippines*

14:50「Respiratory Bacterial Microbiome in Nontuberculous Mycobacteria Pulmonary Disease」
Byung Woo Jhun, *Samsung Medical Center, South Korea*

15:10「Precision practice in NTM infection associated with anti-interferon-gamma autoantibodies」
Doris Un-In Wu, National Taiwan University College of Medicine, Taiwan

15:30-35 short break

Part2 Chair : **Ho Namkoong**, *Keio University, Japan*

15:35「Decoding the *Mycobacterium tuberculosis* (MTB) Genome:
Optimizing Whole Genome Sequencing for Improved Drug-resistance profiling」
Ma. Angelica A. Tujan, *Research Institute for Tropical Medicine, Department of Health, Philippines*

15:55「Genomic epidemiology of Mycobacterium tuberculosis in the Philippines, a high-burden country and overview of NTM genomic diversity study in the Philippines」
Dodge R. Lim, *Research Institute for Tropical Medicine, Department of Health, Philippines*

16:15「An Epidemiological Survey on NTM-PD in Southeast Asia」
Masaki Inoue, *Keio University, Japan*

16:35「Single-cell transcriptomics of blood identified *IFIT1*$^{+}$ Neutrophil subcluster expansion in NTM-PD patients」
Wei Sha, *Shanghai Pulmonary Hospital, School of Medicine, Tongji University, China*

16:55 Closing Remarks
Ho Namkoong, *Keio University, Japan*

Abstracts

Part1
14:05 ～

Genome-wide meta-analysis identifies novel loci for nontuberculous mycobacterial pulmonary disease and a potential causal relationship with body mass index

Kyungtaek Park

Institute of Health and Environment, Seoul National University, Republic of Korea

This study investigates nontuberculous mycobacteria pulmonary disease (NTM-PD), a condition with a rising global incidence. Genome-wide association studies on Korean and Japanese cohorts reveal three genetic loci associated with NTM-PD. These loci impact the splicing patterns of *IL1R1* and expression levels of *PDE8B* and *CHP2* genes in lung tissue. Additionally, the study establishes a significant negative genetic correlation between body mass index (BMI) and NTM-PD. Mendelian randomization analysis suggests a potential causal relationship, with low BMI increasing the risk of NTM-PD. Overall, this research identifies genetic factors and low BMI as potential risk factors for NTM-PD.

14:25 〜

The Philippines NTRL and the TB Laboratory Network

Ramon P. Basilio
Medical Specialist IV
Head, National Tuberculosis Reference Laboratory
Research Institute for Tropical Medicine, Department of Health, Philippines

The National Tuberculosis Reference Laboratory (NTRL) at the Research Institute for Tropical Medicine is pivotal in the Philippines' TB Laboratory Network and the Department of Health's TB eradication strategy. NTRL provides reference services, training, quality assessment, and support for the National TB Control Program. Central to the TB Laboratory Network Strategic Plan, NTRL aims to increase access to quality TB diagnostics, ensure service continuity, strengthen laboratory quality management, and enhance the utilization of TB laboratory information and research. It plays a crucial role in advancing the cascade of care for TB testing and diagnostics in the country's comprehensive strategy to combat tuberculosis.

14:45 ～

Respiratory Bacterial Microbiome in Nontuberculous Mycobacteria Pulmonary Disease

Byung Woo Jhun

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Republic of Korea.

Nontuberculous mycobacteria (NTM) are ubiquitous organisms found in the environment and can cause pulmonary disease (PD), with the global burden increasing. Among the approximately 200 NTM species, *Mycobacterium avium* complex is the most common causative agent, while *Mycobacterium abscessus* is known for its high treatment resistance, making it a significant focus in clinical practice. NTM-PD can occur in immunocompromised individuals, but a significant proportion of NTM-PD patients show no clear evidence of immunodeficiency and exhibit a typical phenotype known as 'Lady Windermere syndrome.' Therefore, NTM-PD is currently recognized as a multifactorial disease, with its pathogenesis assumed to involve interactions between NTM virulence, the host's immune system, and the microbial environment.

Recently, the association between the respiratory tract's microbial environment and diseases has been reported. The microbiome, composed of microbial communities, has been employed to understand the progression and pathogenesis of various respiratory diseases. Especially, culture-independent techniques, such as targeted sequencing of the 16S rRNA gene, aid in identifying bacterial microbes and characterizing the taxa present in a respiratory bacterial microbiome. However, to date, there has been limited data on the microbiome's role in influencing the course of NTM-PD.

In recent times, our research team at my institution has been conducting bacterial microbiome analysis using lung tissue and sputum samples from NTM-PD patients. Some of this data has been published in research papers. Therefore, in this lecture, I will review recent literature on the microbiome in NTM-PD and briefly share and explain the research findings conducted by my research team.

15:05 ～

Precision practice in NTM infection associated with anti-interferon-gamma autoantibodies

Doris Un-In Wu
Associate Professor, Department of Internal Medicine,
National Taiwan University College of Medicine

In addressing the challenge of frequent misdiagnosis in Adult-Onset Immunodeficiency Syndrome associated with neutralizing anti-interferon-gamma autoantibodies (AIGA), Dr. Wu and her research team have undertaken a comprehensive diagnostic approach. They initially validated the QuantiFERON assay for efficient AIGA screening. This, coupled with the identification of distinctive clinicopathological features, aim not only to facilitate the differentiation between AIGA-associated lymphadenopathy and lymphoma, but also to gain insights into the disorder's pathogenesis. Ongoing efforts are directed towards exploring serological and radiographic markers to predict clinical outcomes. This multifaceted approach aims to guide precision management for AIGA patients.

Part2 Chair : Ho NAMKOONG, *Keio University, Japan*

15:35 ～

Decoding the *Mycobacterium tuberculosis* (MTB) Genome: Optimizing Whole Genome Sequencing for Improved Drug-resistance profiling

Ma. Angelica A. Tujan
Senior Science Research Specialist
Head, Research and Development Section
Molecular Biology Laboratory
Research Institute for Tropical Medicine, Department of Health, Philippines

In response to the rising burden of drug-resistant tuberculosis (TB), we are optimizing a whole genome sequencing (WGS) protocol for TB research and clinical applications. By analyzing 31 MTB samples using Oxford Nanopore Technology (ONT) and Illumina sequencing platforms, we achieved >98% genome coverage and identified L1 and L4 lineages. Importantly, this protocol detected mutations conferring resistance to key drugs in 80.6% (25/31) of samples. Notably, the most prevalent resistance mutations were isoniazid (67.7%; 21/31), ethionamide (64.5%; 20/31), streptomycin (48.4%; 15/31), and rifampicin (25.8%; 8/31). These results highlight the potential of WGS for drug resistant profiling and treatment tailoring and with further optimization, this streamlined WGS protocol holds promise for tackling the evolving challenges of TB control.

15:55 〜

Genomic epidemiology of Mycobacterium tuberculosis in the Philippines, a high-burden country and overview of NTM genomic diversity study in the Philippines

Dodge R. Lim
Science Research Specialist II
National Tuberculosis Reference Laboratory
Research Institute for Tropical Medicine, Department of Health, Philippines

The Philippines faces a high burden of tuberculosis (TB), exacerbated by the rise in multi-drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) *Mycobacterium tuberculosis* strains, posing challenges to disease control. Analyzing genome sequencing data from 732 isolates collected during drug resistance surveys (2011-2019), we identified prevalent lineages 1 (72.5%) and 4 (23.8%), notably the Manila strain (lineage 1.2.1.2.1). A substantial proportion exhibited at least MDR-TB (66.0%), with emerging XDR-TB genotypic resistance (0.4%). Transmission clusters revealed prison-based transmission and mutations linked to bedaquiline and previously unreported resistance mutations. Meanwhile, the scientific enigma of non-tuberculous mycobacteria in the Philippines remains unexplored. A proposed collaboration between the Philippines and Japan seeks to unveil this scientific mystery, potentially accelerating TB and NTM research and control strategies.

16:15 ～

An Epidemiological Survey on NTM-PD in Southeast Asia

Masataka INOUE
Keio University, Japan

For many years, NTM pulmonary disease (NTM-PD) has not been considered as seriously as tuberculosis. However, it is a condition that can worsen if not treated properly. In order to establish a system that allows for more accurate diagnosis and appropriate treatment of NTM-PD in Southeast Asia, we conduct a survey using Google Form. Not only does it enable us to estimate the incidence of NTM-PD, it would also reveal how the situations are different among the hospitals. Even if NTM infection is not tested in a hospital, this survey is still important; identifying the reasons for not testing can contribute to the improvement of medical services in the region.

16:35～

Single-cell transcriptomics of blood identified *IFIT1*$^{+}$ Neutrophil subcluster expansion in NTM-PD patients

Wei Sha
Shanghai Pulmonary Hospital, School of Medicine, Tongji University, China

Objective

Non-tuberculous mycobacterial pulmonary disease (NTM-PD) is caused by an imbalance between pathogens and impaired host immune responses. Mycobacterium avium complex (MAC) and Mycobacterium abscessus (MAB) are the two major pathogens that cause NTM-PD. In this study, we sought to dissect the transcriptomes of peripheral blood immune cells at the single-cell resolution in NTM-PD patients and explore potential clinical markers for NTM-PD diagnosis and treatment.

Methods

Peripheral blood samples were collected from six NTM-PD patients, including three MAB-PD patients, three MAC-PD patients, and two healthy controls. We employed single-cell RNA sequencing (scRNA-seq) to define the transcriptomic landscape at a single-cell resolution. A comprehensive scRNA-seq analysis was performed, and flow cytometry was conducted to validate the results of scRNA-seq.

Results

A total of 27,898 cells were analyzed. Nine T-cells, six mononuclear phagocytes (MPs), and four neutrophil subclusters were defined. During NTM infection, naïve T-cells were reduced, and effector T-cells increased. High cytotoxic activities were shown in T-cells of NTM-PD patients. The proportion of inflammatory and activated MPs subclusters was enriched in NTM-PD patients. Among neutrophil subclusters, an IFIT1 + neutrophil subcluster was expanded in NTM-PD compared to healthy controls. This suggests that IFIT1+ neutrophil subcluster might play an important role in host defense against NTM. Functional enrichment analysis of this subcluster suggested that it is related to interferon response. Cell-cell interaction analysis revealed enhanced CXCL8-CXCR1/2 interactions between the IFIT1+ neutrophil subcluster and NK cells, NKT cells, classical mononuclear phagocytes subcluster 1 (classical Mo1), classical mononuclear phagocytes subcluster 2 (classical Mo2) in NTM-PD patients compared to healthy controls.