（報告様式４）

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I. 基本情報

事　業　名：（日本語）地球規模保健課題解決推進のための研究事業　日米医学協力計画
（英　語）Research Program on the Challenges of Global Health Issues: U.S.-Japan Cooperative Medical Sciences Program

研究開発課題名：（日本語）日米医学協力計画を基軸とした抗酸菌症に関する研究
（英　語）Research on mycobacteriosis through U.S.-Japan Cooperative Medical Sciences Program

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II. 成果の概要（総括研究報告）

（日本語）

結核は地球規模で蔓延しており、2015 年には推定約 1040 万人の新規患者と約 180 万人の死亡者を出している最重要感染症の一つである。ハンセン病に関しても、患者数が減少したとは言うものの、未だに全世界では約 22 万人の新規患者を出しているのが現状である。一方、非結核性抗酸菌、特に *Mycobacterium avium* complex (MAC) による感染症の数は先進国において増加傾向にある。したがって、結核のみならず抗酸菌症全般の対策が喫緊の課題となっていると言っても過言ではない。しかしながら、現状の抗酸菌症対策は十分なものと言えず、現在の様な状況になっているものと考えられる。そこで本研究開発では、抗酸菌対策において重要な新規の診断、予防、治療法の開発へと繋げるために、以下の基礎的研究から、応用研究までを網羅的に実施した。

アジアで分離された多剤耐性結核菌の分子遺伝学的解析

ミャンマーで 2010 年と 2012 年に分離された多剤耐性結核菌 277 株の遺伝子型解析により、ミャンマーで分離された北京株多剤耐性結核菌株におけるクラスター形成率が比較的低い事を見いだし、薬剤耐性結核が治療の失敗により出現している事を示すことを明らかにした。

抗酸菌におけるイソニアジド耐性に関わる研究

台湾 CDC で分離された INH 耐性結核菌のうち、*katG* 遺伝子上に既知の遺伝子変異が認められなかった菌株において新規遺伝子変異を同定し、変異を持つ組換え型 KatG を作製してその酵素機能を明らかにした。

薬剤耐性結核菌、結核再治療と関連する宿主遺伝子多型と遺伝子発現様式の検討

ベトナムとの共同研究により、結核再治療時に多剤耐性菌が検出された患者群約100名と多剤耐性を示さない新規結核患者群約100名について、治療開始前の全血中のDNA、RNAを用いて、抗結核応答に関わると考えられる抗結核免疫関連遺伝子の多型頻度、ならびにmRNA発現量を検討し、MBLの多型と多剤耐性結核の関連を見いだした。

ハンセン病起因菌同定に関する研究

らい菌、*M. lepromatosis* および *M. haemophilum* 塩基配列を基に種鑑別可能領域の同定を行い、ハンセン病のもう一つの原因菌として *M. leprae* と近縁の *M. lepromatosis* の疫学調査に応用可能な PCR による判別法を確立した。

ハンセン病早期診断法の開発

中華人民共和国の西南部地域の 77 名のハンセン病患者、家族内接触者 60 名、健常人 53 名の血清を対象として抗 PGL-I IgM 抗体、抗 MMP-II IgG 抗体を測定して、抗 MMP-II IgG 抗体の有用性を証明した。更に、エキソソームがハンセン病の診断法の開発に応用できることを証明した。

非定型抗酸菌症血清診断法の国内外医療機関におけるバリデーション

刀根山病院で肺 MAC 症と診断された患者検体および、台湾で HIV 陽性と診断された血清 586 検体を用いて、“キャピリア MAC 抗体 ELISA”の有効性を証明した。

抗酸菌の感染症モデルの作製及び宿主免疫応答に関する研究

実験的マウスMAC症モデルにおけるクラリスロマイシンの治療成績と臨床での臨床像とに相関がみられるかを検討し、in vitroの感受性と治療効果に必ずしも相関が確認できないことを明らかにした。

新規抗酸菌受容体を介する宿主免疫応答機構の解明

C 型レクチン受容体 DCAR（dendritic cell immunoactivating receptor）が結核菌の AcPIM2、Ac2PIM2 を認識して、結核菌に対する Th1 応答に寄与することを明らかにした。

肺結核における IL-17 サイトカイン･ファミリー依存性免疫応答の解明

BCG 感染肺における IL-17F の発現動態を調べて、IL-17A と IL-17F の機能分担がその局在性により規定される可能性があることを明らかにした。

抗酸菌の遅延発育、長期生存及び薬剤抵抗性におけるヒストン様タンパク質の役割の解明

Mycobacterial DNA-binding protein 1 (MDP1) が抗酸菌の細胞機能をグローバルに制御することによって、菌の遅延発育、長期生存、薬剤への低感受性化など、病態に関連する形質の発現を制御していることを示し、MDP1は既存の抗結核薬との相乗効果が期待できる創薬ターゲットとなる可能性を示した。

部会の運営

2017 年 1 月 17 日に東村山市において日米医学協力計画抗酸菌症部会平成 28 年度研究報告会(班会議)を開催した。更に、2017 年 2 月 9 -10 日に韓国のソウル市において日米合同会議である US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting を共催した。

(英語)

Tuberculosis is spreading globally and is one of the most important infectious diseases with about 10.4 million estimated new cases and about 1.8 million estimated deaths in 2015. Although the number of new cases of leprosy has decreased, there are still about 220,000 new cases worldwide. On the other hand, the number of diseases caused by nontuberculous mycobacteria, especially *Mycobacterium avium* complex (MAC), is increasing in developed countries. Therefore, establishment of proper control measures against not only tuberculosis but also mycobacterosis caused by mycobacteria other than *M. tuberculosis* are urgent issues. However, the current measures against infectious diseases caused by mycobacteria seems not to be satisfactory. Hence, in this program, in order to develop novel diagnostic, preventive and therapeutic methods important for measures against infectious diseases caused by mycobacteria, fundamental to applied research were carried out comprehensively.

Molecular analysis of multidrug-resistant (MDR) *M. tuberculosis* strains isolated in Asia

Genotyping of 277 MDR-*M. tuberculosis* strain isolated in 2010 and 2012 in Myanmar demonstrated that the cluster formation rate in MDR-Beijing strain isolated in Myanmar was relatively low and that MDR-tuberculosis may emerged due to treatment failures.

Study on isoniazid resistance in mycobacteria

Among INH-resistant *M. tuberculosis* isolated by Taiwan CDC, a novel gene mutation was identified in a strain that had no known genetic mutation on the *katG* gene. In addition, recombinant KatGs having mutations were expressed and their enzymatic activities were reviled.

Elucidation of host gene polymorphism and gene expression pattern related to Drug-resistant *M. tuberculosis* and retreatment

In collaboration with Vietnam, DNA and RNA derived from about 100 patients with MDR-tuberculosis and about 100 new TB patients with none-MDR-tuberculosis were examined for allele frequencies and mRNA expression level of anti-tuberculosis immune-related genes and found the relationship between polymorphism in mannose-binding lectin gene and MDR-tuberculosis.

Study on the identification of causative bacteria of leprosy

Species-distinguishable regions were identified based on the comparison of *M. leprae*, *M. lepromatosis* and *M. haemophilum* genome and PCR applicable for epidemiological investigation of *M. lepromatosis* closely related to *M. leprae* was developed.

Development of methods that enable early diagnosis of leprosy

The anti-PGL-I IgM antibody and anti-MMP-II IgG antibody in sera of 77 leprosy patients, 60 household contacts and 53 healthy individuals in the Southwestern region of the People's Republic of China were measured and confirmed the superiority of the measurement of anti-MMP-II IgG antibody. In addition, applicability of exosomes for the development of diagnostic methods of leprosy was confirmed.

Validation of serodiagnosis for the infectious diseases caused by nontuberculous mycobacteria at Japanese and overseas medical facilities

We demonstrated the effectiveness of capillia MAC antibody ELISA using 586 patient sera who were diagnosed as MAC infection at Toneyama hospital or as HIV positive in Taiwan.

Establishment of model of mycobacteriosis and study on host immune response

We established the mouse model of MAC infection and investigated the correlation between the clinical symptom and the effect of clarithromycin and clarified that the correlation between in vitro sensitivity and therapeutic effect could not necessarily be seen.

Elucidation of the mechanism of host immune response via novel mycobacteria receptors

We clarified that the C type lectin receptor DCAR (dendritic cell immunoactivating receptor) recognizes *M. tuberculosis* through AcPIM 2, Ac2PIM2 and contributes to Th 1 response against *M. tuberculosis.*

Elucidation of IL-17 cytokine family dependent immune response in pulmonary tuberculosis

Investigating the expression kinetics of IL-17F in BCG-infected lungs revealed that the localization of IL-17A and IL-17F could regulate the function sharing.

Elucidation of the role of histone-like protein in slow growth, long-term survival and drug resistance in mycobacteria

We elucidated that mycobacterial DNA-binding protein 1 (MDP1) regulated the cellular function of mycobacteria, thereby controlling the expression of traits associated with pathogenicity such as slow growth, long-term survival and reduced susceptibility to drugs and indicated that MDP1 can be a target that can express synergistic effects with conventional anti-tuberculosis drugs.

Management of the Mycobacterial Panel

On January 17, 2017, we held the US-Japan Medical Cooperation Program Mycobacterial Panel domestic meeting at Higashimurayama city. In addition, we held the US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting in Seoul City, Korea, on February 9 - 10, 2017.

III. 成果の外部への発表

（１）学会誌・雑誌等における論文一覧（国内誌　　件、国際誌　　件）

1. Thanda Tun, Khin Saw Aye, Wint Wint Nyunt, Crump JA, Nakajima C, Suzuki Y, Kyi Kyi Thinn, Cook GM, Htin Lin Aung. Genotypic Diversity of *Mycobacterium tuberculosis* strains in Myanmar. Infect Dis, 2017; 49: 237-239
2. Yamaguchi T, Yokoyama K, Nakajima C, Suzuki Y. DC-159a shows inhibitory activity against DNA gyrases of *Mycobacterium leprae.* PLoS Neglect Tropical Dis, 2016; 10: e0005013
3. Matsuba T, Siddiqi U, Hattori T, Nakajima C, Fujii J, Suzuki Y. Antigenic characterisation of dimorphic surface protein in *Mycobacterium tuberculosis*. *FEMS Microbiol Lett,* 2016; 363: fnw082
4. Thuong PH, Tam DB, Sakurada S, Hang NT, Hijikata M, Hong LT, Ngoc PT, Anh PT, Cuong VC, Matsushita I, Lien LT, Keicho N. Circulating granulysin levels in healthcare workers and latent tuberculosis infection estimated using interferon-gamma release assays. BMC Infect Dis. 2016; 16:580.
5. Miyamoto Y, Mukai T, Matsuoka M, Kai M, Maeda Y, Makino M. Profiling of Intracellular Metabolites: An Approach to Understanding the Characteristic Physiology of *Mycobacterium leprae*. PLOS Neglected Tropical Diseases, 2016; 10: e0004881
6. Tsukamoto Y, Maeda Y, Tamura T, Mukai T, Mitarai S, Yamamoto S, Makino M. Enhanced protective efficacy against tuberculosis provided by a recombinant urease deficient BCG expressing heat shock protein 70-major membrane protein-II having PEST sequence. Vaccine. 2016; 34: 6301-6308.
7. Kai M, Fafutis-Morris M, Miyamoto Y, Mukai T, Mayorga-Rodriguez J, Rodriguez-Castellanos MA, Martínez-Guzman MA, Matsuoka M. Mutations in the drug resistance-determining region of *Mycobacterium lepromatosis* isolated from leprosy patients in Mexico. J Dermatol. 2016; 43: 1345-1349.
8. Morimoto K, Hasegawa N, Izumi K, Namkoong H, Uchimura K, Yoshiyama T, Hoshino Y, Kurashima A, Sokunaga J, Shibuya S, Shimojima M, Ato M, Mitarai S. A Laboratory-based Analysis of Nontuberculous Mycobacterial Lung Disease in Japan from 2012 to 2013Ann Am Thorac Soc. 2017; 14: 49-56.
9. Stinson K, Kurepina N, Venter A, Fujiwara M, Kawasaki M, Timm J, Shashkina E, Kreiswirth BN, Liu Y, Matsumoto M, Geiter L. MIC of Delamanid (OPC-67683) against Mycobacterium tuberculosis Clinical Isolates and a Proposed Critical Concentration. Antimicrob Agents Chemother. 2016; 60: 3316-3322.
10. Kerscher B, Dambuza IM, Christofi M, Reid DM, Yamasaki S, Willment JA, Brown GD. Signalling through MyD88 drives surface expression of the mycobacterial receptors MCL (Clecsf8, Clec4d) and Mincle (Clec4e) following microbial stimulation. Microbes Infect. 2016; 18: 505-509.
11. Umemura M, Okamoto-Yoshida Y, Yahagi A, Touyama S, Nakae S, Iwakura Y, Matsuzaki G. Involvement of IL-17A-producing TCR γ δ T cells in late protective immunity against pulmonary *Mycobacterium tuberculosis* infection. Immun Inflamm Dis. 2016; 4: 401-412.
12. Hatano S, Tamura T, Umemura M, Matsuzaki G, Ohara N, Yoshikai Y. Recombinant *Mycobacterium bovis* bacillus Calmette-Guérin expressing Ag85B-IL-7 fusion protein enhances IL-17A-producing innate γ δ T cells. Vaccine. 2016; 34: 2490-2495.
13. Totani T, Nishiuchi Y, Tateishi Y, Yoshida Y, Kitanaka H, Niki M, Kaneko Y, Matsumoto S. Effects of nutritional and ambient oxygen condition on biofilm formation in *Mycobacterium avium subsp. hominissuis* via altered glycolipid expression. Sci Rep. 2017; 7: 41775.

（２）学会・シンポジウム等における口頭・ポスター発表

1. Sero-diagnosis of Mycobatcerium avium complex lung diseases from international collaboration studies.口頭. Ato M, Kitada S, Jou R, Wang J-Y, Kobayashi K. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea,2017/02/09-10, 海外.

2. Polymorphisms and blood expression levels of interleukin 12 receptor subunit beta2 gene in tuberculosis. 口頭. Hijikata M, Matsushita I, Kato-Kogoe N, Hang NTL, Do Tam DB, Cuong VC, Thuong PH, Naoto Keicho. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
3. Efforts to detect paucibacillary leprosy patients by serological methods. 口頭. Maeda Y, Mukai T, Tsukamoto Y, Tamura T. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
4. Current status of delamanid, and a new candidate compound against Tuberculosis. 口頭. Matsumoto M. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
5. HDL cholesterol is the risk for *Mycobacterium tuberculosis* infection and suppresses TLR2 - dependent TNF-α production from *M. tuberculosis*–infected macrophages. 口頭. Matsumoto S. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
6. Molecular characterization of a nicotinate phosphoribosyltransferase from *Mycobacterium tuberculosis* H37Rv. 口頭. Mori S, Kim H, Rimbara E, Shibayama. K. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
7. Production and screening of M. leprae specific antigens for detection of leprosy. 口頭. Mukai T, Maeda Y, Miyamoto Y. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
8. Role of IL-17 producing gamma/delta T cells in the granuloma formation in the lung duringmycobacterial infection. 口頭. Umemura M, Okamoto Y, Yahagi A, Nakae S, Iwakura Y, Matsuzaki G. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
9. Recognition of mycobacterial glycolipids via C-type lectin receptors. 口頭, Yamasaki S. US-Japan Cooperative Medical Science Program Mycobacterial Panel Meeting, Seoul, Republic of Korea, 2017/02/09-10, 海外.
10. Molecular and Phenotypic Surveys for Multidrug-Resistant Tuberculosis in South East Asia. 口頭. Suzuki Y, Aye KS, Aung WW, Phetsuksiri B, Nakajima C. 19th International Conference on Emerging Infectious Diseases in the Pacific Rim, Seoul, Republic of Korea, 2017/02/07-8, 海外.

（３）「国民との科学・技術対話社会」に対する取り組み
該当なし

（４）特許出願
該当なし