Original Article

DRUG RESISTANCE IN RECURRENT CASES OF TUBERCULOSIS

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Abstract [Purpose] Investigate drug resistant rate in recurrent cases after cure, or after drop-out from treatment, and the analysis of the risk factors for acquired drug resistance in these cases.

[Object] Patients who were previously treated for tuberculosis that was drug sensitive or unknown about previous drug sensitivity, and were hospitalized to Fukujuji Hospital to start treatment for recurrent tuberculosis from Jan. 1, 1993 to Dec. 31, 2003. Primary drug resistant cases were excluded and cases were further divided into full sensitive cases and cases with drug sensitivity test results were unknown.

[Method] Chart review.

[Result] Drug resistant rate (any resistance to INH, RFP, SM, EB) in all recurrent cases (N=200) was 16.5%. Availability of previous drug sensitivity results affected on the drug resistant rate in recurrent cases. In previously pan-sensitive cases, drug resistant rate was 4.3% and it was lower than the rate in primary treated cases. No significant risk factor for acquired drug resistance was not found, including poor adherence to medication.

[Conclusion] Every effort should be made to know the previous drug sensitivity results because these results have a major impact on drug resistance rate at recurrence. Doctor's miss managements of tuberculosis patients might be the major factor for acquired drug resistance in recurrent cases, and it is needed for the improvement of tuberculosis control program to implement the measures to control these miss managements.

Key words: Recurrent tuberculosis, Drug resistance, Poor adherence, Drop-out

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Case Report

ACUTE ONSET OF SOMNOLENCE AND AMNESIA DUE TO CEREBRAL INFARCTION OF BILATERAL THALAMUS ACCOMPANIED WITH TUBERCULOUS MENINGITIS: A CASE REPORT

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Abstract A 55-year-old man was admitted to our hospital because of somnolence and aspontaneity. He was hospitalized in the psychopathic ward under the initial diagnosis of depression. Chest X-ray showed infiltration in both upper lobe. Twelve days later, *Mycobacterium tuberculosis* was detected from his sputum and was confirmed by RT-PCR. Cerebrospinal fluid findings showed elevated ADA and mononuclear cells, suggesting the presence of tuberculous meningitis. However, the brain CT revealed no abnormal findings. By applying antituberculous treatment the pulmonary lesion improved but psychological symptoms remained. Three months later follow-up brain MRI was examined. Contrast enhanced granuloma was detected in the ambiens, suprasellar and quadrigeminal cisterns. A strong signal was seen in the left frontal thalamus and a weak enhanced lesion was detected in the right frontal thalamus on a T2 enhanced image. These lesions showed low intensity on a T1 enhanced image, suggesting cerebral infarction affecting the bilateral thalamus. Somnolence and memory disorder was due to cerebral infarction of the bilateral thalamus and tuberculous meningitis contributed to form the intracranial lesion. From the experience of this case, it is needed to consider cerebral infarction (especially the thalamus) due to tuberculous meningitis when we examine the patients with acute onset of psychological symptoms.

Key words: Cerebral infarction, Thalamus, Somnolence, Amnesia, Tuberculous meningitis, Pulmonary tuberculosis

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Case Report

PULMONARY TUBERCULOSIS CASE WITH CONSISTANT FINDINGS OF ENDOBRONCHIAL SPREAD IN CHEST ROENTOGENOGRAPHY FOR ABOUT THREE YEARS : A CASE REPORT

Atsushi HIRANO, Atsuhiko TADA, Nagio TAKIGAWA, Noriko KAWATA, Goro KIMURA, Takuo SHIBAYAMA, Chiharu OKADA, Ryo SODA, and Kiyoshi TAKAHASHI

Abstract We reported a case of pulmonary infection by *Mycobacterium tuberculosis* complicated by endobronchial spread.

Chest roentogenography and CT for an 85-year-old male complaining of cough showed endobrochial spread in right upper lung field. His sputum culture for eight weeks showed 10−20 colonies of *Mycobacterium tuberculosis*. Transbronchial lung biopsy revealed granulomas with caseous necrosis. Findings in chest XP and CT after the therapy with INH, RFP and EB for six months showed much improvement.

Key words : Pulmonary tuberculosis, Endobronchial spread

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The 79th Annual Meeting Symposium

CUTTING EDGE OF BASIC STUDY ON TUBERCULOSIS

Chairpersons: [1]Masao MITSUYAMA and [2]Kazuo KOBAYASHI

Abstract During the past two decades, we have observed outstanding advance in tuberculosis research including immune response, identification of antigens/ligands of *M. tuberculosis* and molecular mechanism of drug resistance. Worldwide genome project enabled the whole genome sequence of *M. tuberculosis* H37Rv and has provided a great impact on the molecular genetics on virulence mechanism. Extensive study by new members is greatly encouraged in our country. Though the number of tuberculosis patients has decreased comparing to some 50 years ago, there are new concerns about the emergence of multi-drug resistant strains and a possible increase due to the increase of immunocompromised population. Now the basic study on the pathophysiology, virulence mechanism and vaccine development are urgently required.

In this symposium, we have invited leading researchers in the field of basic study of tuberculosis/*M. tuberculosis* in order to provide general overview of the cutting edge of basic study. Mycobacterium is rich in lipids or glycolipids that are quite unique in both structure and biological activity, and they contribute a lot to the pathophysiology of tuberculosis. Dr. Ikuya Yano (Japan BCG) reviewed the major classes of glycolipids and presented some applications to clinical and bacteriological diagnosis. *M. tuberculosis* appear to have various genes contributing to the slow growth, escape from macrophage killing and persistence, however, almost nothing has been clarified yet. Dr. Sokichi Matsumoto (Osaka City University) discovered MDP1 as a possible mycobacterial factor responsible for slow growth. He mentioned that MDP1 might be involved in the dormancy during the long course of disease development after primary infection. Dr. Kazuyoshi Kawakami (University of the Ryukyus) presented many interesting data on the importance of various cytokines, especially Th1 cytokines, to resistance against experimental *M. tuberculosis* infection in mice obtained by using cytokine-knock out mice. The relevance of animal data to clinical observation was discussed. Th1-dependent cell-mediated immunity is regarded as the most effective immune response in the protection. Dr. Ikuo Kawamura (Kyoto University) employed animal model in which only viable BCG induces protective Th1 cells while killed BCG does not, and showed that the difference is due to some proteinaceous factor release by viable BCG. He showed preliminary data on the purification of such IFN-γ-inducing factor from the culture filtrate of *M. tuberculosis*. BCG is the most widely employed live vaccine for tuberculosis in the world, however, more effective and safe vaccine is requested urgently because of the serious concern about the efficacy of BCG vaccination. In Japan, Dr. Masaji Okada (Kinki-Chuo Chest Medical Center) has been engaged actively in the development of new tuberculosis vaccines. He showed basic strategy for construction of new candidate vaccines and also presented some preliminary data on the trial using monkeys.

1. Molecular characterization and immunological properties of mycobacterial high molecular weight components: Yukiko FUJITA and Ikuya YANO (Japan BCG Laboratory)

Mycobacterial envelope contains a great variety of wax-like high molecular weight lipids which contribute to its strong hydrophobicity or acid-fastness and also play crucial role against host phagocytic cells at the early step of infection. Cord factor (trehalose 6,6′-dimycolate) and the related mycoloyl glycolipids are one of the most characteristic components in mycobacteria and are recognized to be a key molecule for pathogenicity and immunity. Recent progress of analytical techniques such as MS or NMR reveal the molecular structure-activity relationship. Lipoarabinomannan (LAM), lipomannan (LM) and core phospholipids (PIMx) are recently demonstrated to be one of the virulence factor, however some of which are shown to be IL-12 producer and apoptosis inducer. Mycobacterial sulfolipids play important role as a virulence factor together cord factor, but the mechanism seems to differ from TDM. *M. avium* complex produce serotype specific glycolipid (GPL) which suppresses the humans T cell response. The core structure is unique and carbohydrate shows high antigenicity to determine serotypes. Mycobacterial cell wall skeleton (CWS) plays the central role for maintaining the rigid structure and shows antitumor or infection prevention activities via the stimulation of innate immunity. Although at the present, their molecular structure-activity relationship is not fully understood, above components may play pivotal roles with the protein antigens for host immune responses.

2. Molecular mechanism of *M. tuberculosis* dormancy: Sokichi MATSUMOTO (Department of Host Defense, School of Medicine, Osaka City University)

Mycobacterium tuberculosis has remarkable ability to persist in the human host and infects both macrophages and nonprofessional phagocytes, such as alveolar epithelial cells. Glycosaminoglycans are considered as the component of mycobacterial adherence to epithelial cells. Mycobacterial DNA-binding protein 1 (MDP1) is suggested one of key molecules in latent infection. Here we show that extracellular MDP1 promotes mycobacterial adherence to A549 human lung epithelial cells through hyaluronan. Simultaneous treatment of intratracheal mycobacteria-infected mice with

HA reduced the growth of bacteria *in vivo*. Taken together, anti-MDP1 antibody or hyaluronan has potential as therapeutic and prophylactic interventions in mycobacterial infection.

3. A host defense mechanism against *Mycobacterium tuberculosis* mediated by Th1-related cytokines : Kazuyoshi KAWAKAMI (Division of Infectious Diseases, Department of Internal Medicine, Graduate School and Faculty of Medicine, University of the Ryukyus)

Th1-related cytokines play a central role in the host defense to *Mycobacterium tuberculosis* infection. IL-12 is a key cytokine for development of Th1 cells and IL-18 potentiates this response. Recently, two novel IFN-γ-inducing cytokines, IL-23 and IL-27, have been discovered. In a series of studies, we demonstrated the important roles of IL-12p40 and IL-18 in the host defense to this infection and proposed a possible host protective mechanism mediated by IFN-γ which synthesis is independent of IL-12, IL-18 and IL-23. Thus, host is likely protected from *M. tuberculosis* infection by various Th1-related cytokines in a more complicated manner.

4. Possible involvement of TLR2 ligand derived from *M. bovis* BCG and *M. tuberculosis* to generate protective immunity by inducing endogenous IFN-γ production : Ikuo KAWAMURA (Department of Microbiology, Kyoto University Graduate School of Medicine)

IFN-γ plays a pivotal role for development of protective T cells, and both IL-12 and IL-18 which are produced from macrophages are necessary to induce IFN-γ production. To identify the mycobacterial factor contributing to the Th1 cytokine productions, we prepared a culture filtrate from 1d-culture of *M. bovis* BCG and *M. tuberculosis*, and measured the cytokine-inducing activity. These culture filtrates elicited IL-12p40 production from peritoneal macrophages. They could induce NF-κB activation in HEK293 cells expressing TLR2 and the activity was sensitive to treatment with proteinase K and heating. These results suggest that an early secreted protein from BCG and *M. tuberculosis* activates macrophages to produce IL-12 via TLR2 dependent pathway. It is likely that this is a critical interaction between mycobacteria and macrophages for the generation of protective immunity.

5. Establishment and evaluation of novel vaccine against tuberculosis : Masaji OKADA (National Hospital Organization Kinki-Chuo Chest Medical Center, Clinical Research Center)

HVJ-liposome/HSP65 DNA＋IL-12 DNA vaccination was 100 fold more efficient than BCG on the elimination of *Mycobacterium tuberculosis* (M.TB) in the BALB/c mice. Cytotoxic T cells activity against M. TB was augmented. The recombinant (r) 72f BCG vaccine as well as HSP65＋IL-12 DNA vaccine showed stronger anti-TB immunity than BCG in the mice, and guinea pigs. By using these new vaccines (HSP65 ＋IL-12 DNA, r72f BCG and 72f fusion protein＋BCG) and the cynomolgus monkey models which are very similar to human tuberculosis, the prophylactic effect of vaccines was observed. Thus, these novel vaccines should provide a useful tool for the prevention of human TB infection.

Key words : Tuberculosis, *Mycobacterium tuberculosis*, Basic research

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