IMMUNITY AGAINST *MYCOBACTERIUM TUBERCULOSIS* (INTRODUCTION)

Masaji OKADA

Abstract A third of world's population infected with *Mycobacterium tuberculosis*, and 2 million people die from tuberculosis every year. It is well established that protective to *M. tuberculosis* depends on both $CD4^+$ and $CD8^+$ T cells[1)~8)].

In particular, acquired immunity (cytotoxic T cell, Th1 helper T cell and M ϕ) play an important role for TB infection. Recently, natural immunity also play a very attractive role for the development of TB immunity.

We found that memory CTL is most important for the protection against TB using several kinds of mice. It was demonstrated that DBA/1 mice are more sensitive to TB infection than BALB/c mice (Th2 prone mice). Induction of memory CTL in DBA/1 mice was lower than BALB/c. In contrast, IFN-γ production of DBA/1 mice was higher than BALB/c.

Therefore, famous researchers in the fields of TB immunity reviewed the recent advances of TB immunity, such as (1) T cell immunity and recognition against TB antigen, (2) TLR9 and CpG motif, (3) lipocalin2 and SLPI in natural TB immunity, (4) acquired immunity (CTL) and granulysin. The development novel vaccine (HSP65+IL-12 DNA vaccine), (5) The mechanism of protection against TB, in this mini-review series.

Key words: Immunity against M. TB, T cell, Acguired immunity, Innate immunity

Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center

Correspondence to: Masaji Okada, Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai-shi, Osaka 591-8555 Japan. (E-mail: okm@kch.hosp.go.jp)

T CELL-MEDIATED IMMUNE RESPONSES AND THE RECOGNITION OF TUBERCULOSIS ANTIGENS

[1]Kunio TSUJIMURA and [2]Yukio KOIDE

Abstract T cell-mediated immune responses profoundly contribute to the protection against the re-activation of latently infected *Mycobacterium tuberculosis*. Th1 cells produce IFN-γ to activate infected macrophages and promote the formation of granulomas around infected macrophages. $CD8^+$, $\gamma\delta$ and CD1-restricted T cells also produce IFN-γ and participate the protective responses against bacterial growth. Th17 cells produce IL-17 to promote the mobilization of immunocompetent cells and contribute to the granuloma formation. On the contrary, Th2 cells and Tregs interfere these protective immune responses.

Key words : *Mycobacterium tuberculosis*, Latent infection, Cellular immunity, Th1, IFN-γ

[1]Department of Infectious Diseases, and [2]Executive Director for Education and International Exchange, Hamamatsu University School of Medicine

Correspondence to : Yukio Koide, Executive Director for Education and International Exchange, Hamamatsu University School of Medicine, 1−20−1, Handa-yama, Higashi-ku, Hamamatsu-shi, Shizuoka 431−3192 Japan.
(E-mail: koidelb@hama-med.ac.jp)

Current Topics

CpG MOTIF AND TUBERCULOSIS IMMUNITY

Saburo YAMAMOTO

Abstract A potent immunostimulatory effect of DNA containing an unmethylated CpG motif was found in the course of research on water-soluble components from BCG possessing antitumor activity. Because such CpG motifs are relatively common in bacterial DNA, but rare in mammalian animal and plant DNA, they may be evolutionary adaptation augmenting innate immunity, most likely in response to pathogens that replicate within the host cells, such as viruses and intracellular bacteria. Microbial infection induces innate immunity by triggering pattern-recognition system. The infected cells produce proinflammatory cytokines that directly combat microbial invaders and express costimulating surface molecules, which develop adaptive immunity by inducing distinct T cell differentiation. Bacterial DNA with unmethylated CpG-DNA stimulates vertebrate immature immune cells to induce maturation and to produce Th1 cytokines as well as TNF-α. Therefore, CpG-DNA functions as an adjuvant for regulating the initiation of Th1 differentiation. DNA vaccine including genes encoding mycobacterial proteins either MPB64 or HSP65 was assessed the ability to prevent the growth of bacilli in the lungs and spleens of guinea pigs after pulmonary challenge of virulent *Mycobacterium tuberculosis* H37Rv. Immunization with two constructs such as MPB64 and HSP65 elicited protective responses compared to a vector control or saline control. The roles of immunostimulatory CpG motifs in DNA vaccine developments and therapeutic applications have been discussed.

Key words: CpG, BCG, Oligonucleotide, IFN, DNA

Japan BCG Laboratory

Correspondence to: Saburo Yamamoto, Japan BCG Laboratory, 3−1−5, Matsuyama, Kiyose-shi, Tokyo 204−0022 Japan. (E-mail: yamamoto@bcg.gr.jp)

Current Topics

POTENTIAL OF NOVEL ANTIMYCOBACTERIAL IMMUNE FACTORS, SLPI AND LIPOCALIN 2

Hiroyuki SAIGA and Kiyoshi TAKEDA

Abstract *Mycobacterium tuberculosis*, causing tuberculosis, is the pathogen that invades immune cells, especially macrophages, and evade from the host immune response. Recent studies have reported that *M. tuberculosis* also invade alveolar epithelial cells as well as alveolar macrophages. However, the role of alveolar epithelial cells in the host defense against *M. tuberculosis* remains unknown. In this study, we demonstrate that secretory leukocyte protease inhibitor (SLPI) and lipocalin 2 are secreted into the alveolar space by alveolar macrophages and epithelial cells during the early phase of respiratory mycobacterial infection. SLPI kills mycobacteria by enhancing the membrane permeability, and lipocalin 2 is internalized into the alveolar epithelial cells and inhibits intracellular mycobacterial growth by blocking iron uptake. Taken together, these findings highlight a pivotal role for alveolar epithelial cells during mycobacterial infection.

Key words: *Mycobacterium tuberculosis*, TLR, Alveolar epithelial cell, SLPI, Lipocalin 2

Laboratory of Immune Regulation, Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University

Correspondence to: Hiroyuki Saiga, Laboratory of Immune Regulation, Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, 2−2, Yamadaoka, Suita-shi, Osaka 565−0871 Japan.
(E-mail: saiga@ongene.med.osaka-u.ac.jp)

Current Topics

ANTI-TUBERCULOSIS IMMUNITY BY CYTOTOXIC T CELLS · GRANULYSIN AND THE DEVELOPMENT OF NOVEL VACCINES (HSP-65 DNA+IL-12 DNA)

Masaji OKADA and Yoko KITA

Abstract CDC and ACET in U.S.A. reported that novel vaccines instead of BCG are required for the protection against infection of *Mycobacterium tuberculosis* worldwide. However, no novel vaccine for clinical use has not yet been developed in the world including U.S.A. and Europe.

We have developed a novel tuberculosis (TB) vaccine; a combination of the DNA vaccines expressing mycobacterial heat shock protein 65 (HSP65) and interleukin 12 (IL-12) delivered by the hemagglutinating virus of Japan (HVJ)-envelope and -liposome (HSP65+IL-12/HVJ). This vaccine provided remarkable protective efficacy in mouse 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 also provided therapeutic efficacy against multi-drug resistant TB (MDR-TB) and extremely drug resistant TB (XDR-TB) 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. Furthermore, the BCG priming and HSP65+IL-12/HVJ vaccine (booster) by the priming-booster method showed a synergistic effect in the TB-infected cynomolgus monkey (100% survival). Furthermore, this vaccine exerted therapeutic efficacy (100% survival) and augmentation of immune responses in the TB-infected monkeys. These data indicate that our novel DNA vaccine might be useful against *Mycobacterium tuberculosis* including XDR-TB and MDR-TB for human therapeutic clinical trials.

The review also provides recent advances of the precise studies of induction of immunity including CD8 positive cytotoxic T cells and effector molecules such as granulysin by these vaccines, against multi-drug resistant tuberculosis and extremely drug resistant tuberculosis.

Key words: Killer T cell, Granulysin, New TB vaccine

Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center

Correspondence to: Masaji Okada, Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai-shi, Osaka 591-8555 Japan. (E-mail: okm@kch.hosp.go.jp)

PROTECTIVE IMMUNITY AGAINST *MYCOBACTERIUM TUBERCULOSIS*

Ikuo KAWAMURA

Abstract *Mycobacterium tuberculosis* (MTB) is an intracellular pathogen that has evolved strategies to enable growth in macrophages. The bacterium is able to inhibit fusion of phagosome with lysosome through secretion of some bacterial components and modulation of host cell intracellular signaling pathways. On the other hand, the complex system of protective immunity is expressed to control bacterial burden in host upon MTB infection. However, virulent MTB is capable of surviving in macrophages *in vivo* and persists in host even after acquired immunity has developed. These data suggest that MTB has developed a sophisticated immune evasion mechanism. In this issue, host protective response and the strategies of MTB for intracellular survival and immune evasion, which have been unraveled so far, are shown and the mechanisms are discussed.

Key words: *Mycobacterium tuberculosis*, Macrophage, Phagolysosome, Apoptosis, Necrosis, Cytokine, $CD4^+$ T cell, $CD8^+$ T cell, $\gamma\delta$ T cell

Department of Microbiology, Kyoto University Graduate School of Medicine

Correspondence to: Ikuo Kawamura, Department of Microbiology, Kyoto University Graduate School of Medicine, Yoshidakonoecho, Sakyo-ku, Kyoto-shi, Kyoto 606−8501 Japan. (E-mail: ikuo_kawamura@mb.med.kyoto-u.ac.jp)

Original Article

TUBERCULOSIS INFECTION BY TUBERCULIN SKIN TEST OR QuantiFERON AND RELATED FACTORS IN CONTACT INVESTIGATIONS

Kenji MATSUMOTO, Tomomi TATSUMI, Noriko KAMIYA, Kazuyo ARIMA, Shinichi KODA, and Satoshi HIROTA

Abstract [Objective] We investigated the factors related to secondary infections in contact group investigations.

[Method] From March, 2008 to February, 2009, a total of 457 tuberculosis (TB) notifications were reviewed by the Health Examination Committee of the Health Centers of Osaka City over indications of a contact investigation. A contact investigation was considered necessary for 92 cases with 620 contacts. For the contacts of these cases, the tuberculin skin test (TST) or / and QuantiFERON-TB 2nd Generation (QFT) was used. The contacts testing positive for QFT were classified as "infected". The contacts with "doubtful" QFT were classified as either "infected" or "non-infected" according to the QFT-positive rate of the group as a whole. Those with blisters in TST were classified as "infected".

[Results] Among the total of 84 "infected" cases thus defined, 56 were QFT-positive, 17 were doubtful, and 11 had blisters with TST. On the other hand, among the total of 515 "uninfected" cases, 18 were doubtful on the QFT test, 323 were QFT-negative and 174 were cases with TST of erythema less than 30 mm without QFT test. The relationship between characteristics of index cases and whether a contact was infected or not was as follows: Severe chest X-ray findings, sputum smear positivity, and a cough persisting for more than two months were significantly associated with infection of the contacts. The closeness of contact was also significantly related with a higher risk of infection, so that contact with an index case for more than 100 hours, and contact in a space less than 100 square meters were more likely to cause transmission of infection.

[Conclusion] Severity of chest X-ray findings, degree of smear positivity and period of cough were confirmed to be important in judging whether investigations are necessary. The time and space of contact were also considered to be important factors. We should consider these factors comprehensively in order to decide on indications for a contact investigation.

Key words: Contact examination, Infection risk, QFT, TST, Index case, Contact status

Osaka City Public Health Office

Correspondence to: Kenji Matsumoto, Health and Welfare Center of Nishinari Ward, Osaka City, 1−5−20, Kishinosato, Nishinari-ku, Osaka-shi, Osaka 557−8501 Japan.
(E-mail: ke-matsumoto@city.osaka.lg.jp)

Case Report

A CASE OF ABDOMINAL TUBERCULOSIS RELAPSED AFTER RESECTION

[1]Kazuyoshi NAKAMURA and [2]Tohru YAMANAKA

Abstract Abdominal wall tuberculosis is rare. We report a case of abdominal wall tuberculosis that relapsed after surgery. A 40-year-old man without a past history of tuberculosis visited our hospital complaining of an abdominal wall mass. The mass was resected in the department of orthopedics of our hospital. No bacteriological or histological examination of the resected specimen was done. After 5 months, the patient found swelling of the axillary lymph node. CT revealed left axillary lymph node swelling and chest wall nodules of various sizes. As the pus aspirated from the left axillary lymph node was positive for PCR-TB, the patient was diagnosed with relapsed chest wall tuberculosis and tuberculous lymphadenopathy. Anti-tuberculosis drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) were administered, but resistance to both isoniazid and ethambutol were revealed afterward. So, isoniazid and ethambutol were replaced with levofloxacin and streptomycin. After 6 months of this therapy, the left axillary lymph node decreased remarkably and became scarred. Abdominal tuberculosis should be considered in cases of an abdominal wall mass, regardless of whether the patient has a history of tuberculosis. This case stresses the importance of postoperative anti-tuberculosis treatment, as well as the need for bacteriological and histological examinations of resected specimens.

Key words: Abdominal wall tuberculosis, Drug resistant, Tuberculous lymphadenopathy, Relapse, Hyposensitization

[1]Respiratory Department, Taragi Municipal Hospital, [2]Respiratory Department, National Hospital Organization Kumamoto Minami National Hospital

Correspondence to: Kazuyoshi Nakamura, Respiratory Department, Taragi Municipal Hospital, 4210 Taragi, Taragi-machi, Kuma-gun, Kumamoto 868−0598 Japan.
(E-mail: kazu1124@pj9.so-net.ne.jp)

TUBERCULOSIS ANNUAL REPORT 2008
— Series 8. Treatment of TB (1) —

Tuberculosis Surveillance Center, RIT, JATA

Abstract The standard treatment of tuberculosis (TB) is the key to its control. Here we report on the statistics of treatment history and initial regimen for treating TB in 2008.

The frequency of retreatment among newly notified TB patients might be partly a indicator of previous insufficient treatment. In 2008, 24,760 TB patients were newly notified. Of those, 1,836 cases were reported as having had previous treatment and 424 cases were reported as unknown treatment history. The proportion of retreatment was 7.5%, excluding those of unknown treatment history. The proportion of retreatment among newly notified TB patients increased with age from their 20s (4.0%) to their 70s (9.4%).

Regarding the year of previous treatment, the number of cases having received previous treatment in 2007 was the most cases (n−187). The total number of cases whose previous treatment had begun in 2007 or 2008 was 220, i.e. 12.0% of all retreatment cases in 2008. On the other hand, the number of cases having received previous treatment in 1950s was also significant (n=234, 12.7%).

As initial treatment regimen, the combination of INH, RFP, PZA+EB/SM is recommended by Japanese Society for Tuberculosis. This regimen was initially used in 79.1% of all forms of TB patients aged 15–79 years old, excluding those cases whose treatment regimen was unknown.

The data on duration of having actually received PZA was adopted to add to the central TB surveillance database from 2007. The number of cases who started TB treatment including PZA in 2007 was 15,282. Of those, 11,817 cases had completed TB treatment by the end of 2008, but 8.4% of them could not take PZA fully for 2 months.

Key words: Tuberculosis, Age, Treatment history, New treatment, Retreatment, Regimen, PZA

Research Institute of Tuberculosis, JATA

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