慶長直人	No evidence for association between the interferon regulatory factor 1 (IRF1) gene and clinical tuberculosis.	Tuberculosis	89(1)	71-76	2009
慶長直人	Identification of MICA asa susceptibility gene for pulmonary Mycobacterium avium complex infection.	J Infect Dis			in press
慶長直人	HLA-A, -B, -C, -DRB1 and -DQB1 alleles and haplotypes in the Kinh population in Vietnam.	Tissue Antigens	71(2)	127-134	2008
竹田 潔	Lipocalin 2-dependent inhibition of mycobacterial growth in alveolar epithelium.	J. Immunol	181	8521-8527	2008
竹田 潔	Toll-like receptor (TLR) 2 and dectin-1 contribute to the production of IL-12p40 by bone marrow- derived dendritic cells infected with Penicilliummarneffei.	Microbes Infect	10	1223-1227	2008
竹田 潔	ATP drives lamina propria TH17 cell differentiation.	Nature	455	802-812	2008
竹田 潔	Targeted disruption of Hsp110/105 gene protects against ischemic stress.	Stroke	9	2853-2859	2008
竹田 潔	Inefficient phagosome maturation in infant macrophages.	Biochem. Biophys. Res. Commun.	375	113-118	2008
竹田 潔	STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury.	J. Neurosci	28	7231-7243	2008
竹田 潔	Stat3is required in hypothalamic Agrp/Npy neuronsfor normal energy homeostasis.	Endocrinology	149	3346-3354	2008
竹田 潔	Stat6-independent tissue inflammation occurs selectively on the ocular surface and perioral skin of IkBz-/- mice.	Invest. Ophthalmol. Vis. Sci.	49	3387-3394	2008
竹田 潔	Class-specific regulation of pro-inflammatory genes by MyD88 pathways and IkBz.	J. Biol. Chem	283	12468-12477	2008
竹田 潔	Deoxynucleic acids from Cryptococcus neoformans activate myeloid dendritic cells via a TLR9-dependent pathway.	J. Immunol	180	4067-4074	2008
竹田 潔	Potent antimycobacterial activity of mouse secretory leukocyte protease inhibitor.	J. Immunol	180	4032-4039	2008
竹田 潔	Malaria parasites require TLR9 signaling for immune evasion by activating regulatory T cells.	J. Immunol	180	2496-2503	2008

竹田 潔	STAT3 is indispensable to IL-27-mediated cell proliferation but notto IL-27-induced Th1 differentiation and suppression of proinflammatory cytokine production.	J. Immunol.	180	2903-2911	2008
竹田 潔	Role of nuclear IkB proteins in the regulation of host immune responses.	J. Infect. Chemother.	14	265-269	2008
坂谷光則	Novel prophylactic and therapeutic vaccine against Tuberculosis.	Vaccine			in press
坂谷光則	The study of Novel Vaccination using Granulysin transgenic mice.	Vaccine			in press
坂谷光則	Identification of MICA as a susceptibility gene for pulmonary Mycobacterium avium complex infection.	J.Infect. Dis.			in press
坂谷光則	Evaluation of the discrepant Mycobacterium tuberculosis strains between any ordinary susceptibility testing and rpoB gene analysis by the line probe assay.	Kekkaku	83	577-583	2008
坂谷光則	An adolescent case of pulmonary MAC infection, found 3 years later from bone marrow transplantation for myelodysplastic syndrome.	Kekkaku	83(8)	585-590	2008
坂谷光則	Serodiagnosis of *Mycobacterium avium*- Complex Pulmonary Disease Using an Enzyme Immunoassay Kit.	Am. J. Respir. Crit. Care Med.	177	793-797	2008
坂谷光則	遺伝子を用いた抗酸菌鑑別同定試薬INNO-LiPA MYCOBACTERIA v2の有用性の検討	結核	84(1)	15-21	2009
坂谷光則	MGITの前処理液BBLマイコプレップと2% NaOH処理との比較	結核	83(6)	471-473	2008
坂谷光則	非結核性抗酸症	Mebio	25(1)	68-77	2008
坂谷光則	クオンティフェロンTB-2G検査の意義	臨床検査	52 (10)	1139-1143	2008
中島俊洋	Novel prophylactic and therapeutic vaccine against Tuberculosis.	Vaccine			2009 in press
鈴木克洋	Serodiagnosis of Mycobacterium avium- Complex Pulmonary Disease Using an Enzyme Immunoassay Kit.	Am. J. Respir. Crit. Care Med.	177	793-797	2008
鈴木克洋	Population structure analysis of the Mycobacterium tuberculosis Beijing family indicates an association between certain sublineages and multidrug resistance.	Antimicrob Agents Chemother	52 (10)	3805-3809	2008
鈴木克洋	Biological and Molecular Characteristics of Mycobacterium tuberculosis Clinical Isolates with Low-level Resistance to Isoniazid in Japan.	J. Clin. Microbiol.	46	2263-2268	2008

鈴木克洋	遺伝子を用いた抗酸菌鑑別同定試薬INNO-LiPA MYCOBACTERIAの有用性の検討	結核	84	15-21	2009
鈴木克洋	肺MAC症の診断と治療	MEDICANENT NEWS	1969	4	2009
鈴木克洋	質疑応答「多剤耐性結核の定義・現状と対策」	日本医事新報	4424	96-97	2009
鈴木克洋	MGITの前処理液BBLマイコプレップと2% NaOH処理との比較	結核	83(6)	471-473	2008
鈴木克洋	薬剤感受性検査でRFP感受性、line probe assayでRFP耐性となる結核菌の検討	結核	83	577-583	2008
鈴木克洋	骨髄異型性症候群で骨髄移植後に3年をへて発症した若年者肺MAC症の1例	結核	83	585-590	2008
鈴木克洋	非結核性抗酸菌症	小児科診療	71(1)	83-88	2008
鈴木克洋	非結核性抗酸菌症	Mebio	25(1)	68-77	2008
鈴木克洋	肺炎の画像所見	日本医事新報	4367	69-72	2008
鈴木克洋	多剤耐性結核	結核	83(1)	39-42	2008
鈴木克洋	非結核性抗酸菌症	Medical Technology	36(2)	165-169	2008
鈴木克洋	肺真菌症の画像所見	日本医事新報	4371	53-56	2008
鈴木克洋	肺癌の画像所見	日本医事新報	4375	53-56	2008
鈴木克洋	その他の画像診断	日本医事新報	4380	53-56	2008
鈴木克洋	非結核性抗酸菌症と化学療法	呼吸器科	13(1)	62-67	2008
鈴木克洋	日本結核病学会非結核性抗酸菌症対策委員会、日本呼吸器学会感染症・結核学術部会:肺非結核性抗酸菌症診断に関する指針—2008	結核	83	525-526	2008
鈴木克洋	日本結核病学会非結核性抗酸菌症対策委員会:肺非結核性抗酸菌症に対する外科治療の指針	結核	83	527-528	2008
鈴木克洋	クオンティフェロンTB-2G検査の意義	臨床検査	52 (10)	1139-1143	2008
鈴木克洋	変貌する感染症ー人類の備えは十分か?結核予防法から新感染症法へ	総合臨床	57 (11)	2621-2624	2008
露口一成	薬剤感受性検査でRFP感受性、line probe assayでRFP耐性となる結核菌の検討	結核	83(8)	577-583	2008
露口一成	骨髄異形成症候群で骨髄移植後に3年をへて発症した肺MAC症の1例	結核	83(8)	585-590	2008
露口一成	多剤および超多剤耐性結核の全国調査(2006年)	結核	83 (12)	773-777	2008

菅原　勇	Protective efficacy of rBCG Tokyo[Ag85A] in rhesus monkeys infected intratracheally with H37Rv M. tuberculosis.	Tuberculosis	89	62-67	2009
菅原　勇	Higher susceptibility of type 1 diabetic rats to Mycobacterium tuberculosis infection.	Tohoku J. Exp. Med.	216	363-370	2008
菅原　勇	Disruption of Nrf2 enhances sususceptibility to airway inflammatory responses induced by low-dosediesel exhaust particles in mice.	Clin. Immunol	128	366-373	2008
菅原　勇	Promising loci of variable numbers of tandem repeats for typing Beijing family Mycobacterium tuberculosis.	J. Med. Microbiol.	57	873-880	2008
菅原　勇	Identification of 23 mycobacterial species by invader assay with targeting 16S rRNA gene and ITS-1 region-comparison with DDH method in clinical isolates.	Kekkaku	83	487-496	2008
阿部千代治	Biological and molecular characteristics of Mycobacterium tuberculosis clinical isolates with low-level resistance to isoniazid in Japan.	J Clin Microbiol	46	2263-2268	2008
阿部千代治	今回の結核菌検査指針改訂のポイント.	結核	83	46-47	2008
阿部千代治	結核菌のバイオハザード対策	Medical Technology	36	137-141	2008
阿部千代治	16S rRNA遺伝子およびITS-1領域をターゲットとしたInvader法による23菌種の抗酸菌の同定	結核	83	487-496	2008
阿部千代治	世界の薬剤耐性結核の現状と迅速結核菌検査	モダンメディア	54	223-232	2008
赤川清子	Erythromycin-derivatives EM201 and EM703 inhibit HIV-1 replication in macrophages through modulation of MAPK activity to induce small isoform of C/EBPβ.	Proc NatAcad Sci	105 (34)	12509-12514	2008
赤川清子	Unique CD14-positive intestinal macrophages contribute to the pathogenesis of Crohn's disease via IL- 23/IFN-γ axis.	J. Clin. Inve	118	2269-2280	2008
赤川清子	肺胞マクロファージの分化とGM-CSF--PPAR-γの発現と抗炎症作用	医学のあゆみ	224	857-860	2008

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Paper title

Novel prophylactic and therapeutic vaccine against Tuberculosis

Author's name

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Abstract

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 therapeutic efficacy as well as remarkable protective efficacy via $CD8^+$ T and $CD4^+$ T cells in murine models compared with the saline controls, on the basis of CFU of number of multi-drug resistant TB (MDR-TB), and survival of extremely drug resistant TB(XDR-TB) challenged mice. Furthermore, we extended our studies to a cynomolgus monkey model, which is currently the best animal model of human tuberculosis. This vaccine exerted therapeutic efficacy (survival and 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.

keywords

1. HSP65+IL-12DNA vaccine
2. Tuberculosis
3. Therapeutic effect

abbreviated article title, for use as a running headline

Therapeutic effect of novel TB vaccine

1. Introduction

Tuberculosis is a major global threat to human health, with about 2 million people dying every year from *Mycobacterium tuberculosis* (TB) infection. The only tuberculosis vaccine currently available is an attenuated strain of *Mycobacterium bovis* BCG (BCG), although its efficacy against adult TB disease remains controversial. Furthermore, multi-drug resistant tuberculosis (MDR-TB) and extremely drug resistant TB (XDR-TB) are becoming big problems in the world. In such circumstances, the development of therapeutic vaccine against TB as well as prophylactic vaccine against TB is required. Therefore, we have recently developed a novel TB vaccine, a DNA vaccine expressing mycobacterial heat shock protein 65 (HSP65) and interleukin-12 (IL-12) delivered by the hemagglutinating virus of Japan (HVJ)-liposome (HSP65 + IL-12/HVJ). This vaccine was 100 fold more efficient than BCG in the murine model on the basis of the elimination of *M. tuberculosis* mediated by the induction of CTL [1,2]. A nonhuman primate model of TB will provide information for vaccine development. In fact, in the previous study we evaluated the protective efficacy of HSP65 + IL-12/HVJ in the cynomolgus monkey model, which is an excellent model of human tuberculosis [1,3]. Furthermore, we observed the synergistic effect of the HSP65 + IL-12/HVJ and BCG using a priming-booster method in the TB-infected cynomolgus monkeys. The combination of the two vaccines showed a very strong prophylactic efficacy against *M. tuberculosis* (100% survival) as we have seen previously in the murine model of TB [4]. In the present study, we evaluated therapeutic effect and prophylactic effect of this vaccine on the MDR-TB infection and XDR- TB infection in murine and monkey models.

2. Materials and Methods

DNA vaccines encoding *M.tuberculosis* HSP65 and human IL-12 were encapsulated into HVJ-Envelope or HVJ-liposomes [5]. CTL activity was assessed by ^{51}Cr-release [1,6].

At 5 and 10 weeks after intravenous challenge of *M.Tuberculosis* H37RV, the number of CFU in the lungs, spleen, and liver were counted and therapeutic efficacy of HVJ-Envelope DNA vaccines was evaluated [1]. Therapeutic efficacy was also evaluated by chronic TB infection model of mice using aerosol challenge of TB (15CFU/mouse: Madison aerosol exposure chamber, University of Wisconsin). 5 weeks after aerosol infection of TB, the vaccine was administered to mice 6 times in 3 weeks.

Cynomolgus monkeys were housed in a BL 3 animal facility of the Leonard Wood Memorial Research

Center. The animals were vaccinated nine times with the HVJ-envelope with expression plasmid of both HSP65 and human IL-12 (HSP65 + hIL-12/HVJ: 400ug i.m.), one week after the challenge with the *M.tuberculosis* Erdman strain (5×10^2) by intratracheal instillation. Immune responses and survival were examined as described in our previous studies [2,4].

3. Results

The purpose of this study was to elucidate the therapeutic efficacy of a TB vaccine we have developed in a murine and nonhuman primate TB model[1, 3].

The in vivo necessity of CD8 positive T cells as well as CD4 positive T cells to exert the prophylactic efficacy of the HVJ-envelope/HSP65 DNA + IL-12 DNA vaccine was demonstrated in mice. Anti-CD8 antibody alone or anti-CD4 antibody alone treatment during the whole immunization period induced the increase in the number of TB in the mice immunized with the vaccine(Fig.1). Both anti-CD8 antibody and anti-CD4 antibody treatment increased in the number of TB synergistically.

Fig.2 shows the survival of vaccinated mice after XDR-TB (extremely drug resistant TB). All mice in the control group died of TB within 160 days after XDR-TB infection. In contrast, mice treated with HVJ-Envelope/HSP65 DNA+IL-12 DNA prolonged the survival periods significantly by statistical analysis($p<0.05$). It was demonstrated that this vaccine had a therapeutic activity against XDR-TB.

At 5 and 10 weeks after intravenous challenge of MDR-TB, the CFU in the lungs, spleen, and liver were counted and therapeutic efficacy of HVJ-Envelope DNA vaccine was evaluated.

As shown in Fig.3, HVJ-Env/HSP65 DNA +IL-12 DNA vaccine treatment significantly reduced the bacterial loads as compared to saline control group($P<0.05$).

Therapeutic efficacy of HVJ-Envelope/HSP65 DNA + IL-12 DNA was also observed, using in vivo humanized immune models of IL-2 receptor γ-chain disrupted NOD-SCID mice constructed with human PBL (SCID-PBL/hu)[7,8].

Fig.4 shows the results of bacterial loads 5 weeks after TB infection. Therapeutic vaccination with HVJ-Env/HSP65 DNA+IL-12 DNA group resulted in significantly therapeutic activity even in SCID-PBL/hu mice which exerted human T cell immune responses.

Furthermore, the therapeutic activity of this vaccine was evaluated in a nonhuman primate model infected with *M.tuberculosis*.

Fig.5A shows the results of immune responses of cynomolgus monkey at 11 weeks after challenge of

M.tuberculosis Erdman strain (5×10^2) by intratracheal instillation. The proliferation of PBL in therapeutic vaccination of monkeys in the group with HVJ-Env/HSP65 DNA +IL-12 DNA was augmented. This vaccine also improved the survival of monkeys, compared to the saline (control) group, during the period between 0 weeks and 19 weeks after TB challenge(Fig.5B).

4. Discussion

The HSP65+hIL-12/HVJ vaccine exerted a significant therapeutic effect against TB, as indicated by: (1) extension of survival of mice infected with XDR-TB, (2) decrease in the CFU of TB in lungs, liver and spleen of mice infected with MDR-TB as well as drug-sensitive TB(H37RV), (3) decrease in the CFU of TB in these organs of mice challenged with TB in the in vivo humanized immune model of SCID-PBL/hu, (4) augmentation of immune responses, in a cynomologus monkey model which closely mimics human TB disease. It is important to evaluate the survival of monkey [6, 7]. During the period between 0 weeks and 19 weeks after TB challenge, increase in the survival rate of the monkeys treated with this vaccine were observed, compared to the control monkeys treated with saline.

MDR-TB and XDR-TB are becoming big problems in the world. About 500,000 new patients with MDR-TB are shown every year. However, the effective drugs against MDR-TB are few.

The HVJ-Envelope/HSP65DNA+IL-12DNA vaccine exerted the therapeutic activity even against XDR-TB, which is resistant to RFP, INH, SM, EB, KM, EVM, TH, PAS, LEFX, PZA and only sensitive to CS. Thus, our results with the HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine in the murine therapeutic model and cynomologus monkey therapeutic model should provide a significant rationale for moving this vaccine into clinical trial. Furthermore, we have established chronic TB disease model using mouse infected with TB in the aerosol chamber (data not shown)[9]. By using this model, therapeutic efficacy of this vaccine was also observed.

Thus, we are taking advantage of the availability of multiple animal models to accumulate essential data on the HVJ-envelope DNA vaccine in anticipation of a phase I clinical trial.

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Culture, Sports, Science and Technology Japan, and Grant of Osaka Tuberculosis Foundation..

References

[1] Yoshida S, Tanaka T, Kita Y, Kuwayama S, Kanamaru N, Muraki Y, et al. DNA vaccine using hemagglutinating virus of Japan-liposome encapsulating combination encoding mycobacterial heat shock protein 65 and interleukin-12 confers protection against *Mycobacterium tuberculosis* by T cell activation. Vaccine 2006;24:1191-1204.

[2] Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Evaluation of a novel vaccine(HVJ-liposome/ HSP65 DNA+IL-12 DNA) against tuberculosis using the cynomologus monkey model of TB. Vaccine 2007; 25(16):2990-3

[3] Walsh GP, Tan EV, dela Cruz EC, Abalos RM, Villahermosa LG, Young LJ, et al. The Philippine cynomolgus monkey provides a new nonhuman primate model of tuberculosis that resembles human disease. Nat Med 1996;2(4):430-6.

[4] Kita Y, Tanaka T, Yoshida S, Ohara N, Kaneda Y, Kuwayama S, et al. Novel recombinant BCG and DNA-vaccination against tuberculosis in a cynomolgus monkey model. Vaccine 2005;23:2132-2135.

[5] Saeki Y, Matsumoto N, Nakano Y, Mori M, Awai K, Kaneda Y. Development and characterization of cationic liposomes conjugated with HVJ (Sendai virus). Hum Gene Ther. 1997;8(17):2133-41.

[6] Okada M, Yoshimura N, Kaieda T, Yamamura Y, Kishimoto T. Establishment and characterization of human T hybrid cells secreting immunoregulatory molecules. Proc Natl Acad Sci U S A. 1981; 78(12):7717-21.

[7] Okada M, Okuno Y, Hashimoto S, Kita Y, Kanamaru N, Nishida Y, et al. Development of vaccines and passive immunotherapy against SARS corona virus using SCID-PBL/hu mouse models. Vaccine 2007;25:3038-3040.

[8] Tanaka F, Abe M, Akiyoshi T, Nomura T, Sugimachi K, Kishimoto T, et al. The anti-human tumor effect and generation of human cytotoxic T cells in SCID mice given human peripheral blood lymphocytes by the in vivo transfer of the Interleukin-6 gene using adenovirus vector. Cancer Res 1997;57(7):1335-43.

[9] Flynn JL, Chan J. Immunology of tuberculosis. Annu Rev Immunol. 2001; 19:93-129.

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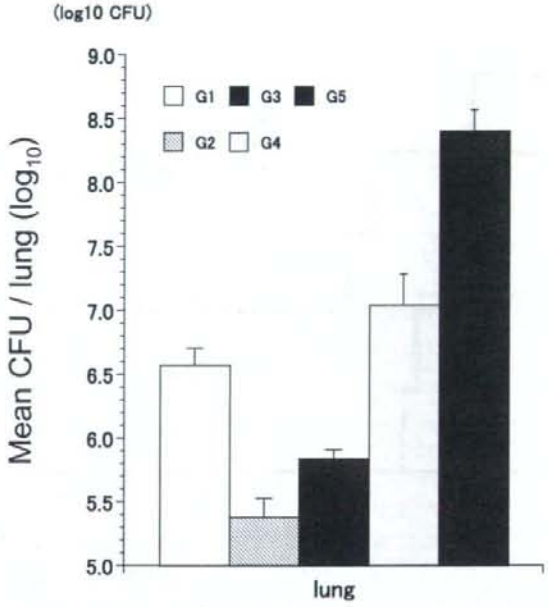
Fig.1



* p<0.05 G2 vs G3,4
* * p<0.01 G2 vs G5

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Fig.2

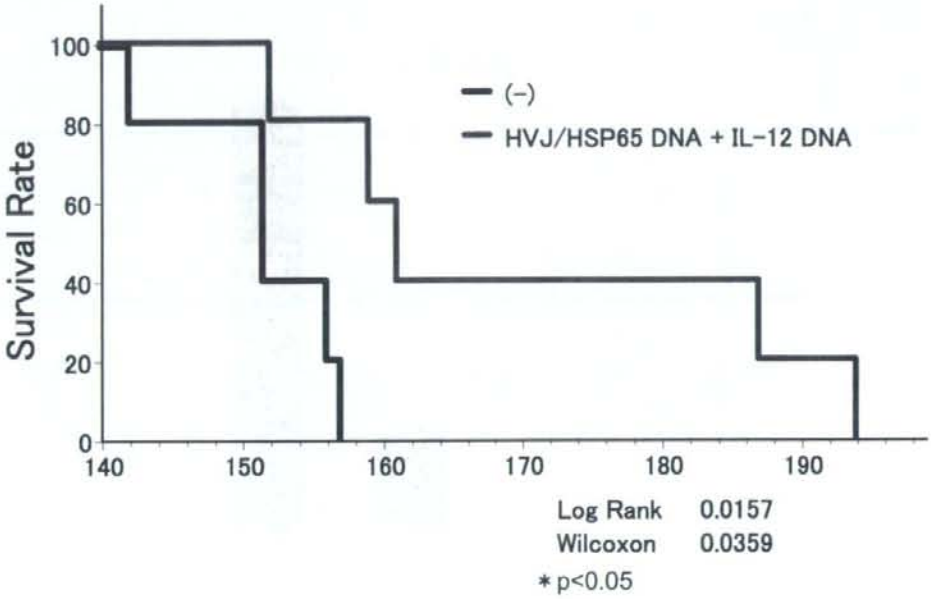

Fig.3

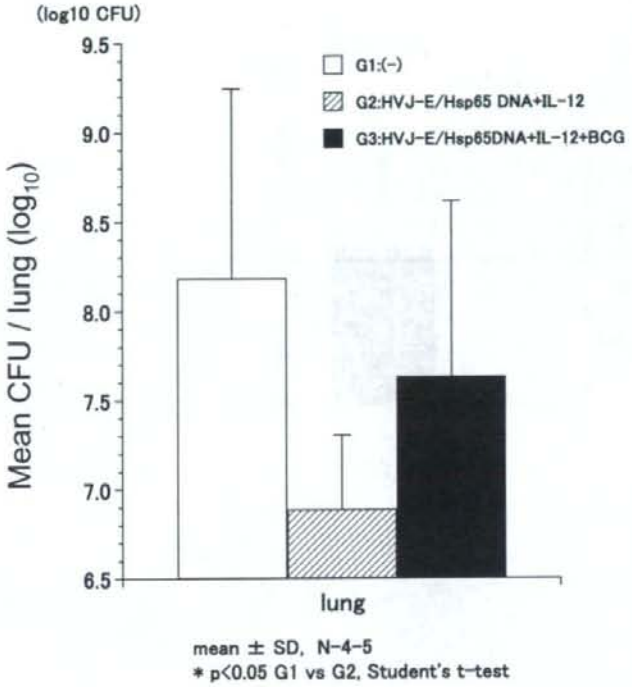
(log10 CFU)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
Mean CFU / lung (log_{10})
G1:(-)
G2:HVJ-E/Hsp65 DNA+IL-12
G3:HVJ-E/Hsp65DNA+IL-12+BCG
lung
mean ± SD, N-4-5
* p<0.05 G1 vs G2, Student's t-test

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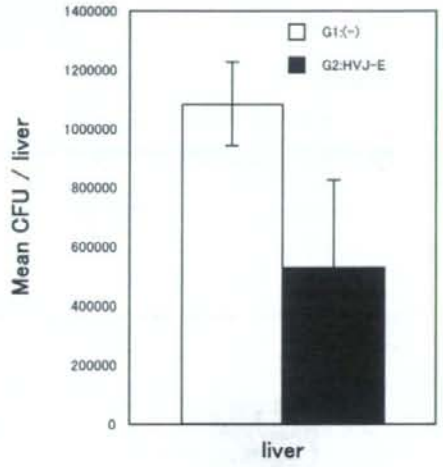
Fig.4



* p<0.05 G1 vs G2

Fig.5A

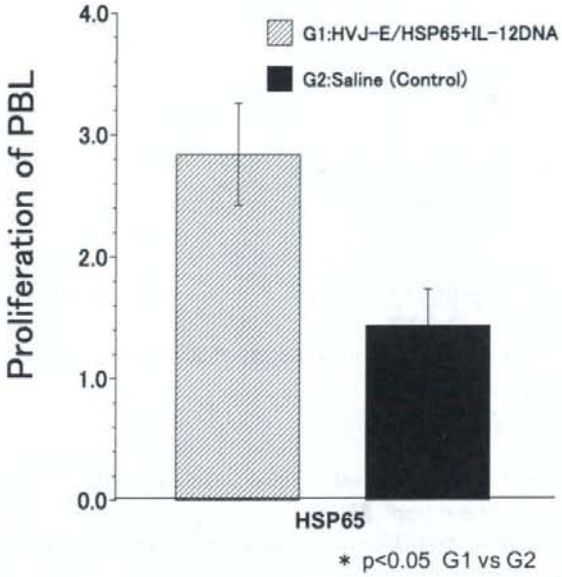
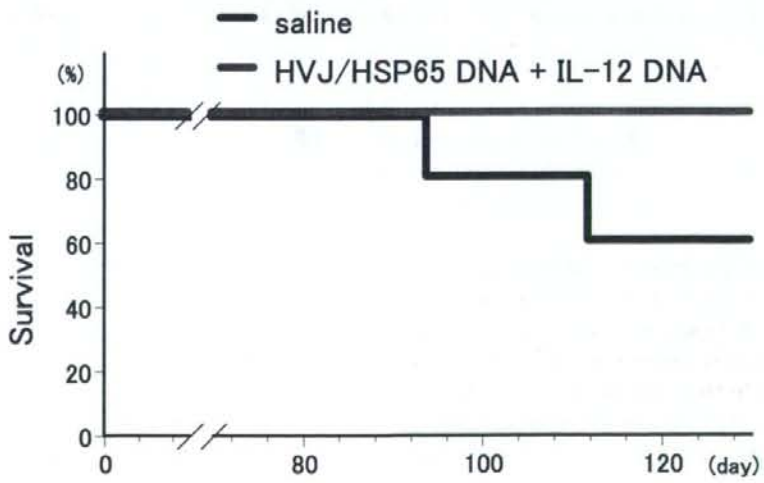

G1:HVJ-E/HSP65+IL-12DNA
G2:Saline (Control)
Proliferation of PBL
4.0
3.0
2.0
1.0
0.0
HSP65
* p<0.05 G1 vs G2

Fig.5B


saline
HVJ/HSP65 DNA + IL-12 DNA
(%)
100
80
60
40
20
0
Survival
0
80
100
120
(day)

Captions to Fig.1.

The in vivo necessity of CD8 positive T cells and CD4 positive T cells for prophylactic efficacy of the HVJ-envelope/HSP65 DNA + IL-12 DNA vaccine.

Anti-CD8 antibody and/or anti-CD4 antibody were injected i.p. every 5 days after the challenge of TB. BCG was used as a priming vaccine and this DNA vaccine was immunized two times (HVJ-Envelope/HSP65DNA 50ug+IL-12DNA 50ug) as booster vaccine. 4weeks after last immunization, 5×10^5 H37RV were challenged i.v. into mice.

G1: without vaccine (□)

G2: vaccine (▨)

G3: vaccine + anti-CD8 antibody (■)

G4: vaccine + anti-CD4 antibody (⬚)

G5: vaccine + anti-CD8 antibody + anti-CD4 antibody (■)

(G2-G3:$p< 0.05$) (G2-G4:$p<0.05$) (G2-G5:$P<0.01$)

Captions to Fig.2.

Therapeutic efficacy of HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine on the extremely drug resistant *Mycobacterium tuberculosis* (XDR-TB).

The survival of DBA/1 mice treated with HVJ-Envelope/HSP65 DNA(50ug)+IL-12 DNA vaccine(50ug) three times after 5×10^5 XDR-TB, injection i.v..

XDR-TB is resistant to RFP, INH, SM, EB, KM, EVM, TH, PAS, LEFX, PZA.

XDR-TB is only sensitive to CS.

Survival rate of mice treated with HVJ-Envelope/HSP65 DNA+IL-12DNA (■).

Survival rate of control mice without treatment(■).

Kaplan-Meier's Method (Log rank test and Wilcoxon) was used to compare the survival of each group. (G1-G2 : Log rank 0.0157 Wilcoxon 0.0359)

Captions to Fig.3.

Therapeutic efficacy of HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine on MDR-TB TNF R gene disrupted DBA/1 mice were treated with HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine three times after 5×10^5 MDR-TB injection i.v..

CFU of MDR-TB in the lungs of mice, 4 weeks after MDR-TB injection, were assessed as described in Material and Method.

G1: (-)(□)

G2: treated with HVJ-Envelope/HSP65 DNA+IL-12DNA(▨)

G3: treated with HVJ-Envelope/HSP65 DNA+IL-12DNA and BCG(■)

student's t-test was used to compare the CFU of TB of each group. (G1-G2 : $p<0.05$)

Captions to Fig.4.

Therapeutic efficacy of HVJ-Envelope/HSP65 DNA + IL-12 DNA, using in vivo humanized immune models of IL-2 receptor γchain disrupted NOD-SCID mice (SCID-PBL/hu).

Groups of animals were treated with three times with HVJ-Envelope/HSP65 DNA + IL-12 DNA (50μg i.m) 10 days after the third vaccination, mice were sacrificed and CFU of TB in the liver of mice were accessed as described in Materials and Methods (1, 2).

1×10^7 PBL from a healthy human volunteer were injected i.p. into IL-2 receptor γchain disrupted NOD-SCID mice. 21 days after injection of PBL, mice were challenges with 5×10^5 H37RV i.v. and then treated with the vaccine.

G1: (-) control (□)
G2: treated with HVJ-Envelope/HSP65 DNA + IL-12 DNA(■)
student's t-test was used to compare the CFU of TB of each group. (G1-G2 : $p<0.05$)

Captions to Fig.5A.

Therapeutic efficacy of HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine using cynomolgus monkey.
Five cynomolgus monkeys were treated with HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine 9 times after 5×10^2 M.TB intratracheal instillation.
Stimulation index of the proliferation of PBL from these monkeys and that from five control monkeys(saline injected) were assessed by the stimulation with HSP65 antigen.
G1; HVJ-Envelope/HSP65 DNA+IL-12 DNA treatment (▨)
G2; saline(control) (■)
Tukey-Kramer's HSD tests were used to compare proliferative responses of PBL between groups. (G1-G2 : $p<0.05$)

Fig.5B.

Survival periods of 5 monkeys treated with HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine 9 times after TB challenge were shown (■).
Survival periods of 5 monkeys treated with saline (control) were shown(■).

特集：特異抗原をターゲットとした Immunotherapy
総　説

新しい結核ワクチンの開発

岡田全司

The development of novel vaccines against Tuberculosis

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summary

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)-liposome or-envelope (HSP65+IL-12/HVJ). This vaccine provided remarkable protective efficacy in mouse and guinea pig models compared to the BCG vaccine, on the basis of an induction of the CD8 positive CTL activity against TB antigens and improvement of the histopathological tuberculosis lesions, respectively. The Elispot assay showed that HSP65+IL-12 DNA/ HVJ vaccine induced a greater number of IFN-γ producing T cells than BCG in the mouse model. 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 production , and lymphocyte proliferation of cynomolgus monkey). The combination of HSP65+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). These data indicate that our novel DNA vaccine might be useful against Mycobacterium tuberculosis for human clinical trials.

Key words——Mycobacterium Tuberculosis; HSP65 DNA+IL-12 DNA; Cytotoxic T-cell; Cynomologus monkey; DNA vaccine and recombinant BCG

抄　録

1998年，米国CDC及び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．我々はBCGを凌駕する強力な結核予防ワクチン（HVJ-エンベロープ（又はリポソーム）/HSP65+IL-12 DNAワクチン）を開発した．結核免疫を強く誘導するヒト結核菌由来のHSP65蛋白をコードするDNAを用いた．プライム・ブースター法を用い，HSP65 DNA+IL-12 DNA（HVJ-エンベロープベクター）のワクチンはBCGよりも1万倍強力な結核予防ワクチンであり，CD8陽性キラーT細胞の分化，IFN-γ産生T細胞の分化を増強した．肺の結核病理像を改善した．このワクチンは多剤耐性結核菌に対しても治療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，このワクチンの強力な有効性を得た．カニクイザルにワクチン接種後ヒト結核菌を経気道投与し，1年以上経過観察した．免疫反応増強及び胸部X線所見・血沈，体重の改善効果が認められた．また，生存率改善・延命効果も認められた．

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．この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．

I. はじめに

いまだに世界の1/3の20億人が結核菌に感染しており，その中から毎年880万人の結核患者が発症し，200万人が毎年結核で死亡している，最大の感染症の一つである（WHOレポート2007年（図1））[1~4]．本邦でも1998年から結核罹患率の増加・横ばいが認められ，1999年“結核緊急事態宣言”が厚生省より出された．結核症に対する宿主の免疫

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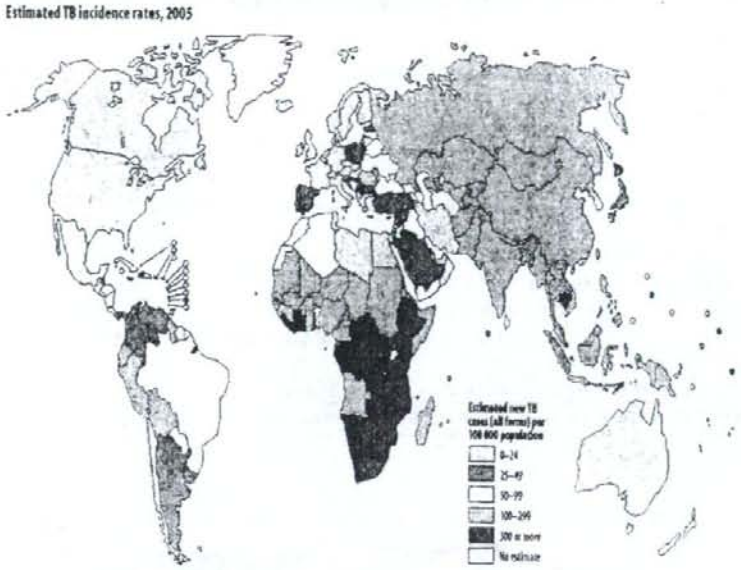

図1

抵抗性はT細胞性免疫といって過言ではない．特に獲得免疫（キラーT細胞とTh1ヘルパーT細胞）が重要であり，最近では自然免疫の結核への関与が再び重要視されている．1998年，米国CDCは結核に対し，政府・学術機関・企業が一体となって新世代の結核ワクチン開発の必要性を強く主張する発表をした．又，ACETは国民の健康に対する大敵である結核撲滅のためには，BCGに代わる有効なワクチンが必要であることを示した．しかしながら，BCG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．我々はBCGよりもはるかに強力なDNAワクチンやリコンビナントBCGワクチンの開発に成功した．（表1，図2）[5~8]新しい抗結核ワクチン開発と結核感染免疫におけるキラーTの機能解明についても述べる[9,10]．

II. 結核と免疫

1. 自然免疫と結核

1) マクロファージ（Mφ）

結核菌の増殖場所はMφ内である．一方，Mφは異物貪食能と細胞内殺菌能及び抗原提示能をもつ．したがって結核菌が優位に立つか，ヒト（生体）が優位に立つかの戦争でもある．（詳細は岡田結核文献[1,2]参照）

2） Toll-like Receptor 及び Pathogen Recognition Receptor とマクロファージ・樹状細胞活性化

最近発見されたToll-like receptor（TLR）ファミリーがinnate immunityの重要な役割を果たしている[11]．

TLR（TLR1～TLR10）はそのリガンドによって大きく3つに分類される．

このうち菌体膜由来の糖脂質を認識するTLRとしては，TLR1, TLR2, TLR4, TLR6, TLR9である．

結核菌のcell wall（LAM, mAGP, total lipid）による応答はTLR2を介する．一方，結核生菌に対する反応にはTLR2とTLR4が必要である．病原株のM. tuberculosis由来のMan LAMはMφを活性化しないが，非病原性の抗酸菌は異なるglycolipid Ara LAMよりなり，これはTLR2を介してMφを活性化する．この差が発病の差となる可能性もある．結核菌体成分19kDaのlipoproteinが－/TLR2を介してMφを活性化する．また，抗酸菌DNAから見いだされたCpGモチーフ（パリンドローム配列）は感染防御免疫能増強することが示されていたが，CpGレセプターに対するTLR9が審良らによりクローニングされた．

TLR2の場合，細胞内領域の2つの変異（Arg753GlnとArg677Trp）が認められ，Arg753Gln

表1 新しい結核ワクチンの開発

(1) DNA ワクチン	BCG より有効
HV J-liposome/HSP65 DNA＋IL-12 DNA	（マウス，モルモット，カニクイザル）
(2) DNA ワクチン	BCG よりはるかに有効（マウス）
HVJ-エンベロープ/HSP65 DNA＋IL-12 DNA	
(3) リコンビナント BCG ワクチン	BCG より有効
①リコンビナント 72f BCG	（マウス，モルモット，カニクイザル）
②リコンビナント（Ag85A＋85B＋MPB51）BCG	BCG より有効（マウス）
(4) 治療ワクチン	
IL-6 related DNA（マウス）	
(5) Priming-Booster Method	
BCG (priming)＋新しいワクチン（booster）（カニクイザル）	
(6) 遺伝子ノックアウト attenuated リステリアを用いた新しい結核ワクチン（経口）	
(7) 新しいベクター	
AAV ベクター（1000 倍発現効率↑），Adenovirus ベクター	
→WHO STOP TB Partnership 及び WHO STOP TB Vaccines Working Group に選出	

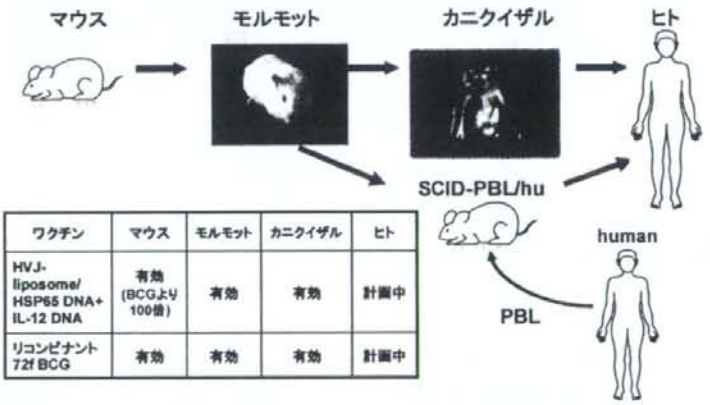

図2 新しい結核ワクチンの開発

は敗血症にかかりやすく，Arg677Trp はアジア人において *M.leprae* による結節性ハンセン症と関連している．

TLR はそれぞれ病原微生物由来の構成成分を認識する．TLR シグナルを介するシグナル伝達経路には MyD88 を介する MyD88 依存的経路と MyD88 を介さない MyD88 非依存的経路の 2 つが存在する．主に前者は全ての TLR を介した炎症性サイトカインの産生を，後者は主に TLR3・TLR4 を介したインターフェロン（IFN）および IFN 誘導性遺伝子群の産生を担う．

この MyD88 非依存的経路を担うアダプター分子が TRIF である．TRIF が TLR3 と TLR4 の MyD88 非依存的経路に共有されているのに対し，TRAM は MyD88 非依存的（TRIF 依存的）経路を TLR4 シグナルに特異的にだけ与えるアダプター分子である．また，TIRAP はすべての TLR に共有された MyD88 依存的経路を，TLR1/2/6 と TLR4 シグナル特異的に与える役割をもつ．竹田らは TRIF(−/−)×MyD88(−/−)ダブルノックアウトマウスを用い，結核菌に対する易感染性を解析しつつある．

TLR 以外にも PRR（pathogen recognition receptor）として DC-SIGN，NOD ファミリー，マンノース受容体，スカベンジャー受容体，dectin-1 があげられる．HIV や *M. tuberculosis* は DC-SIGN に結合して樹状細胞に入り込むが，その際，その TLR による自然免疫機構の活性化を抑制し，これらの病原体の生存を有利にする機構が働いていることが示された．NOD1，NOD2 を中心とする CARD ファ