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A Novel Therapeutic and Prophylactic Vaccine against Tuberculosis Using the Cynomolgus Monkey Model and Mouse Model

Okada M[a]*, Kita Y[a], Nakajima T[b], Kanamaru N[a], Kaneda Y[c], Saunderson P[d], Tan E.V.[d], N.McMurray D[e]

[a] Clinical Research Center, National Hospital Organization Kinki-chuo Chest Medical Center, 1180 Nagasone, Kitaku, Sakai, Osaka 591-8555, Japan
[b] Ikeda Laboratory, GenomIdea Inc.,1-8-31, Midorigaoka, Ikeda, Osaka 530-0043, Japan
[c] Division of Gene Therapy Science, Graduate School of Medicine, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871
[d] Leonard Wood Memorial, Jagobiao, Mandaue City, Cebu 6000, Philippines
[e] Texas A & M University, System Health Science Center, College of Medicine, College Station, TX 77843-1114, USA

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 remarkable protective efficacy in mouse model compared to the BCG. 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 BCG prime and HSP65+IL-12/HVJ vaccine (boost) by the prime-boost method showed a synergistic prophylactic effect in the monkey. Furthermore, this vaccine exerted therapeutic efficacy (100% survival) and augmentation of immune responses in the TB-infected monkeys.HVJ-Envelope/HSP65 DNA+IL-12 DNA vaccine increased the body weight of TB-infected monkeys, improved the ESR, and augmented the immuneresponses (proliferation of PBL and IL-2 production). The enhancement of IL-2 production from monkeys treated with this vaccine was correlated with the therapeutic efficacy of the vaccine. 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: HSP65＋IL-12DNA vaccine; Tuberculosis; Monkey; Therapeutic vaccine

* Corresponding author. Tel.: +81-722-52-3021; fax: +81-722-51-2153.
E-mail address: okm@kch.hosp.go.jp

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 or HVJ-envelope (HSP65 + IL-12/HVJ) [1, 2]. The HSP65 + IL-12/HVJ vaccine using HVJ-envelope was 10,000 fold more efficient than BCG in the murine TB-prophylactic model. 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 [1,4]. In the present study, we observed the synergistic effect of the HSP65 + IL-12/HVJ and BCG using a prime-boost 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 [2,5]. Moreover, we evaluated therapeutic effect of this vaccine on the MDR-TB infection and XDR-TB infection in murine and monkey models, indicating that the vaccine exerts therapeutic efficacy against TB, MDR-TB and XDR-TB. This vaccine exerted strong therapeutic efficacy (100% survival) and augmentation of immune responses in the TB-infected monkeys. Moreover, the combination of Ag85A DNA, Ag85B DNA and MPT51 DNA vaccine augmented the therapeutic efficacy of this HSP65 DNA + IL12 DNA vaccine.

2. Method for the evaluation of the efficacy of vaccines on the *M.tuberculosis*-infected mice

DNA vaccines encoding M.tuberculosis HSP65 and human IL-12 were encapsulated into HVJ-Envelope or HVJ-liposomes [6]. CTL activity was assessed by 51Cr-release assay[1,7]. 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).

3. Method for the evaluation of the efficacy of the vaccine on the *M.tuberculosis*-infected monkeys

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 (5×102) by intratracheal instillation. Immune responses and survival were examined as described in our previous studies [2,5].

4. Results and Discussion

4.1. Prophylactic efficacy

All 4monkeys in the control group (saline) died within 8 months, while 50% (2monkeys out of 4) of monkeys in the HSP65+hIL-12/HVJ group survived more than 14 months post-infection (the termination period of the experiment)(data not shown).

Furthermore, the protective efficacy of the HSP65+IL-12 /HVJ and BCG using the prime-boost method in the TB infected cynomolgus monkeys was very strong. All four monkeys from the group of BCG-prime and the DNA vaccine (HVJ-liposome/HSP65+IL-12 DNA vaccine) boost were alive more than 12 months post-infection (data not shown). In contrast, only 2 monkeys out of 6 from the BCG Tokyo group were alive (33% survival). 50% of the monkeys from the saline control group and DNA vaccine-prime and the BCG Tokyo vaccine boost group, respectively, were alive more than 12 months in the study. In addition, HSP65+hIL-12/HVJ improved both ESR and chest X-ray findings. IL-2 and IL-6 production were augmented in the group vaccinated with BCG vaccine-prime and the DNA vaccine-boost (Table1). Furthermore, proliferation of PBL was strongly enhanced. Taken together, these results clearly demonstrate that BCG prime and the HSP65+hIL-12/HVJ boost could provide extremely strong protective efficacy against *M.tuberculosis* in the cynomolgus monkey model.

Table 1. Efficacy of HSP65 + IL-12 DNA Vaccine in Monkey

Efficacy of HSP65 + IL-12 DNA in Monkeys	
Survival (100% surrival: 1 year)	⇑⇑⇑
Chest X-p (improvement)	⇑
Erythrocyte Sedimentation Rate (improvement)	⇑⇑
Body Weight	⇑
Immune Responses	
IFN-γ	⇑⇑
IL-6	⇑⇑
IL-2	⇑⇑
Proliferation of T cell	⇑

4.2. Therapeutic efficacy

The survival of vaccinated mice after XDR-TB (extremely drug resistant TB) was investigated. 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($p<0.05$). (data not shown) It was demonstrated that this vaccine had a therapeutic activity against XDR-TB (Table 2).

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

HVJ-Envelope/HSP65 DNA +IL-12 DNA vaccine treatment significantly reduced the bacterial loads of MDR-TB as compared to saline control group($P<0.05$) (Table2).

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)[8,9]. Therapeutic vaccination with HVJ-Envelope/HSP65 DNA+IL-12 DNA group resulted in significantly therapeutic activity even in SCID-PBL/hu mice which exerted human T cell immune responses (Table 2).

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

Table 2. The Development of Novel Vaccines for M.tuberculosis using animal model

Vaccine	efficacy	mouse	guinea pig	monkey	SCID-PBL/hu	human
HVJ-E/Hsp65DNA+IL-12DNA	Prophylactic Effect	10,000 fold effective than BCG	effective	effective		Plan (phase I , II)
	Therapeutic Effect	effective	plan	effective	effective	
	Therapeutic Effect against MDR-TB XDR-TB	effective	plan	plan		
HVJ-liposome/Hsp65DNA+IL-12DNA	Prophylactic Effect	100 fold effective than BCG		effective		

Immune responses of cynomolgus monkey at 11 weeks after challenge of M.tuberculosis Erdman strain by intratracheal instillation were augmented. The proliferation of PBL in therapeutic vaccination of monkeys in the group with HVJ-Envelope/HSP65 DNA +IL-12 DNA was augmented (data not shown). This vaccine also improved the survival of monkeys, compared to the saline (control) group, after TB challenge (Fig.1). All five monkeys from the group of HVJ-Envelope/HSP65DNA+IL-12DNA vaccine were alive (100% survival). In contrast, 3 monkeys out of 5 from the saline control group were alive (60% survival). In addition HSP65+hIL-12/HVJ improved ESR compared to control (Fig.2). IL-2 production was strongly augmented in the group vaccinated with HSP65+hIL-12/HVJ by the stimulation with killed TB H37Ra antigens (Fig.3). Furthermore, proliferation of PBL from vaccinated monkeys was enhanced by the stimulation with HSP65 antigen, H37Ra-killed TB antigens, and PPD antigens (data not shown). These results demonstrate that HVJ-Envelope/HSP65DNA+IL-12DNA vaccine could provide strong therapeutic efficacy against TB, in the cynomolgus monkey models as well as murine models.
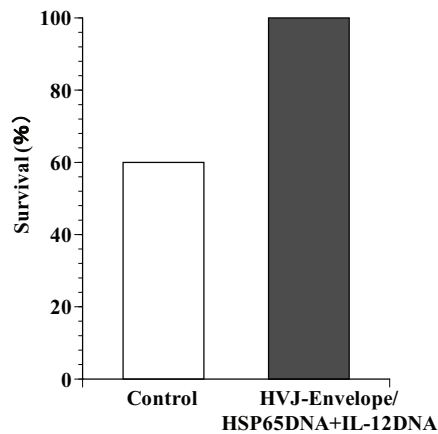

Fig. 1. Therapeutic efficacy (survival) of HVJ-Envelope/HSP65DNA+IL-12DNA vaccine 130 days after TB infection using cynomolgus monkey models.
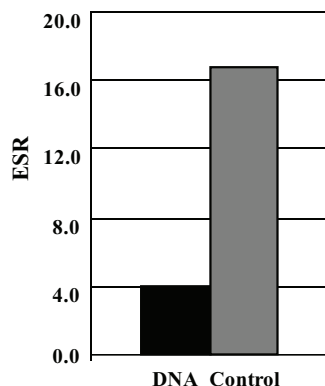
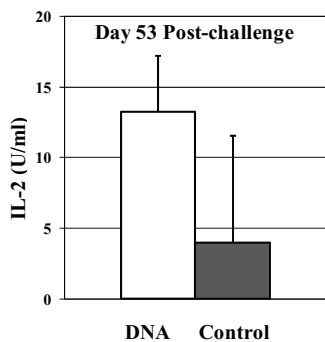Fig. 2. ESR of monkeys treated with HVJ-Envelope/HSP65DNA+IL-12DNA vaccine 11 weeks after TB infection

Fig. 3. Augmentation of IL-2 production from PBL by killed H37Ra stimulation in the monkeys treated with HVJ-Envelope/Hsp65 DNA+IL-12 DNA vaccine.

5. Discussion

The HSP65+hIL-12/HVJ vaccine exerted a significant prophylactic effect against TB, as indicated by: 1) extension of survival for over a year; 2) improvement of ESR and chest X-ray findings; 3) increase in the body weight; 4) augmentation of immune responses, in a cynomolgus monkey model which closely mimics human TB disease. It is very important to evaluate the long survival period in a monkey model, as human TB is a chronic infection disease. Furthermore, the decrease in the body weight of TB patients is usually accompanied by a progression of the disease. [10]

DNA vaccines are a relatively new approach to immunization for infectious diseases. [1,2,5,11-14]

Prophylactic and therapeutic DNA vaccines were established by using several kinds of vectors such as (1) HVJ-liposome, (2) HVJ-envelope, (3) adenovirus vector, (4) adeno-associated virus vector (AAV), (5) lenti-virus vector. [1,3,9]

We have developed a hemagglutinating virus of Japan envelope (HVJ-Envelope) using inactivated Sendai virus, as a nonviral vector for drug delivery.[15-17] It can deliver very efficiently DNA, siRNA,

proteins and anti-cancer drugs into cells both in vitro and in vivo.[15,18,19] Therefore, HVJ-Envelope was used as an efficient and safe vector for DNA vaccine against TB in the present study.

In the present study, it was demonstrated that BCG vaccine prime and HSP65+h IL-12/HVJ boost could provide extremely strong (100% survival) efficacy against M.tuberculosis compared to BCG alone (33% survival) in the cynomolgus monkey model. In Japan and other countries, the BCG vaccine is inoculated into human infants (0~6months after birth). Therefore, BCG prime in infants and HSP65+h IL-12/HVJ boost for adults (including junior high school students, high school students and old persons) may be required for the significant improvement of clinical protective efficacy against TB.

Furthermore, 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 cynomolgus monkey model which closely mimics human TB disease. It is important to evaluate the survival of monkey [7,8]. Increases in the survival rate of the monkeys treated with this vaccine were observed, compared to the control monkeys treated with saline.   Increase in the survival rate of the monkeys treated with HVJ-Envelope/HSP65DNA+IL-12DNA+Ag85B   DNA+Ag85A   DNA   vaccine   or   HVJ-Envelope/HSP65DNA+IL-12DNA+Ag85B DNA+Ag85A DNA+MPT51 DNA vaccine was also strongly observed in the therapeutic models of monkeys (Table 3, Fig.4, 5). In the recent study, it is demonstrated that granulysin vaccine shows therapeutic efficacy against TB in mice (Table 3) [20].   Therefore, the combination of these therapeutic vaccines might be useful in the future.

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.

Table 3. The Development of the combination of Novel Vaccines for M.tuberculosis using animal model.

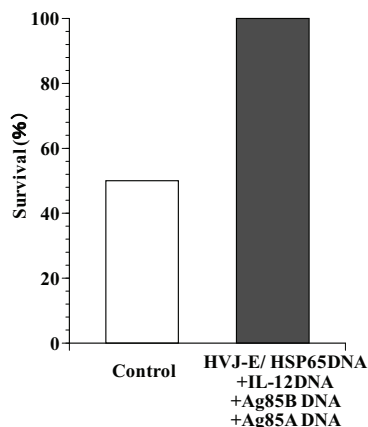| Vaccine	mouse	monkey	human
HVJ-E /Hsp65DNA+IL-12 DNA+ Ag85B DNA+Ag85A DNA	plan	Therapeutic Effect	
HVJ-E/Hsp65DNA+IL-12 DNA +Ag85BDNA+Ag85ADNA+ MPT51DNA	plan	Therapeutic Effect	plan
15K granulysin+HVJ-E/ Hsp65 DNA+IL-12DNA	Therapeutic Effect	plan	

Fig. 4. Therapeutic efficacy (survival) of HVJ-Envelope/HSP65DNA+IL-12DNA +Ag85BDNA +Ag85ADNA vaccine 200 days after TB infection using cynomolgus monkey models.
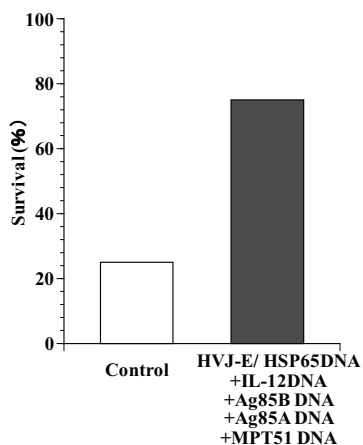

Fig. 5. Therapeutic efficacy (survival) of HVJ-Envelope/HSP65DNA+IL-12DNA +Ag85BDNA+Ag85ADNA+MPT51DNA vaccine 200 days after TB infection using cynomolgus monkey models.

   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, LVFX, 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 cynomolgus 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). 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.

Acknowledgements

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