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A Novel Therapeutic and Prophylactic Vaccine (HVJ-Envelope / Hsp65 DNA + IL-12 DNA) against Tuberculosis Using the Cynomolgus Monkey Model

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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). An IL-12 expression vector (IL-12DNA) encoding single-chain IL-12 proteins comprised of p40 and p35 subunits were constructed. This vaccine provided remarkable protective efficacy in mouse and guinea pig models 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) (prolongation of survival time and the decrease in the number of TB in the lung) 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. All monkeys in the control group (saline) died within 8 months, while 50% of monkeys in the HSP65+hIL-12/HVJ group survived more than 14 months post-infection (the termination period of the experiment). 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). In contrast, 33% of monkeys from BCG Tokyo alone group were alive (33% 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.

Keywords HSP65□IL-12DNA vaccine□ Tuberculosis□Monkey□Therapeutic 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

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on the basis of the elimination of *M. tuberculosis* mediated by the induction of CTL [1,2]. Futhermore 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 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 [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.

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 $^{51}C_r$-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 (5×10^2) by intratracheal instillation. Immune responses and survival were examined as described in our previous studies [2,5].

4. Results and Discussion

(a) 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).

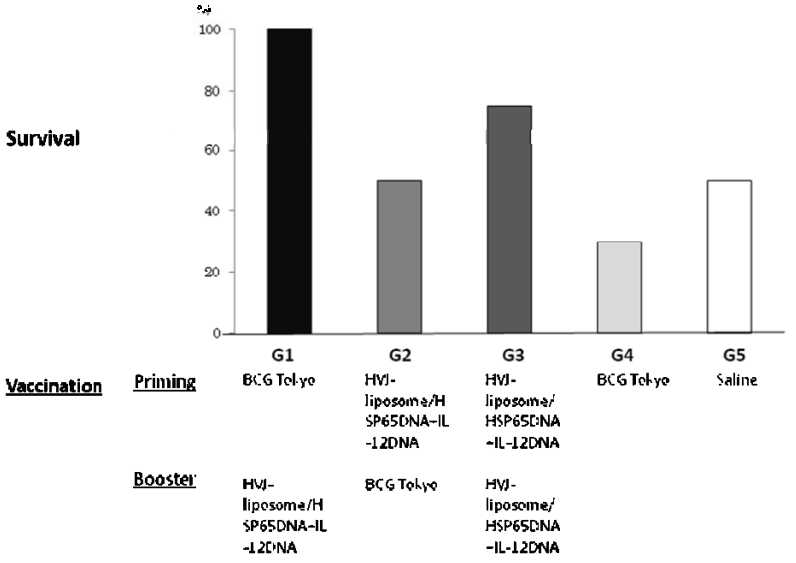

Fig 1. Protective efficacy (survival) of HSP65+IL-12/HVJ and BCG using priming- booster method against TB challenged cynomolgus monkey 350 days after TB using cynomolgus monkey models.

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

Efficacy of HSP65 + IL-12 DNA in Monkeys	
Survival	. . .
Chest X-p (improvement)	.
Erythrocyte Sedimentation Rate (improvement)	. .
Body Weight	.
Immune Responses	
IFN-γ	. .
IL-6	. .
IL-2	. .
Proliferation of T cell	.

Furthermore, using 32 monkeys, the protective efficacy of the HSP65+IL-12 /HVJ and BCG using the priming-booster method in the TB infected cynomolgus monkeys was very strong. All four monkeys from the group of BCG-priming and the DNA vaccine (HVJ-liposome/HSP65+IL-12 DNA vaccine) booster were alive more than 12 months post-infection (Fig.1). 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-priming and the BCG Tokyo vaccine booster 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-priming and the DNA vaccine-booster (Table1). Furthermore, proliferation of PBL was strongly enhanced. Taken together, these results clearly demonstrate that BCG priming and the HSP65+hIL-12/HVJ booster could provide extremely strong protective efficacy against *M.tuberculosis* in the cynomolgus monkey model.

(b)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 2A).

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 2A).

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

Vaccine	mouse	guinea pig	monkey	SCID-PBL/hu	human
HVJ-Envelope/ Hsp65 DNA + IL-12 DNA	prophylactic Effect 10,000 fold effective than BCG	effective	effective	effective	Plan (phase. , .)
	Therapeutic Effect	plan	effective		
	Therapeutic Effect against MDR-TB XDR-TB	plan	plan		
HVJ-liposome/ Hsp65 DNA + IL-12 DNA	Prophylactic Effect 100 fold effective than BCG	effective	effective (100% survival)		

Vaccine	mouse	guinea pig	monkey	SCID-PBL/hu	human
HVJ-Envelope/ Hsp65 DNA + IL-12 DNA + Ag85B DNA + Ag85A DNA	plan	plan	Therapeutic Effect	plan	
15Kgranulysin recombinant 15K granulysin	Therapeutic Effect		plan		
15K granulysin DNA	Therapeutic Effect		plan		

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

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.2). 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). These results demonstrate that HVJ-Envelope/HSP65DNA+ IL-12DNA vaccine could provide strong therapeutic efficacy against TB, MDR-TB or XDR-TB in the cynomolgus monkey models as well as murine models

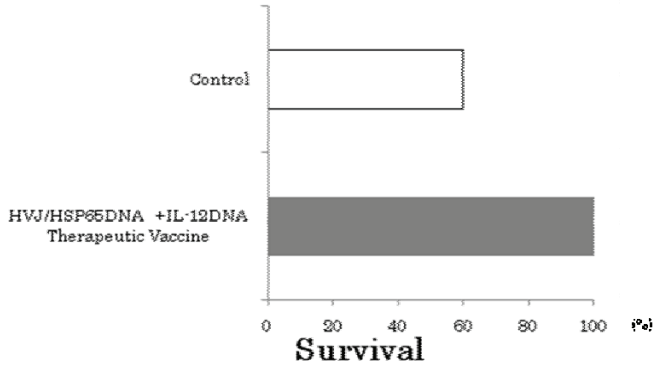

Fig 2. Therapeutic efficacy (survival) of HVJ-Envelope/HSP65DNA+IL-12DNA vaccine 130 days after TB infection using cynomolgus monkey models.

(c) 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 vaccine 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.[1,2,9]

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 guinea pig model, HSP65+gpIL-12/HVJ provided better protection against the pulmonary pathology caused by pulmonary infection with TB than BCG vaccination (data not shown). In the present study, it was demonstrated that BCG vaccine priming and HSP65+h IL-12/HVJ booster could provide extremely strong (100% survival) efficacy against *M.tuberculosis* compared to BCG alone (33% survival) in the cynomologus monkey model. In Japan and other countries, the BCG vaccine is inoculated into human infants (0~6months after birth). Therefore, BCG priming in infants and HSP65+h IL-12/HVJ boosters 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 cynomologus 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 was also strongly observed in the therapeutic models of monkeys(Table 2B). In the recent study, it is demonstrated that granulysin vaccine shows therapeutic efficacy against TB in mice(Table 2B). 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.

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.

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