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Novel recombinant BCG and DNA-vaccination against tuberculosis in a cynomolgus monkey model

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Abstract

We have developed two novel tuberculosis (TB) vaccines: a DNA vaccine combination expressing mycobacterial heat shock protein 65 (Hsp65) and interleukin-12 (IL-12) by using the hemagglutinating virus of Japan (HVJ)-liposome (HSP65 + IL-12/HVJ) and a recombinant BCG harboring the 72f fusion gene (72f rBCG). These vaccines provide remarkable protective efficacy in mouse and guinea pig models, as compared to the current by available BCG vaccine. In the present study, we extended our studies to a cynomolgus monkey model, which is currently the best animal model of human tuberculosis, to evaluate the HSP65 + IL-12/HVJ and 72f rBCG vaccines. Vaccination with HSP65 + IL-12/HVJ as well as 72f rBCG vaccines provided better protective efficacy as assessed by the Erythrocyte Sedimentation Rate, chest X-ray findings and immune responses than BCG. Most importantly, HSP65 + IL-12/HVJ resulted in an increased survival for over a year. This is the first report of successful DNA vaccination and recombinant BCG vaccination against *M. tuberculosis* in the monkey model.

Keywords: HSP65 DNA + IL-12 DNA vaccine; Tuberculosis; Monkey

1. Introduction

Tuberculosis (TB) is a major global threat to human health, with more than 3 million people dying each year from *M. tuberculosis* (TB) infections. The only tuberculosis vaccine currently available is an attenuated strain of *M. bovis* BCG (BCG), although its efficacy against adult TB disease remains controversial. Therefore, we have recently developed two novel TB vaccines: a DNA vaccine combination expressing mycobacterial heat shock protein 65 (Hsp65) and interleukin-12 (IL-12) by using the hemagglutinating virus of Japan (HVJ)-liposome (HSP65 + IL-12/HVJ) and a recombinant BCG harboring the 72f fusion gene (r72f BCG). The former vaccine was 100-fold more efficient than BCG in the elimination of *M. tuberculosis* in mice by the induction of CTL (Yoshida et al., submitted for publication).

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Researchers have recognized that a nonhuman primate model of TB will be able to provide critical information for vaccine development. However, several TB vaccine candidates who appear to protect better than BCG against virulent *M. tuberculosis* in mice, have rarely been tested in the nonhuman primate model because of cost and limited facilities.

In the present study, we evaluated the protective efficacy of HSP65 + IL-12/HVJ and r72f BCG in the cynomolgus monkey model, which is an excellent model of human tuberculosis [1]. These vaccines provided a strong prophylactic effect in monkeys challenged with *M. tuberculosis* as we have seen previously in mice.

2. Materials and methods

DNA vaccines encoding *M. tuberculosis* HSP65, mouse IL-12 and guinea pig IL-12 were encapsulated with HVJ-liposomes [2]. Groups of animals (mice and guinea pigs) were vaccinated intramuscularly with HVJ-liposome DNA vaccines. CTL activity was assessed by ^{51}Cr-release and IFN-γ activity [3,4]. A total of 16 cynomolgus monkeys were housed in a BL 3 animal facility of the Leonard Wood Memorial. Groups of animals were vaccinated three times with either the HVJ-liposome combination with HSP65 DNA plus human IL-12 DNA (HSP65 + hIL-12/HVJ: 400 μg i.m.), r72f BCG (1×10^6 CFU i.d.), BCG Tokyo (1×10^6 CFU i.d.) or saline. One month after the third vaccination, monkeys were challenged with the *M. tuberculosis* Erdman strain (5×10^2) by intratracheally instillation, Erythrocyte Sedimentation Rate (ESR), body weight, chest X-ray, immune responses, DTH reaction against PPD and survival periods were examined during 14 months [1].

3. Results

Mice vaccinated with HSP65 + mIL-12/HVJ had significantly reduced numbers of CFU [5] in the lungs, liver and spleen as compared with mice vaccinated with BCG (Yoshida et al., submitted for publication). CTL activity correlated with the protective efficacy of vaccination. The fusion protein Mtb72f (Mtb39 + Mtb32) vaccine was developed by Skeiky et al. [6]. To improve its vaccine efficacy, a recombinant BCG harboring the 72f fusion gene (r72f BCG) was generated [7]. The ELISPOT assay showed that r72f BCG induced a greater number of IFN-γ producing T-cells than BCG in the mouse model. In the guinea pig model, r72f BCG as well as HSP65 + gpIL-12/HVJ provided better protection against the pulmonary pathology caused by pulmonary challenge with TB than BCG vaccination (data not shown).

The purpose of this study was to evaluate two TB vaccines we have developed in a nonhuman primate model of *M. tuberculosis* infection. To this end, a total of 16 monkeys were vaccinated either with HSP65 + hIL-12/HVJ, r72f BCG, BCG or saline, followed by TB challenge by intratracheally instillation. Table 1 shows survival periods of vaccinated monkeys after TB challenge. All four monkeys in the control (saline) group died of TB infection within 8 months. In contrast, three and two monkeys from the 72f rBCG and HSP65 + hIL-12/HVJ groups, respectively, were alive more than 14 months post-infection (the termination period of the experiment). Survival periods of the remaining monkeys in the both groups were much longer than those of saline control group. In addition, both HSP65 + hIL-12/HVJ and r72f BCG significantly improved ESR and chest X-ray findings (Table 2). Body weights of the HSP65 + hIL-

Table 1
Survival of cynomolgus monkeys immunized with HVJ-liposome/HSP65 DNA + IL-12 DNA vaccine and recombinant 72f BCG vaccine

Vaccination	Total monkeys	Survival	Dead	% Survival
HVJ-liposome/HSP65 DNA + IL-12 DNA	4	2	2	50
Recombinant 72f BCG	4	3	1	75
BCG Tokyo	4	2	2	50
Saline	4	0	4	0

Cynomolgus monkey (4 monkeys/group) were immunized three times (every 3 weeks) with (1) HVJ-liposome/ HSP65 DNA + IL-12 DNA vaccine, (2) r72f BCG vaccine, (3) BCG Tokyo and (4) saline as control group as described in Section 2. One month after last immunization, M.TB (Erdman strain 5×10^2) was challenged by intratracheally instillation. Survival was studied more than 14 months.

Table 2
Improvement of Erythrocyte Sedimentation Rate (ESR) in the cynomolgus monkeys immunized with HVJ-liposome/HSP65 DNA + IL-12 DNA vaccine and recombinant 72f vaccine

Vaccination	ESR (nm/h)	Mean ± S.D.	Statistical significance *P*-value compared to saline group (Student *t*-test)
HVJ-liposome/HSP65 DNA + IL-12 DNA	2	3.5 ± 1.9	<0.01
	6		
	4		
	2		
Recombinant 72f BCG	3	6.75 ± 8.9	Not significant
	1		
	20		
	3		
BCG Tokyo	22	11.25 ± 11.3	Not significant
	2		
	20		
	1		
Saline	50	29.75 ± 18.1	
	14		
	15		
	40		

Cynomolgus monkey (4 monkeys/group) were immunized and challenged as described in Table 1. Elevation of Blood Sedimentation Ratio (BSR) of all monkeys was evaluated every month and maximum values of BSR in each monkey were shown.

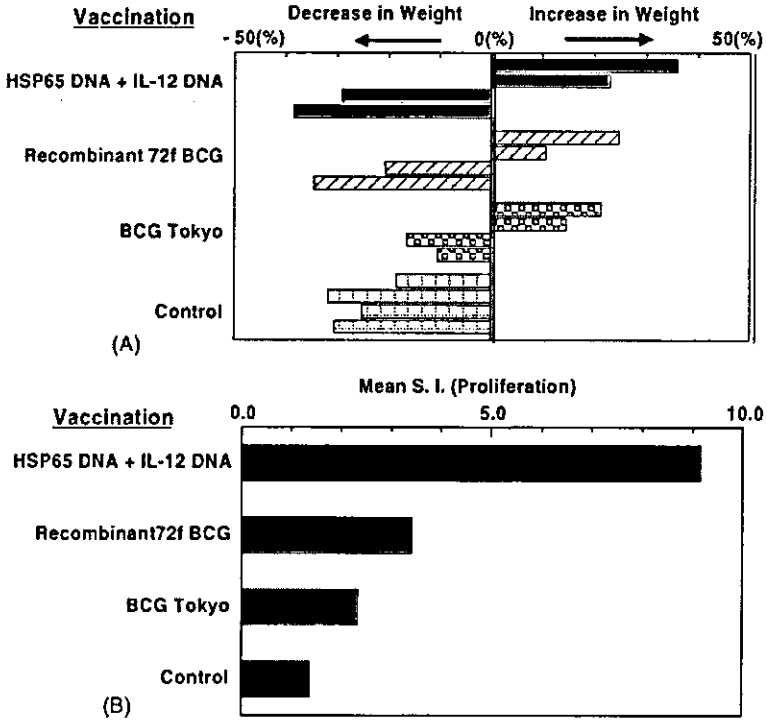

Fig. 1. (A) Increase in body weight: the prophylactic effect of novel vaccines (HSP65 DNA + IL-12 DNA, recombinant 72f BCG) on *M. tuberculosis* infection of cynomolgus monkeys. Percent of increase or decrease in body weight of monkeys immunized with (1) HSP65 DNA + IL-12 DNA (■), (2) recombinant 72f BCG (▨), (3) BCG Tokyo vaccines (▩) and (4) saline (control) (□) and challenged with *M. tuberculosis*, compared to the weight of pre-immunized monkeys. (B) Lymphocyte proliferation activity (LPA) against recombinant HSP65 protein in the peripheral blood (whole blood) from the cynomolgus monkeys immunized with novel vaccines and challenged with *M. tuberculosis*. Peripheral blood lymphocytes (whole blood) 4 weeks after TB challenge were cultured with 10 μg/ml of recombinant HSP65 antigen in a 96-microwell plate for 5 days at 37 °C and then pulsed with 1 μCi of [3H] thymidine per well for the final 16–18 h of incubation. Results are expressed as a stimulation index (S.I.) and compared to the pre-immune LPA from the same monkey.

12/HVJ group also increased significantly, as compared to saline control group (Fig. 1A). IL-2 and IFN-γ production were augmented in the two groups vaccinated with HSP65 + hIL-12/HVJ and r72f BCG (data not shown). Furthermore, proliferation of PBL was strongly enhanced in the group vaccinated with HSP65 + hIL-12/HVJ in response to HSP65 protein 4 weeks after TB challenge (Fig. 1B). Taken together, these results clearly demonstrate that both HSP65 + hIL-12/HVJ and r72f BCG could provide protective efficacy against *M. tuberculosis* in the cynomolgus monkey model.

4. Discussion

HSP65 + hIL-12/HVJ vaccine as well as r72f BCG vaccine exerted the significant prophylactic effect against TB, as indicated by: (1) prolongation of survival for over a year, (2) improvement of ESR and chest X-ray findings, (3) increase in the body weight and (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 with TB is usually accompanied by progress of TB disease. Suppression of IFN-γ production, CTL activity and T-cell proliferation has also been observed in patients with TB [8].

Our results with the HSP65 + hIL-12/HVJ vaccine in the cynomolgus monkey model should provided a significant rational for moving this vaccine into clinical trials. In fact, the 72f fusion protein vaccine entered Phase I testing after its evaluation in cynomolgus monkeys in Leonard Wood Memorial [4] by Reed and Skeiky. Thus, we are taking advantage of the availability of multiple animal models (mouse, guinea pig, and monkey) to accumulate essential data on the HVJ-liposome DNA vaccine in anticipation of a Phase I clinical trial.

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Novel (Recombinant BCG- and DNA-) Vaccination Against Tuberculosis using cynomolgus monkey

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Summary

HVJ-liposome / HSP65 DNA + IL-12 DNA vaccination were 100 fold more efficient than BCG on the elimination of Mycobacterium tuberculosis (M.TB) in lungs, liver, and spleen in the BALB/c mice. Cytotoxic T cells activity against M.TB in the mice was augmented. The recombinant(r) 72f BCG vaccine as well as HSP65 DNA + IL-12 DNA vaccine showed stronger anti-TB immunity than BCG in the mice, and guinea pigs. By using these new vaccines (HSP65 DNA + 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 (survival, Erythrocyte Sedimentation Rate, chest X-P finding, immune responses) of vaccines was observed. Thus, these novel vaccines should provide a useful tool for the prevention of human TB infection.