2014 International Vaccine Meeting

Immunogenicity and therapeutic effects of Ag85A/B chimeric DNA vaccine in mice infected with *M. tuberculosis*

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Institute of Tuberculosis Research,

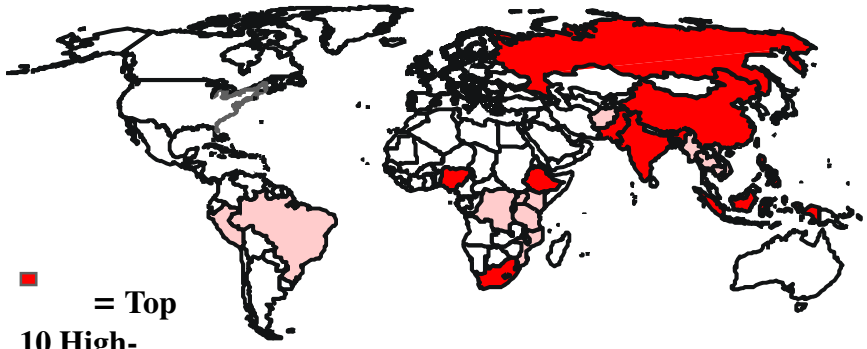
The 309th Hospital of Chinese PLA,

Beijing 100091,

China

Report on surveillance of drug resistance in *M. tuberculosis* in China in 2008

- **In the patient with pulmonary TB**
  - **MDR-rate is 8.32%**
  - **XDR-rate is 0.68 %**



= Top 10 High-burden countries

India China Indonesia Nigeria Bangladesh Pakistan Philippines Ethiopia South Africa Russian Federation DR Congo Viet Nam Kenya Brazil UR Tanzania Thailand Mozambique Myanmar Uganda Afghanistan Zimbabwe Cambodia Peru

The difficulty of MDR-TB treatment

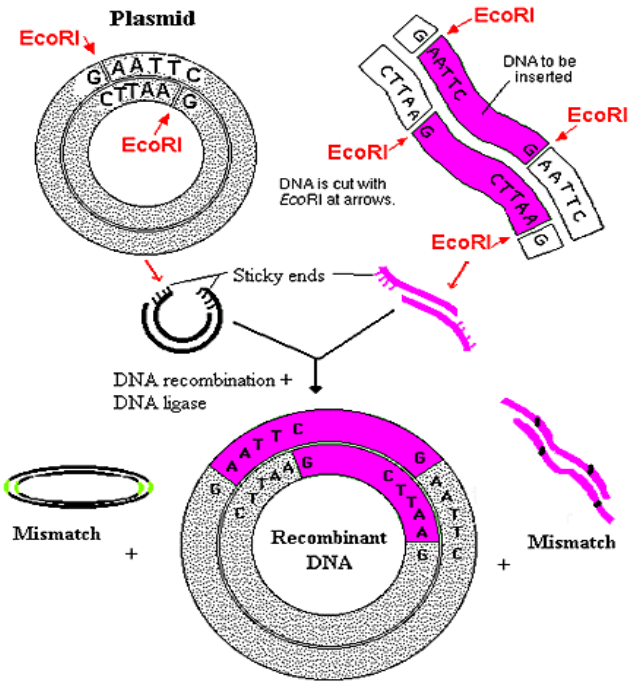
- **The strategy of DOTS is achieving substantial progress in the control of TB worldwide.**
- **MDR-TB, XDR-TB has emerged as a new challenge, especially in the developing countries.**
- **Second-line anti-TB drugs are expensive, less effective and more toxic.**
- **It is mainly due to lacking of the funding to support the treatment of MDR-TB with second line anti-TB drugs.**

Immunotherapy

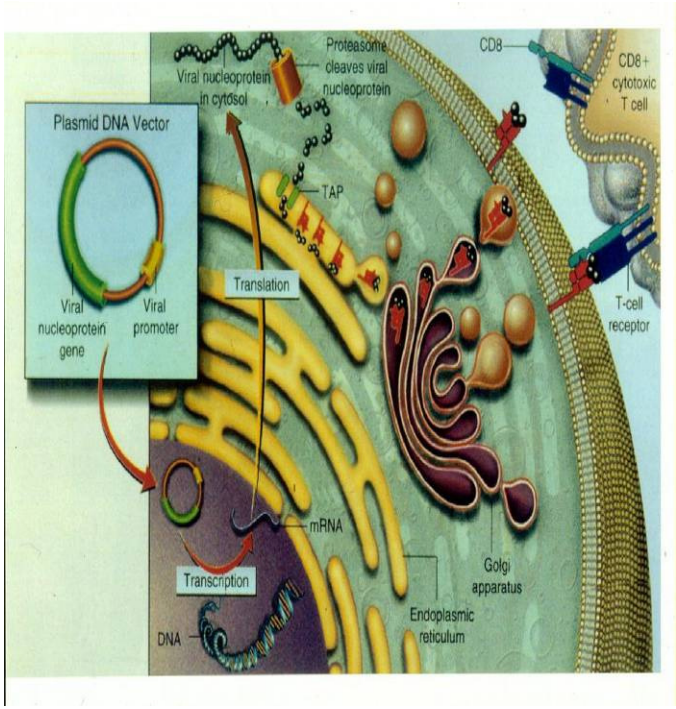
- boost the efficacy of the immune system
- an alternative way for the treatment of tuberculosis
- assist anti-TB chemotherapy to shorten curable duration in patients.

DNA vaccine

- elicit both humoral and cellular immune responses,
- to confer protective and therapeutic effects on TB in animal models.
- a novel and potentially powerful agent to prevent and treat disease

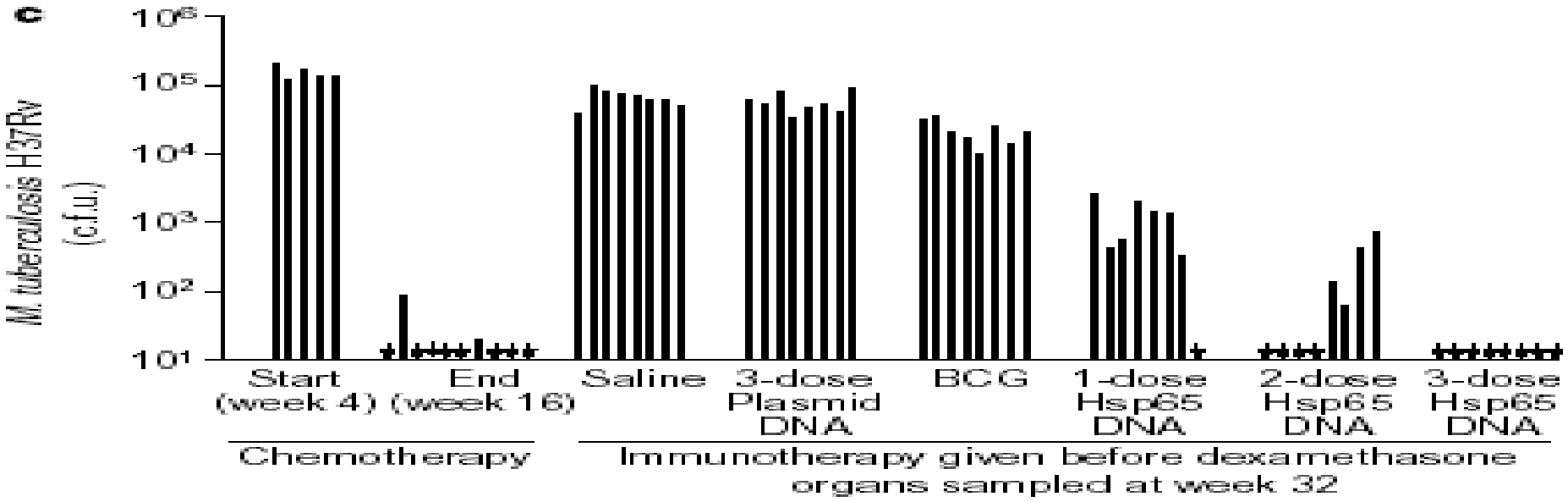


Inserting a DNA Sample into a Plasmid



Lowrie DB, Tascon RE, et al.
Therapy of tuberculosis in mice by DNA vaccination.
Nature 1999; 400:269-271.

- For the first time used the DNA vaccine in the infected mice
- found that hsp65 DNA could eliminate residual *M.tb* in the organs from infected mice and had remarkable therapeutic actions.

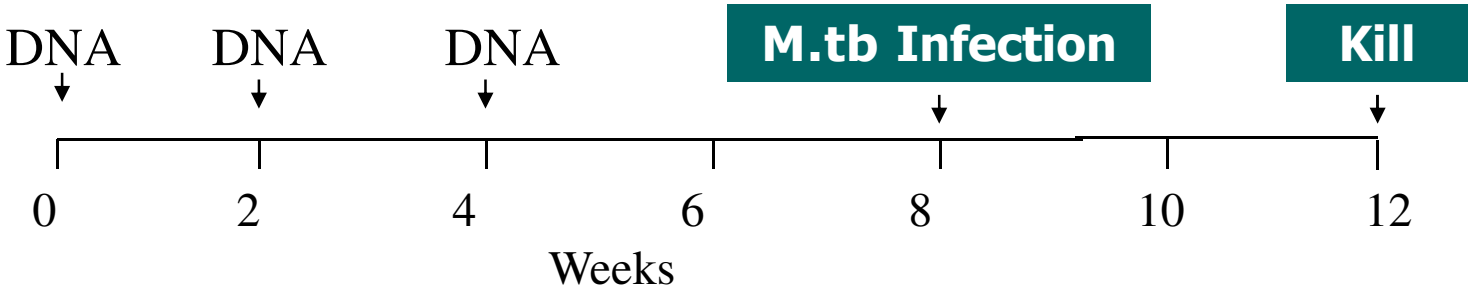


Objective

- **compared the therapeutic efficacy of Ag85A DNA, Ag85B DNA and Ag85A/Ag85B DNA singly or in combination with chemotherapy in the animal model.**
- **To obtain a new therapeutic agents or regimen to treat tuberculosis, especialy MDR-TB**

1. Immunogenicity and protective efficacy of Ag85A and Ag85B DNA vaccines

C57BL/6 mouse TB model and DNA immunization

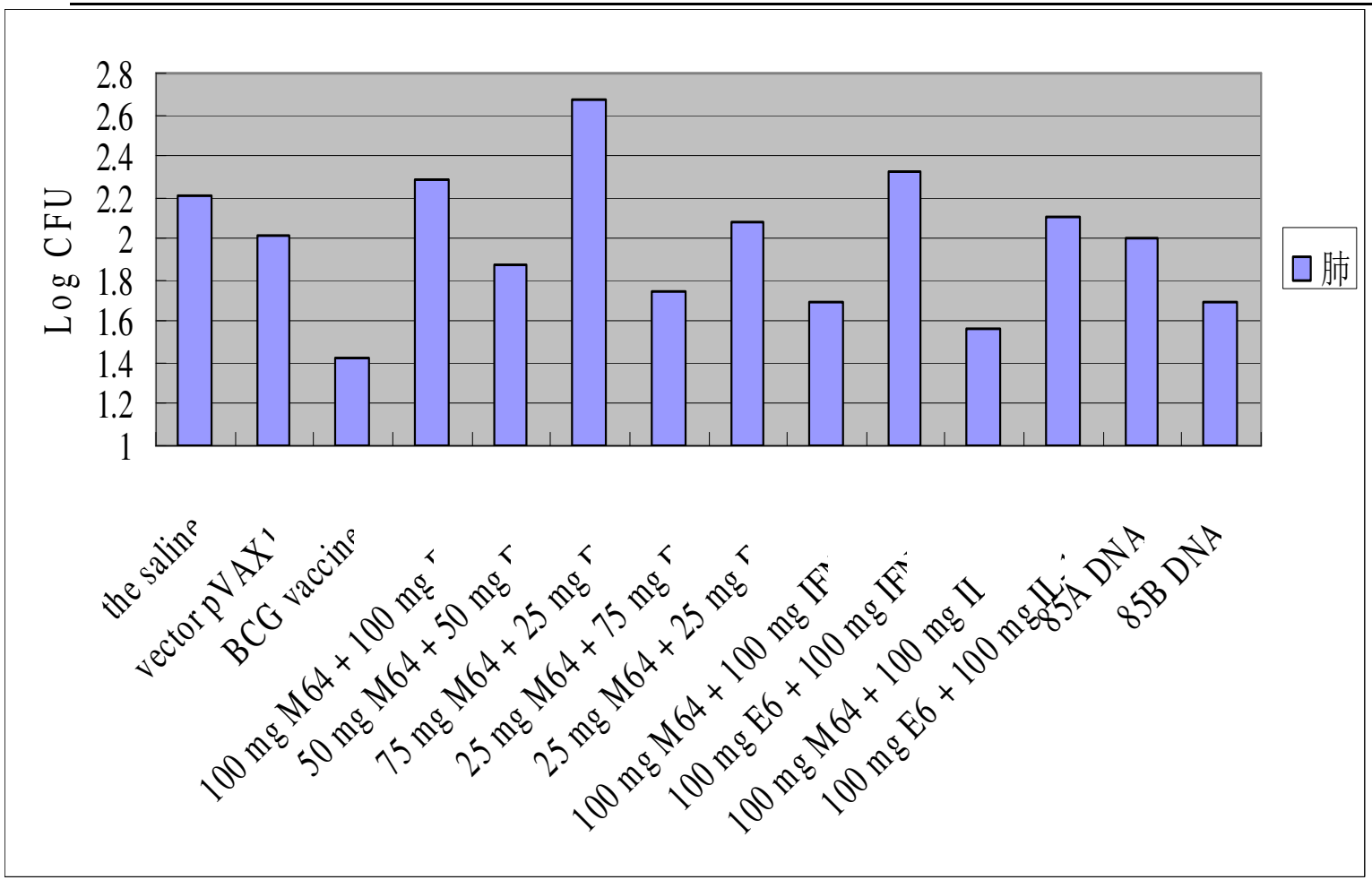


1. Saline
2. Vector pVAX1 100 μg
3. BCG vaccine
4. M64 100 μg + E6 100 μg
5. M64 50 μg + E6 50 μg
6. M64 75 μg + E6 25 μg
7. M64 25 μg + E6 75 μg
8. M64 25 μg + E6 25 μg
9. M64 100 μg + IFNγ 100 μg
10. E6 100 μg + IFNγ 100 μg
11. M64 100 μg + IL-12 100 μg
12. E6 100 μg + IL-12 100 μg
13. 85A 100 μg
14. 85B 100 μg

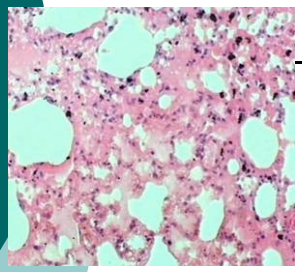
Antibodies detection in the sera by ELISA

Group	Ag85A-specific antibody (X±SD)			Ag85B-specific antibody (X±SD)		
	IgG	IgG1/ IgG2a	IgG2b/ IgG1	IgG	IgG1/ IgG2a	IgG2b/ IgG1
saline	0.166±0.05	1.00	23.16	0.242±0.07	0.46	32.53
vector pVAX1	0.204±0.05	0.90	18.68	0.306±0.09	0.51	25.21
BCG vaccine	0.179±0.09	1.19	20.84	0.259±0.11	0.57	28.24
85A DNA	0.382±0.15	1.91	8.94			
85B DNA				0.924±0.95	2.13	5.92

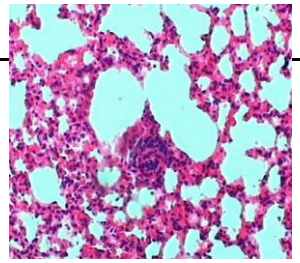
The numbers of viable bacteria in the lungs at 4 weeks after M.tb infection



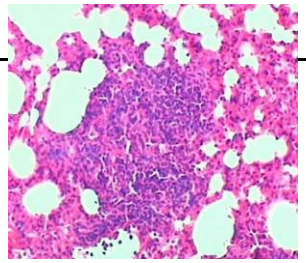
Histopathologies of lungs each group at 4 weeks after M.tb infection



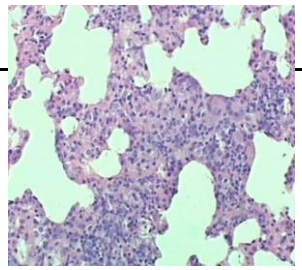
生理盐水



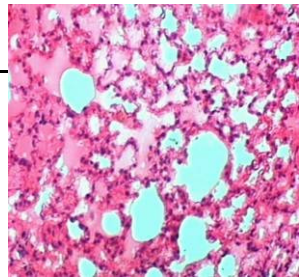
pVAX1



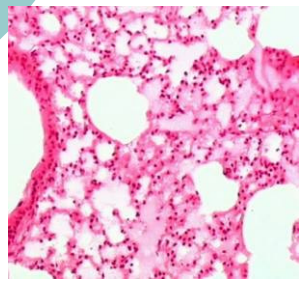
卡介苗



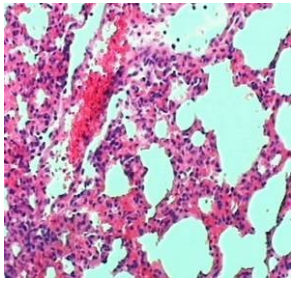
M64 100－E6 100



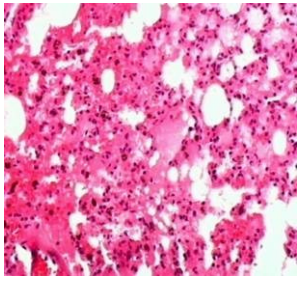
M64 50－E6 50



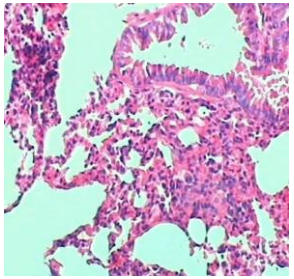
M64 75－E6 25



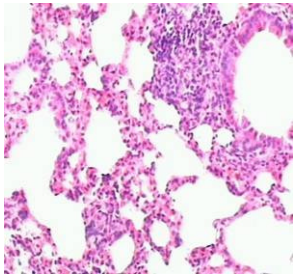
M64 25－E6 75



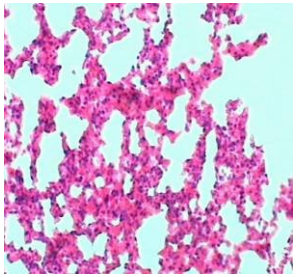
M64 25－E6 25



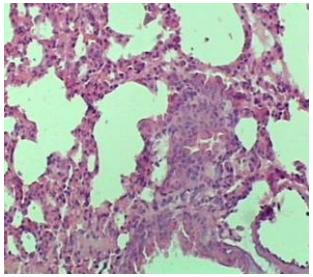
M64－IFNγ



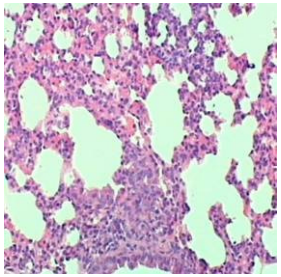
E6－IFNγ



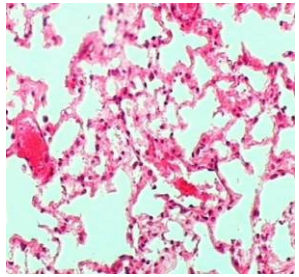
M64－IL12



E6－IL12



85A



85B

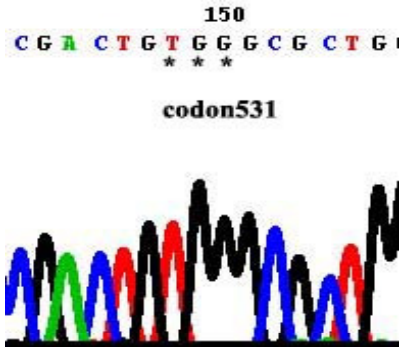
Conclusion (1)

- **Ag85A DNA could alleviate lung lesions in the mouse TB model.**
- **Ag85B DNA could decrease the viable bacteria in the lungs.**
- **Ag85A and Ag85B DNA all have some protective effects on TB.**

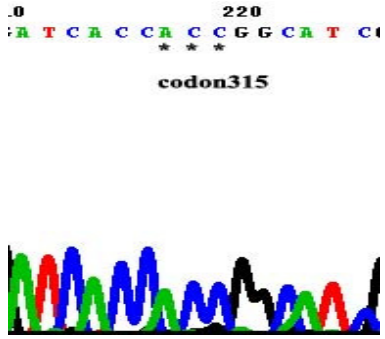
2. The immunotherapeutic effects of Ag85A and Ag85B DNA vaccines

Strain

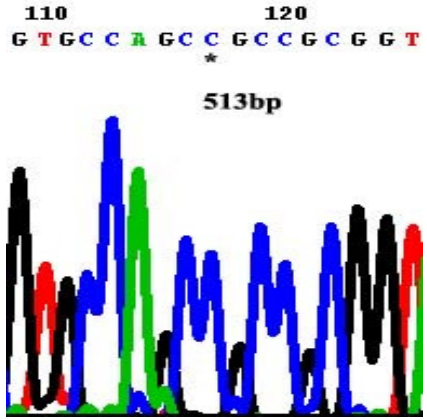
- *M. tuberculosis* isolates HB361
- Conventional drug susceptability testing: RFP 250 μg/ml
  - INH 1μg/ml
  - SM 10μg/ml
- Molecular test: gene mutations



rpoB



katG



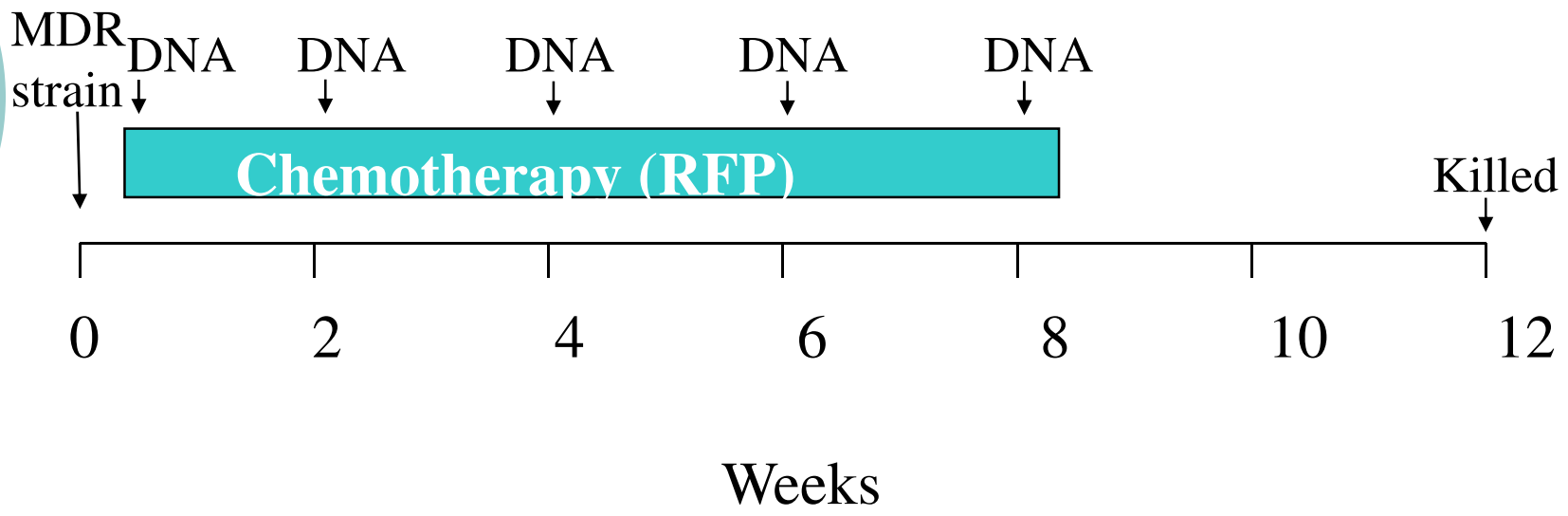
rrs

Establishment of MDR-TB mouse model

- 80 female Balb/c mice of 6-8 weeks were challenged intravenously by tail vein with 220000 CFU/0.4ml of clinical isolate HB361 suspensions.



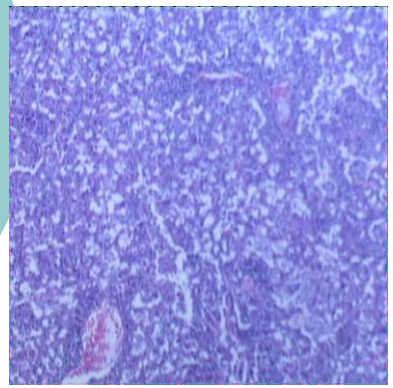
Mouse TB model and DNA immunization



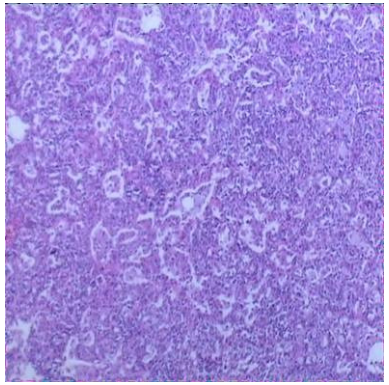
(1) Saline
(2) Plasmid vector pVAX1
(3) 0.4 mg RFP
(4) 100 μg hsp65 DNA

(5) 0.4 mg RFP + 100 μg Ag85A DNA
(6) 100 μg Ag85A DNA
(7) 0.4 mg RFP + 100 μg ESAT6/Ag85A DNA
(8) 100 μg ESAT6/Ag85A chimeric DNA

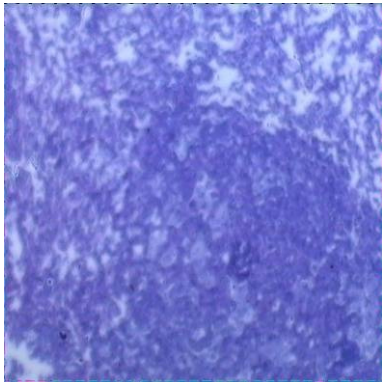
Histopathologies of lungs each group



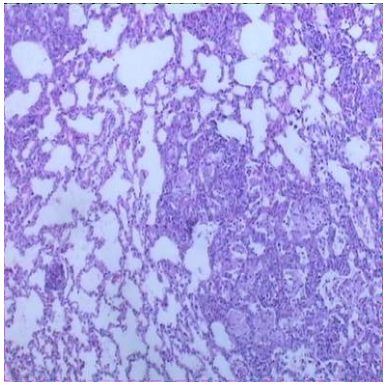
Saline



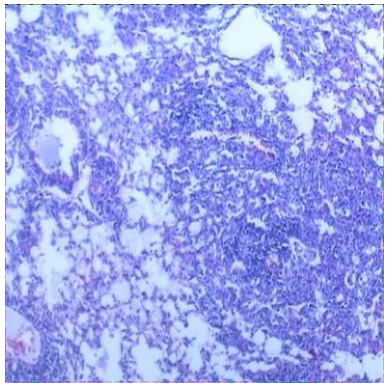
vector



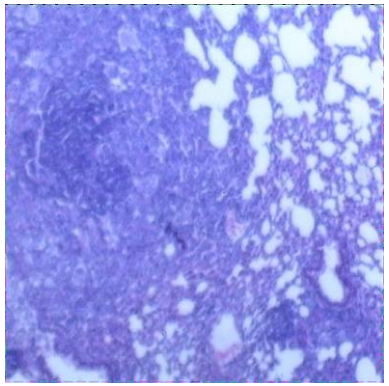
RFP



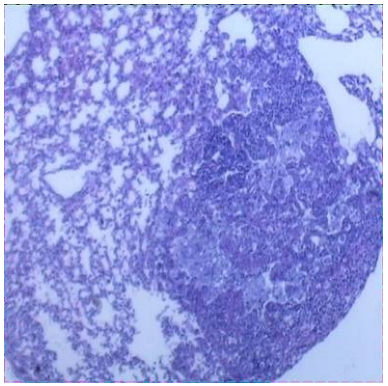
Hsp65



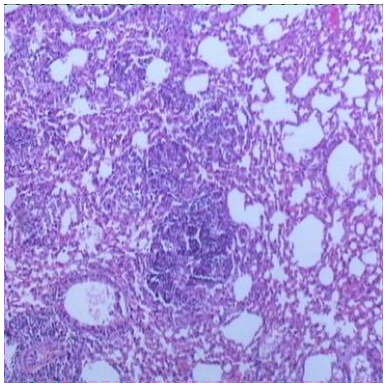
RFP+85A



85A DNA

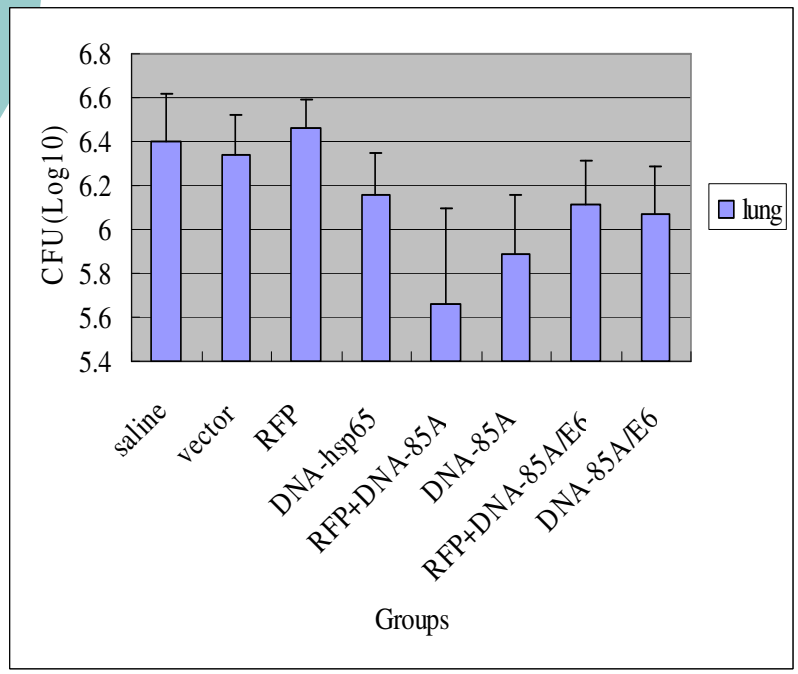


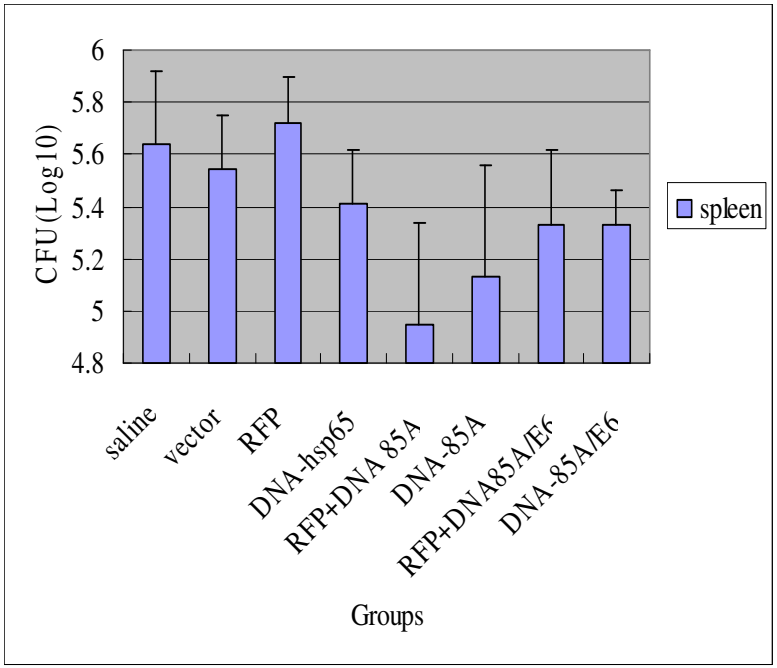
RFP+85A/E6



85A/E6

The numbers of viable bacteria

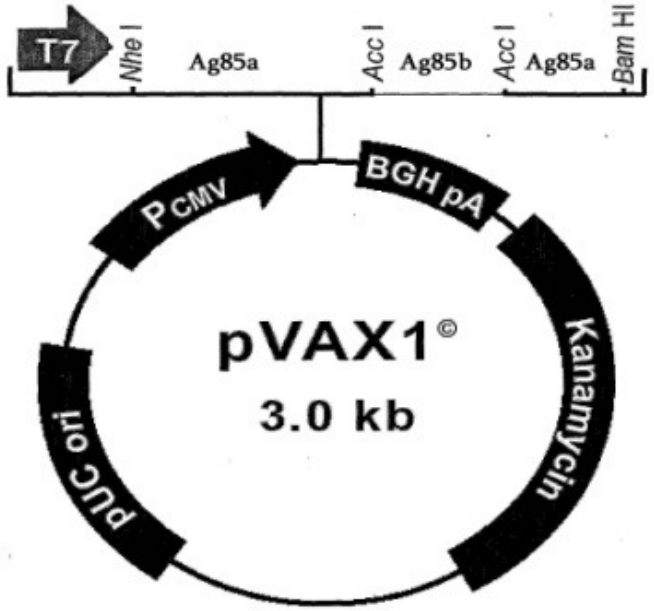




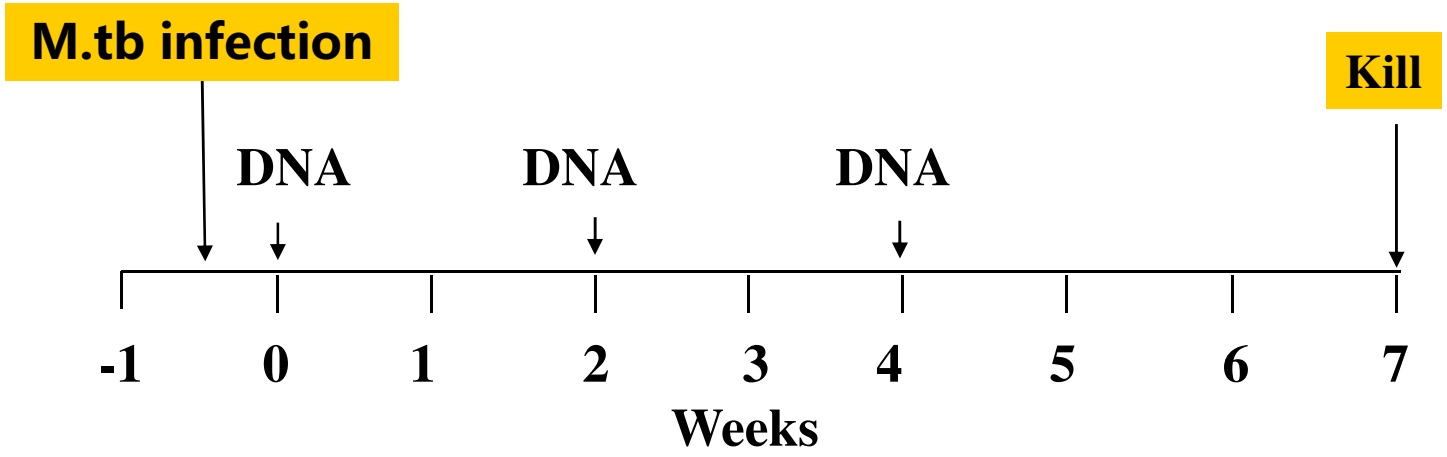
Conclusion (2)

- **Ag85A DNA singly or combined with RFP or PZA had best effect of treatment on MDR-TB infected mice.**
- **The hypothesis is that the immune system was stimulated by Ag85A DNA vaccination and resulted in the enhancement of drug effect of RFP or PZA to kill the MDR-bacteria.**

3. The immunotherapeutic effects of Ag85A/B chimeric DNA



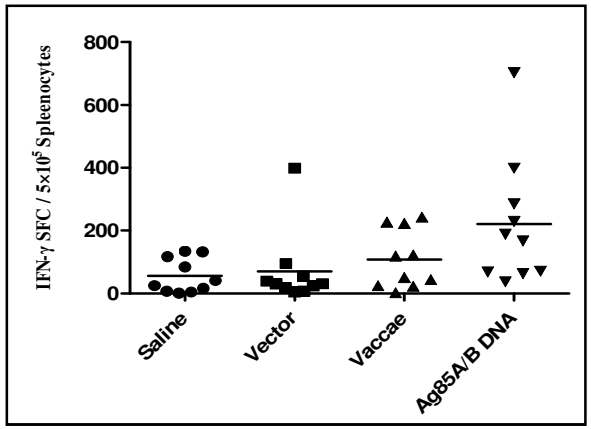
Mouse TB model and DNA immunization

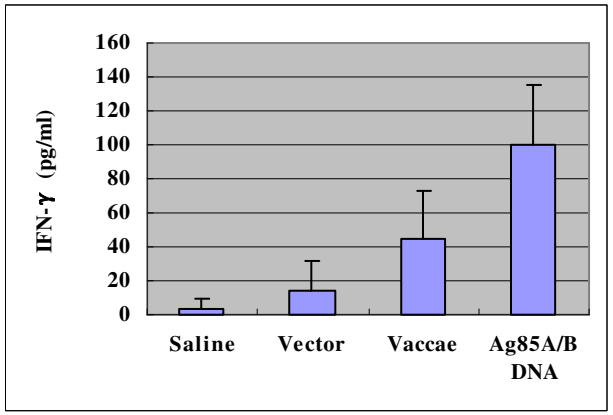


(1) Saline;
(2) 25μg vector pVAX1control;
(3) 50μg vector pVAX1;
(4) 100μg vector pVAX1;
(5) *M. Phlei* F.U.36 injection;

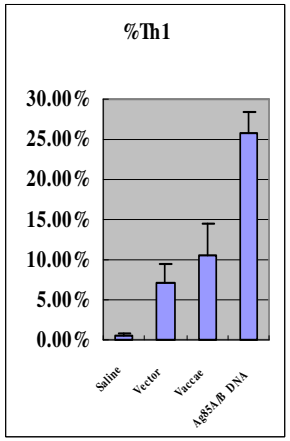
(6) 25μg Ag85A DNA;
(7) 50μg Ag85A DNA;
(8) 100μg Ag85A DNA;
(9) 25μg Ag85A/B DNA;
(10) 50μg Ag85A/B DNA;
(11) 100μg Ag85A/B DNA.

The Cellular immune response in the mice immunized with Ag85A/B chimeric DNA

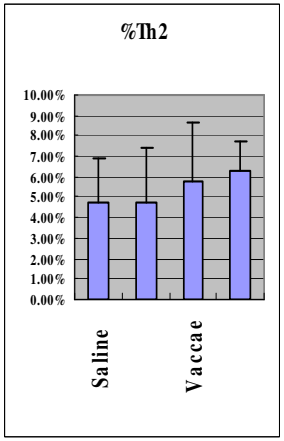




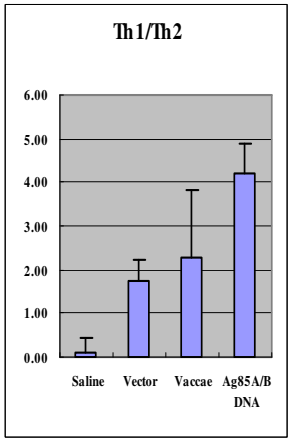
A



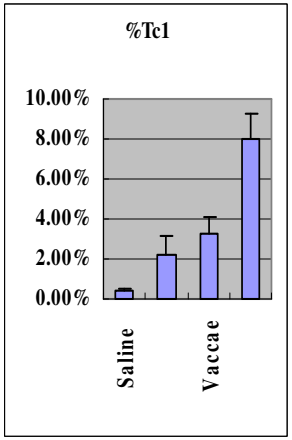
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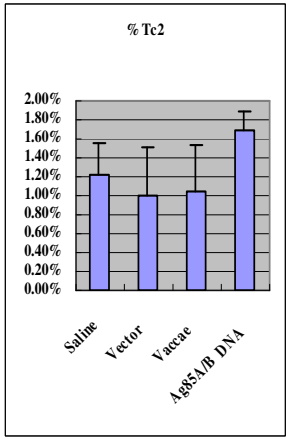
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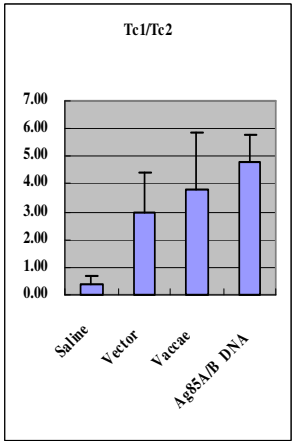
D



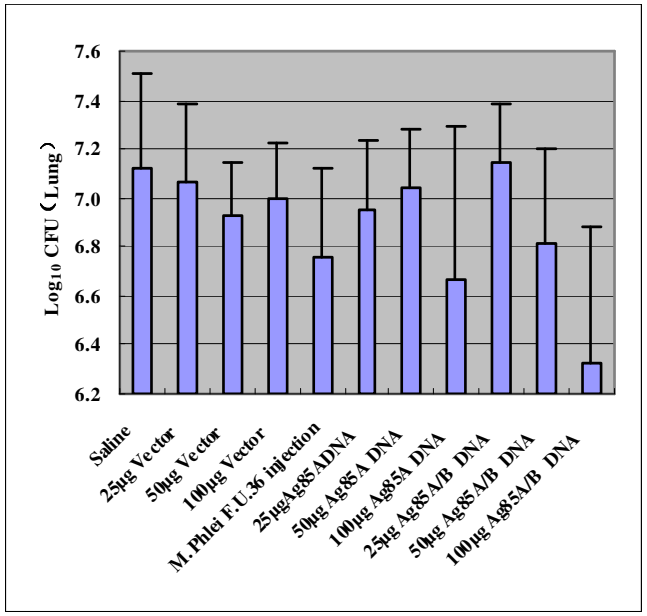
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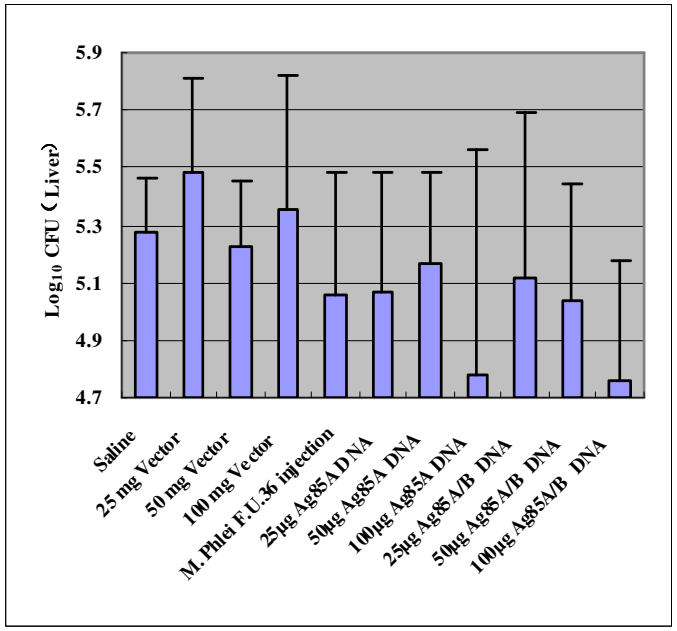


F

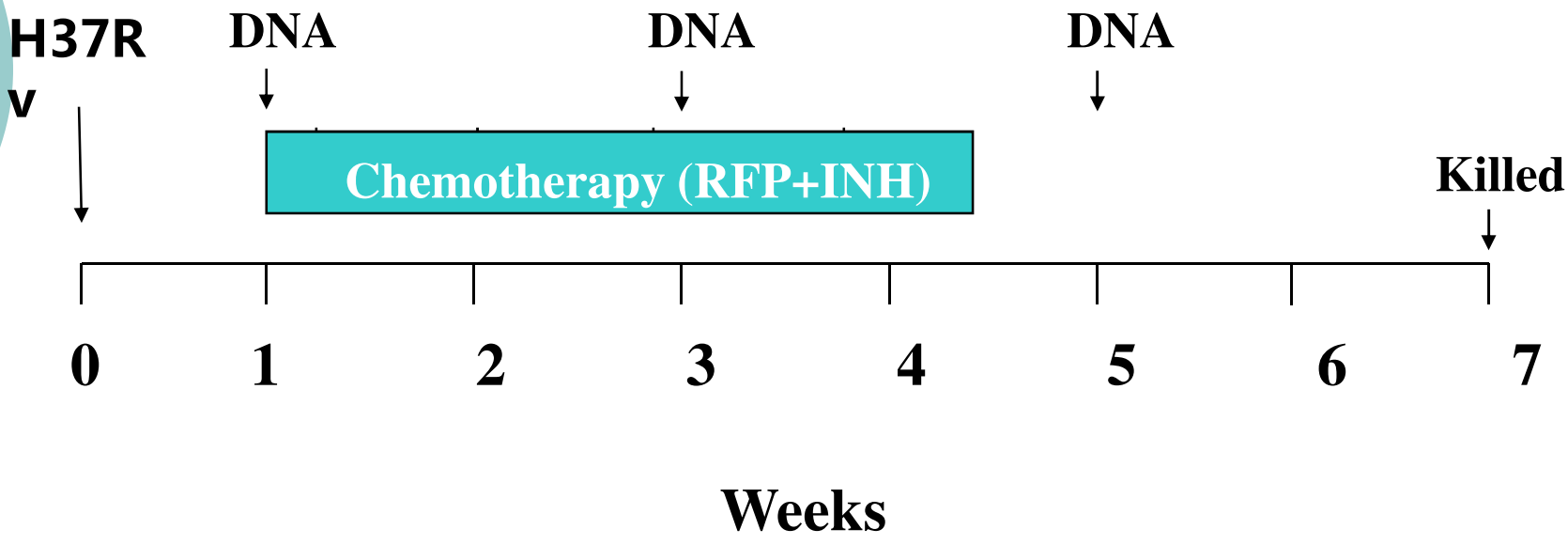


The numbers of viable bacteria in the lungs and the livers



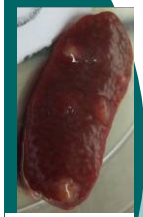


Immunotherapy with Ag85AB DNA vaccine in Guinea pigs TB model



(1)Saline
(2) Vector pVAX1 （150 u g）
(3) chemotherapy: RFP+INH
(4) Ag85A DNA （150 u g）
(5) Ag85AB DNA（150 u g）

Gross pathologies of spleens each group


No.19: 10


No.61: 10


No.23: 20


No.21: 20


No.18: 35


No.22: 35


No.20: 35


No.59: 35


No.60: 35

Saline


No.79: 10


No.45: 10


No.77: 10

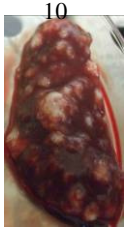

No.78: 10


No.56: 20


No.46: 20


No.57: 20


No.58: 35


No.44: 35

150μg vector


No.16: 0


No.17: 0


No.42: 0

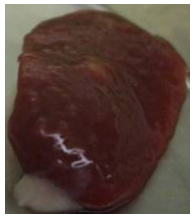

No.43: 0


No.50: 0

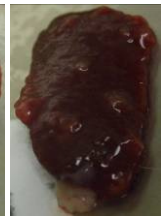

No.51: 0


No.52: 0

RFP+INH


No.27: 10


No.67: 10


No.29: 20


No.68: 10


No.66: 10


No.69: 20


No.65: 35


No.70: 35


No.28: 35

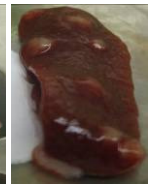
150μg Ag85A DNA


No.40


No.10


No.11


No.12


No.39


No.41

No.24

No.25

No.26

150μg Ag85AB DNA

Conclusion (3)

- **Ag85A/B chimeric DNA vaccine had obvious clear immunotherapeutic effects on TB model.**
- **Inserting Ag85B DNA into Ag85A DNA increased the immunogenicity and immunotherapeutic effect of Ag85A DNA.**
- **Chimeric DNA is a helpful strategy for the development of better TB vaccines.**
- **In one word, therapeutic DNA vaccine is a promising and affordable strategies for the treatment of tuberculosis in developing countries.**

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Animal model in the negative pressure BSL-II Lab.

- **Mouse TB model. challenged by intravenous injection or aerosol using a Middlebrook Airborne Infection Apparatus.**
- **Guinea pig model. challenged by intraperitoneal injection or injection around inguinal lymph nodes.**








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