# GUINEA PIGS, THE PERFECT MODEL FOR EXPERIMENTAL TUBERCULOSIS

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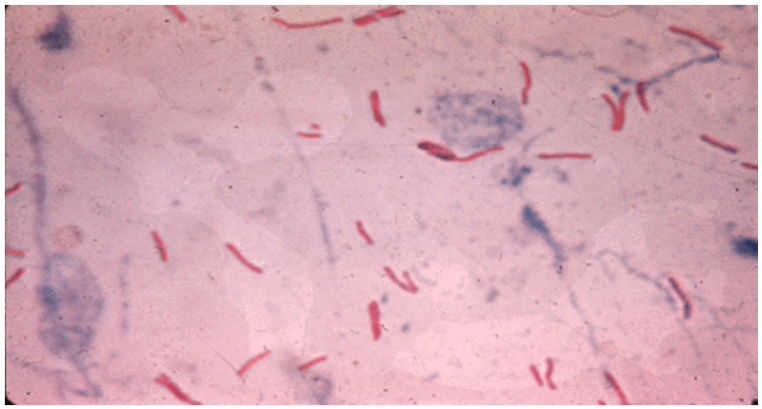
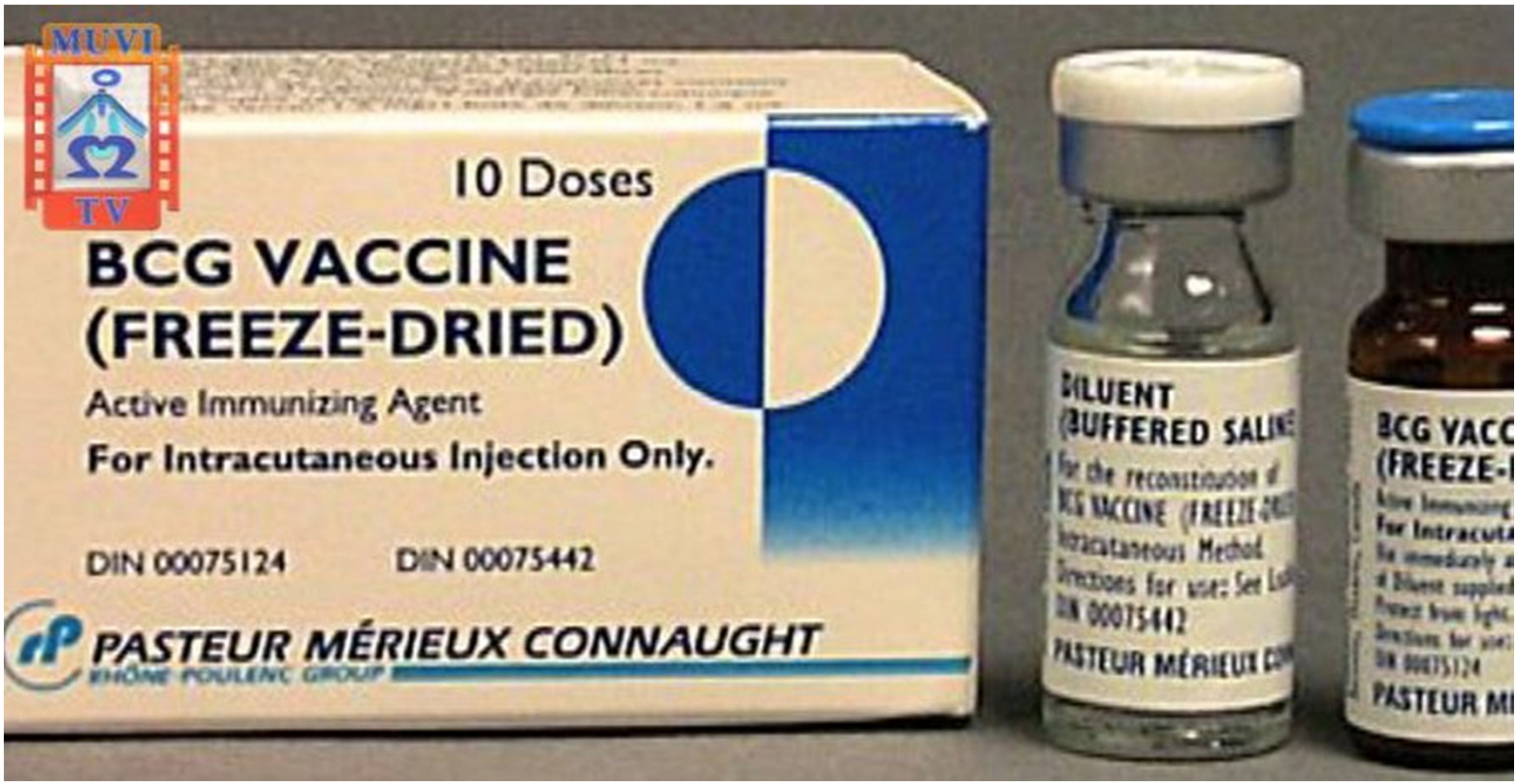
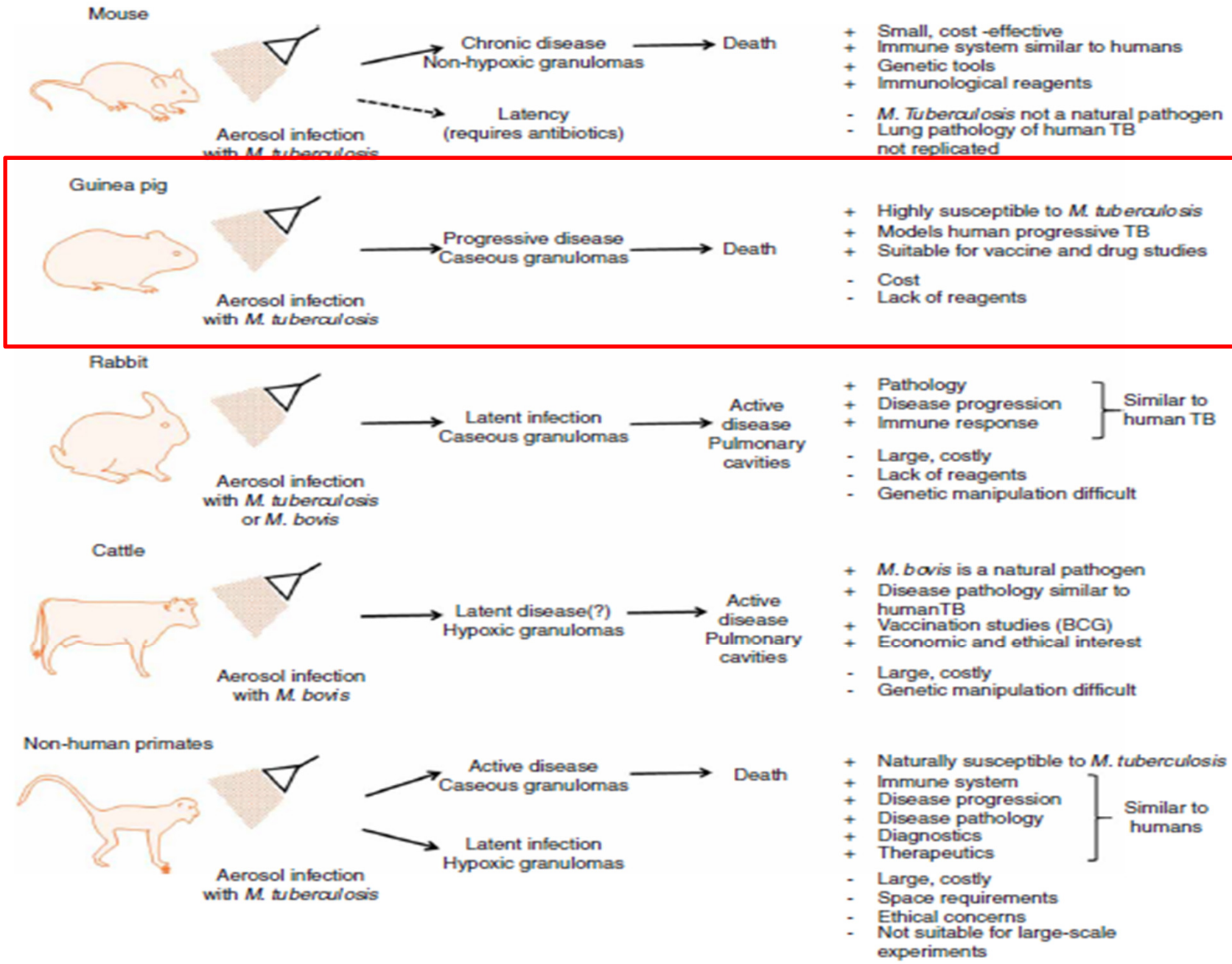
**Tuberculosis** (TB) is still the world's second deadliest infectious disease, with an estimated 9.0 million new cases and 1.5 million deaths in 2013. New drugs and vaccines are urgently needed, and relevant animal models are required to speed up the process.

The almost 100-year-old **Bacille-Calmette-Guerin (BCG)** is currently the only vaccine available for the prevention of TB. Although it protects against a severe form of TB in children (TB meningitis and miliary TB), its efficacy in adults is variable and its protective effect declines over time.



The development of new vaccines against TB is focused on inventing vaccines that would either **replace BCG**, or could be used **to enhance the protection** provided by BCG ('booster' vaccines).






H. Myllymaki *et al.*,2015

## Desirable Attributes

❖Attributes of the guinea pig that make them a desirable research animal include their tractable disposition and their size, which is convenient for many procedures.

❖They are readily available, relatively inexpensive and easy to maintain.

❖Similarity to humans, Guinea pigs have anatomical and physiological features that make them excellent models for specific studies.

❖**Guinea pigs and humans share several features**, including a need for dietary vitamin C, similar placentation and hormonal control of pregnancy, DTH and susceptibility to tuberculosis.

Other research uses of the guinea pigs include

❖immunological studies

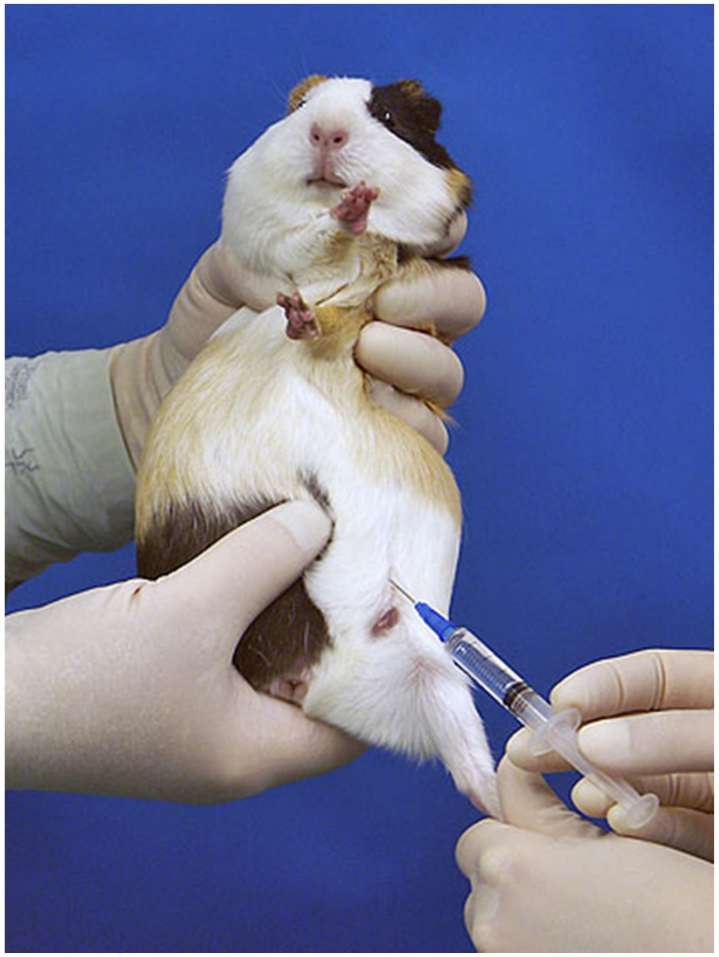
❖auditory research

❖ teratology and toxicity research

❖The guinea pig is also being used as a model for spontaneous diabetes mellitus, Complement 4 deficiency and cryptosporidiosis.

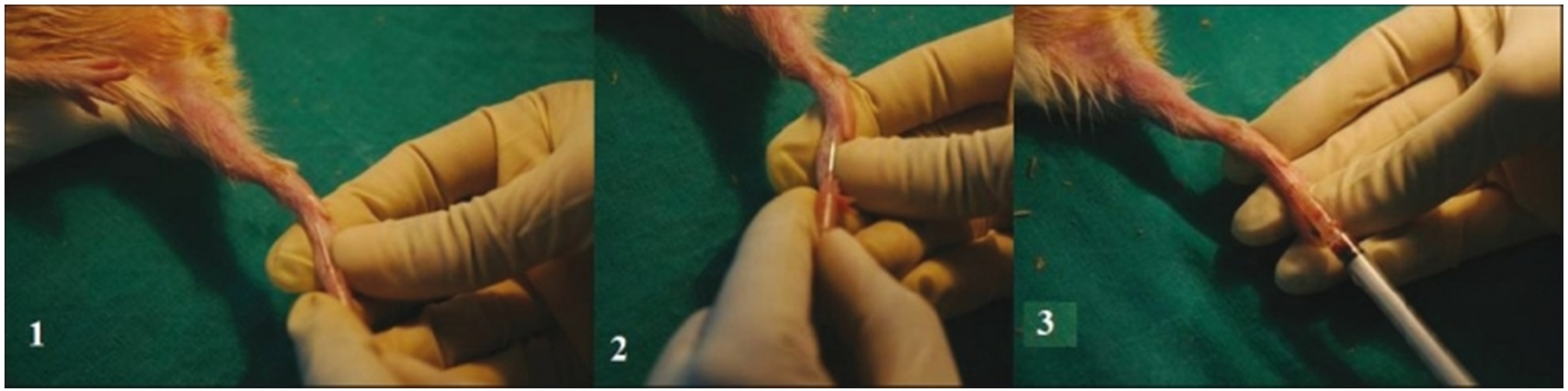
## Handling

Guinea pigs are easy to handle as they do not move quickly, seldom bite and do not inflict injury by kicking or scratching.



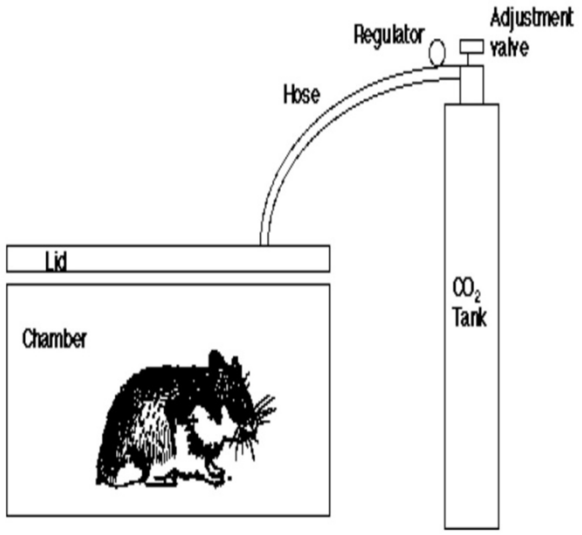
## Collection of blood

- ❖Saphenous vein
- ❖Blood vessel cannulation
- ❖Tarsal vein
- ❖Cardiac puncture
- ❖Orbital sinus



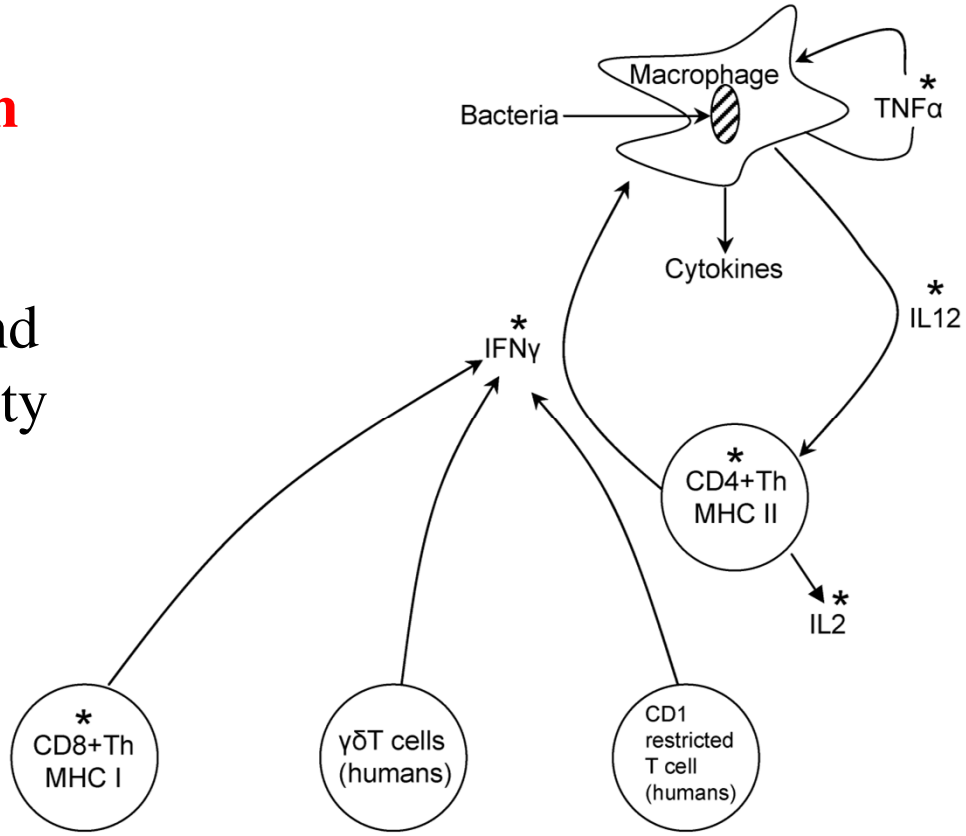
## Euthanization

Guinea pigs may be euthanatized in a carbon dioxide chamber that is pre-charged prior to use. An alternative method is intravenous or intra-peritoneal injection of pentobarbital sodium (150 mg/kg).

## Similar Immune System to Man

Guinea Pig immune system possesses a similar antigen-macrophage interaction to man and delayed cutaneous hypersensitivity reaction.

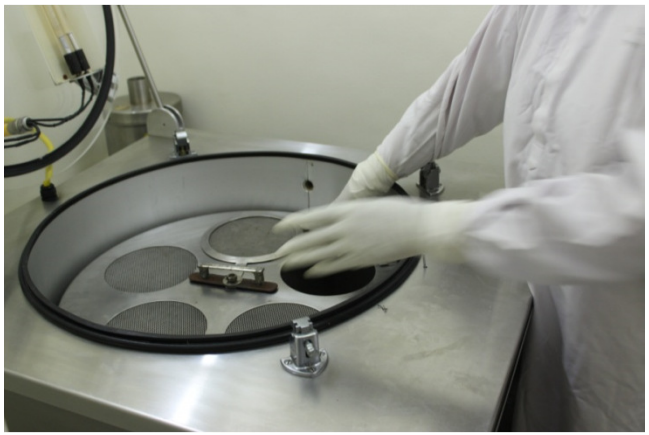


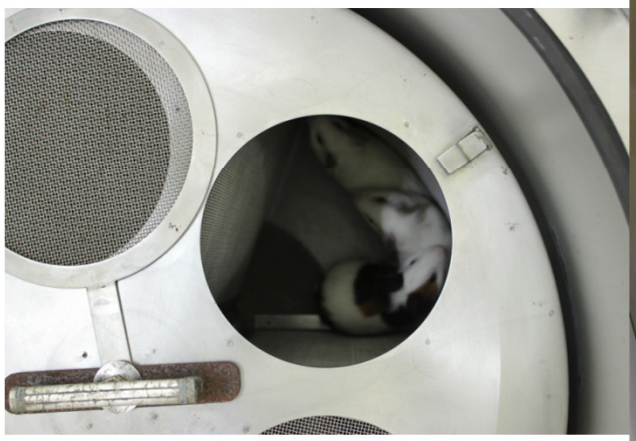

Cascade of immunologic events occurring in the guinea pig model of primary tuberculosis

**DJ Padilla-Carlin *et al*., 2008**

Guinea pigs are well suited to study airborne TB transmission due to their exceptional vulnerability to infection with as little as a few inhaled mycobacteria.

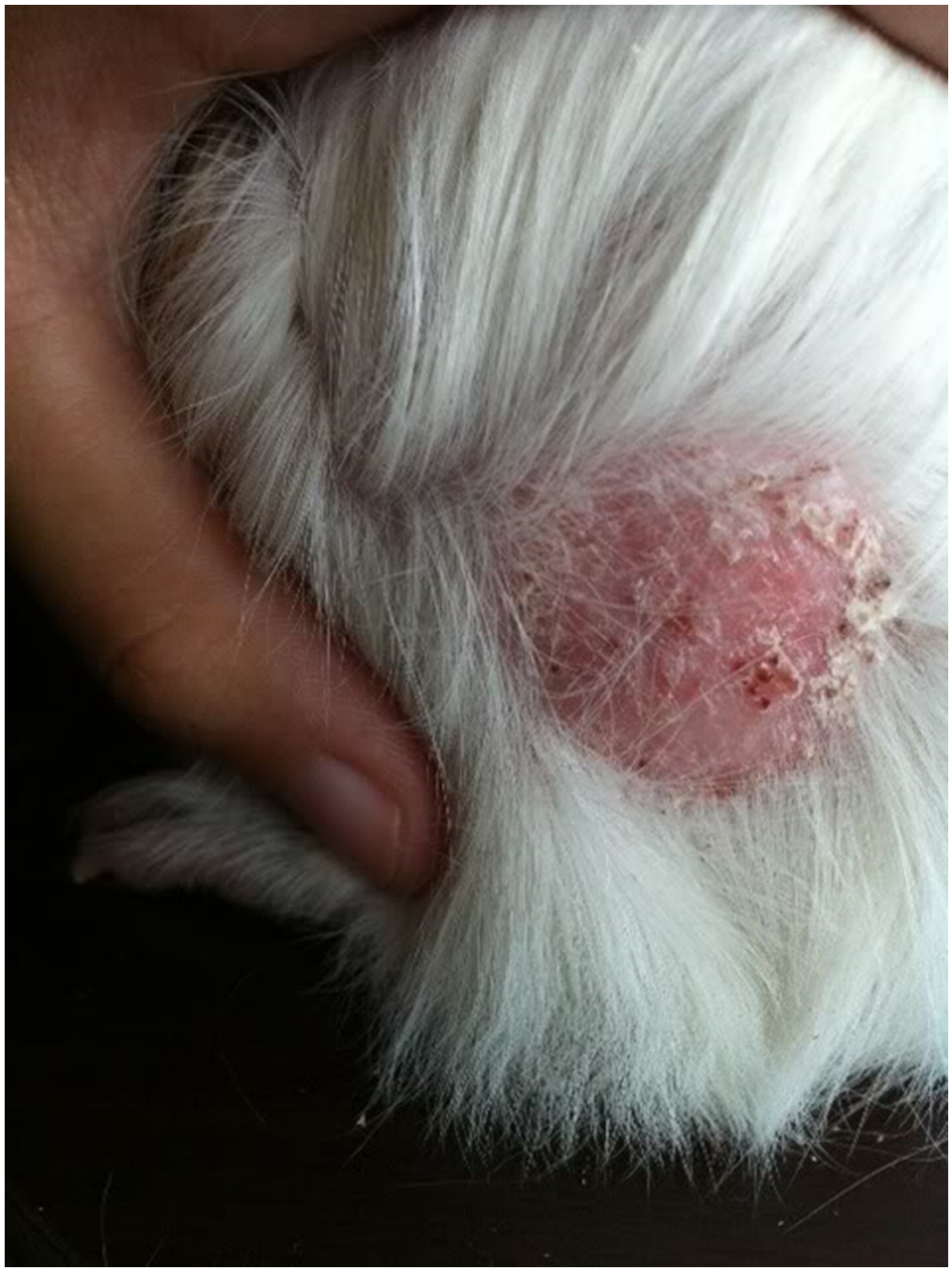
Many aspects of TB infection in humans (especially childhood TB and TB in immunosuppressed hosts), including the formation of granulomata, primary and hematogenous pulmonary lesions, dissemination, and caseation necrosis.



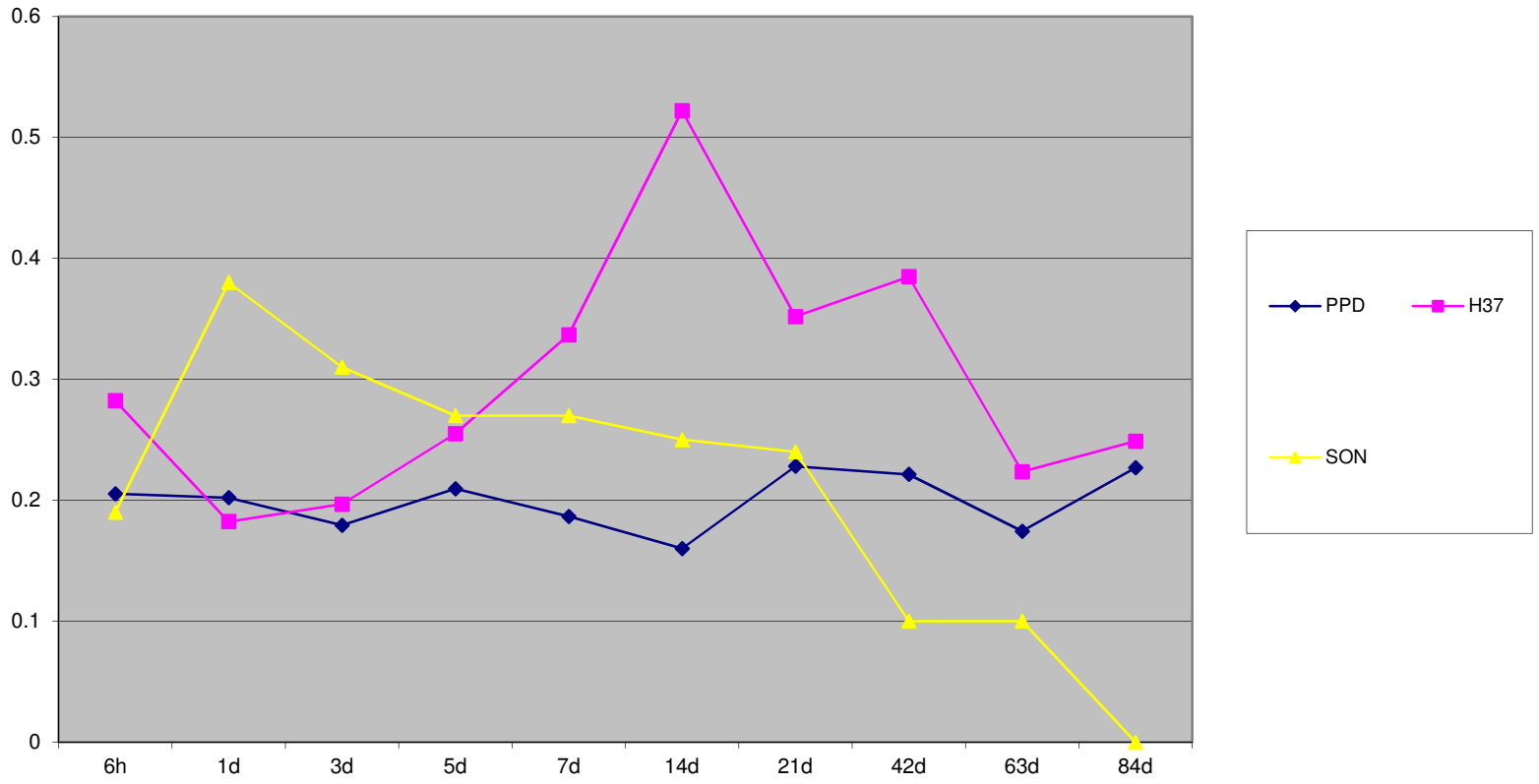


Our experiments with guinea pigs

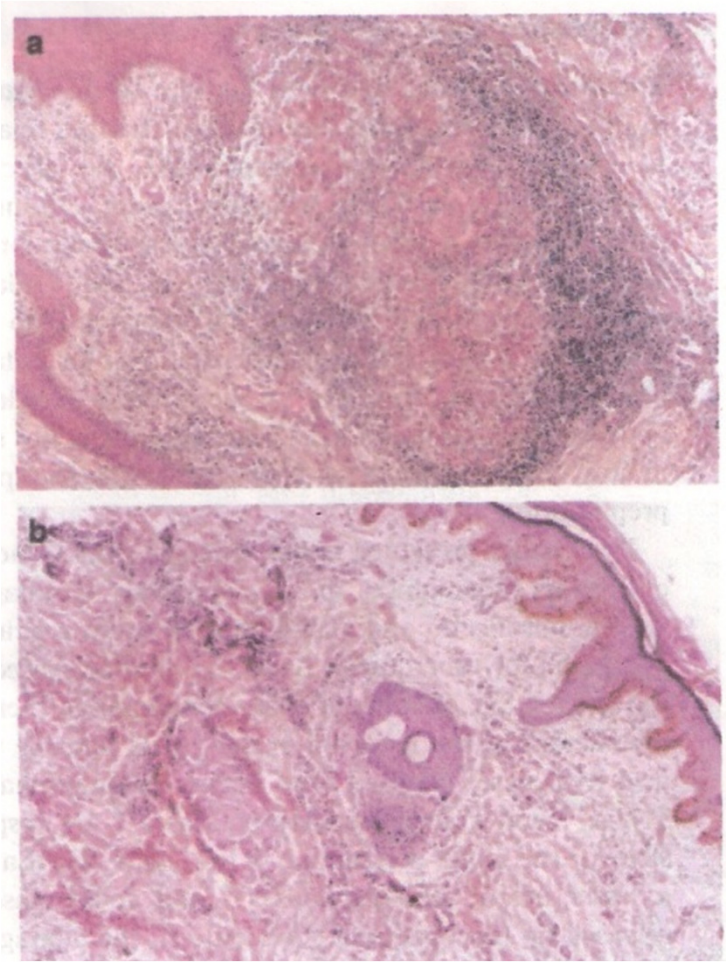
INDURATION



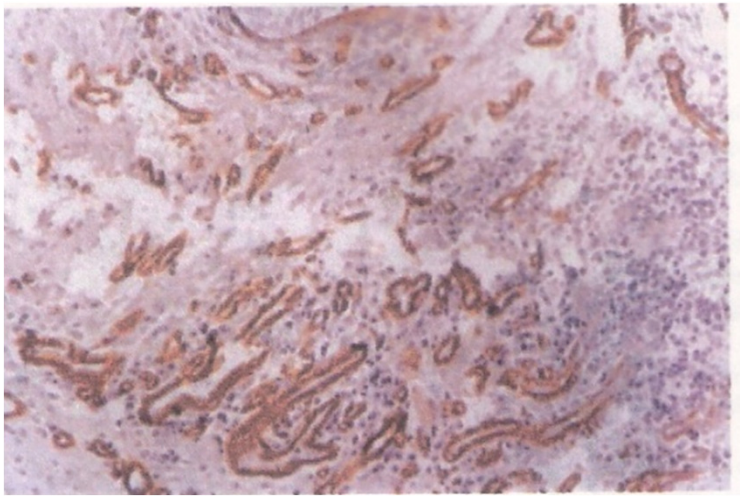
# INDURATION (DERMAL GRANULOMA) INDUCED BY DIFFERENT ANTIGENS



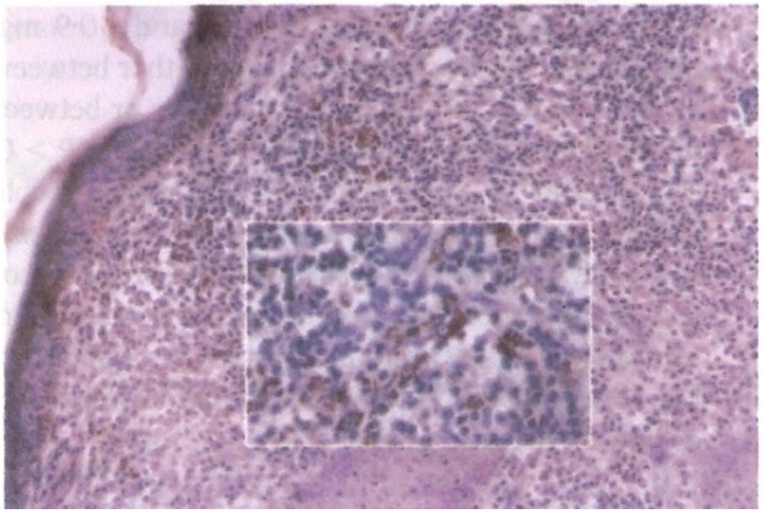
Well organized granuloma comprising
of epithelioid cells, giant cells and lymphocytes



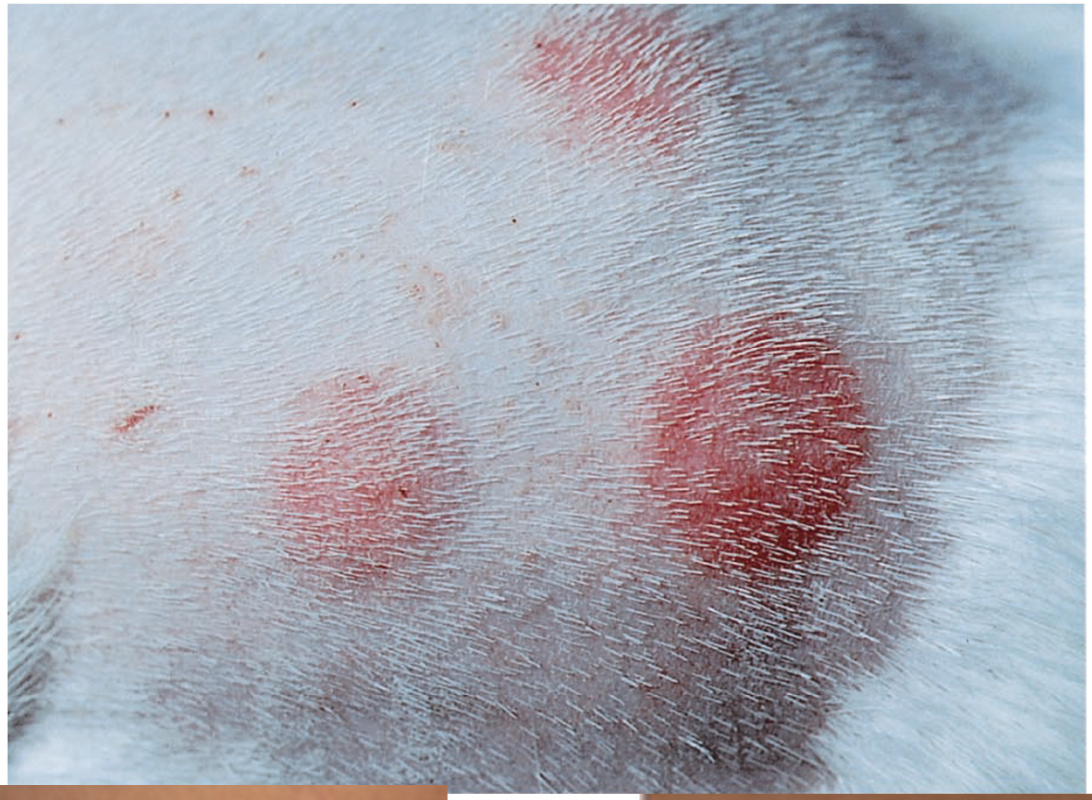

Necrotic granuloma consisting of few lymphocytes and macrophages.



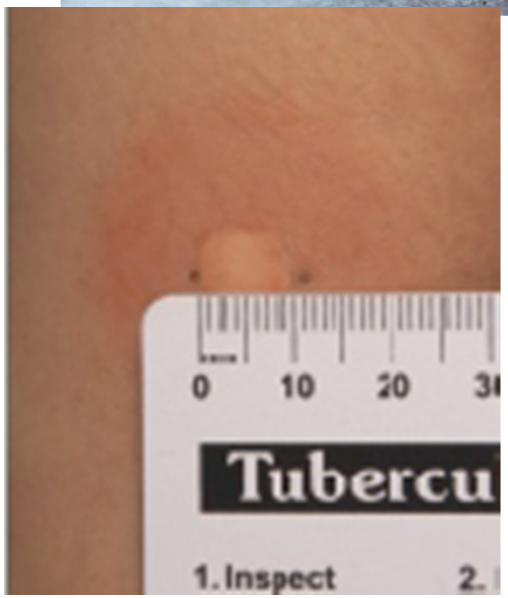
Anti TGF b antibody staining in the granuloma

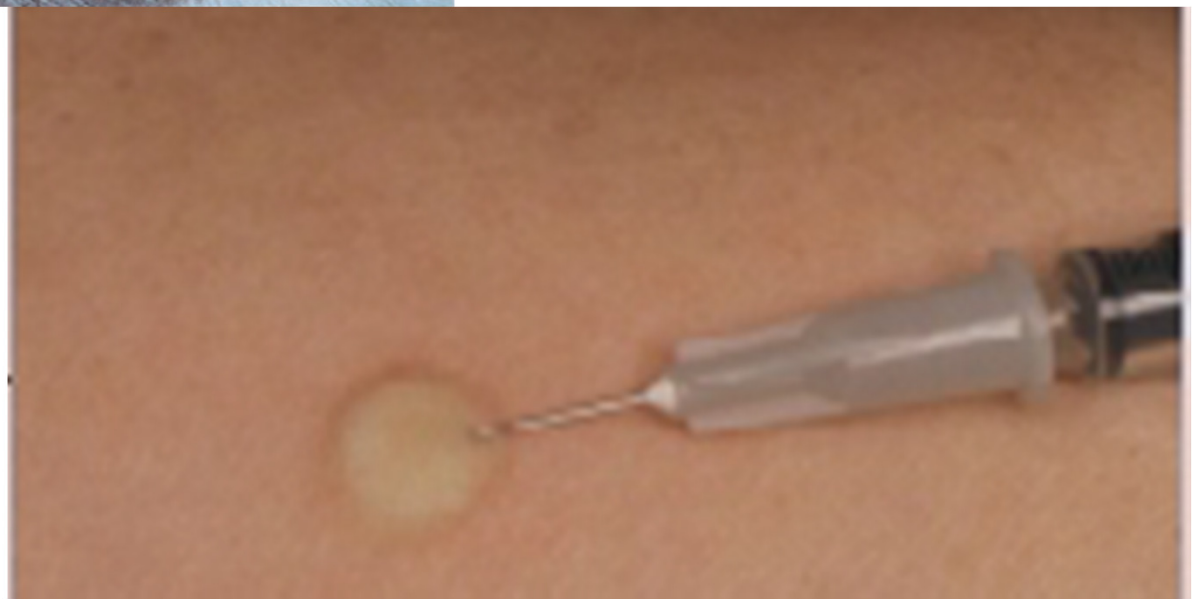


Anti gIFN staining in the granuloma



PPD TESTING COMPARED BETWEEN GUINEA PIG AND HUMAN



Indian J Med Res 110, July 1999, pp 4-10

# The clearance of tubercle bacilli & mycobacterial antigen *vis a vis* the granuloma in different organs of guineapigs

H. Shakila, K. Jayasankar* & V.D. Ramanathan

*Departments of Pathology & *Biochemistry, Tuberculosis Research Centre (ICMR), Chennai*

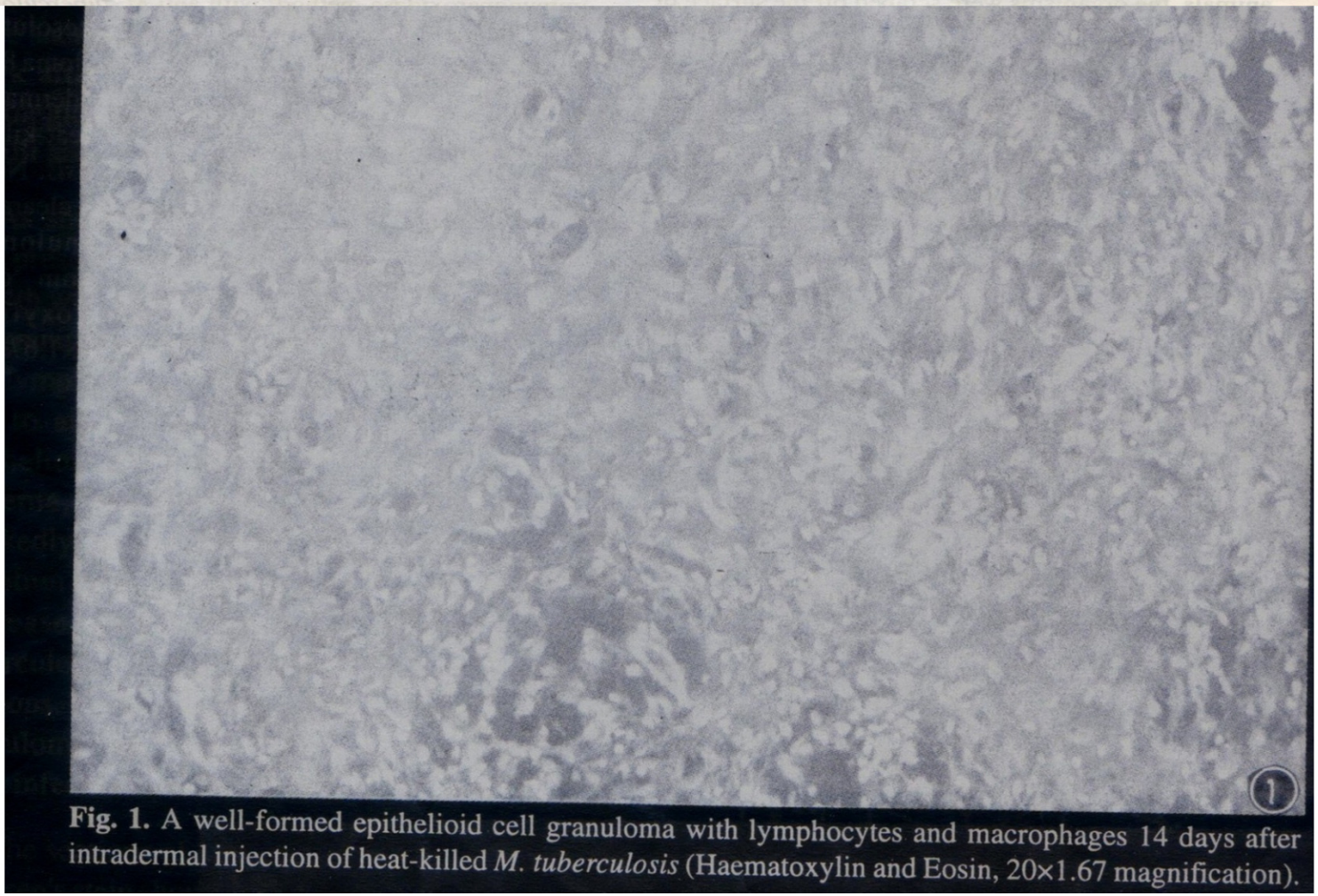


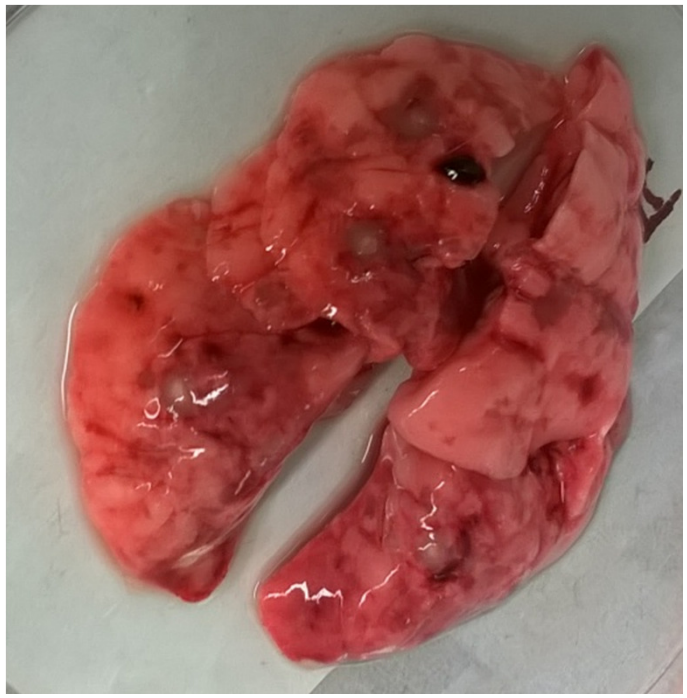
**Fig. 1.** A well-formed epithelioid cell granuloma with lymphocytes and macrophages 14 days after intradermal injection of heat-killed *M. tuberculosis* (Haematoxylin and Eosin, 20×1.67 magnification).

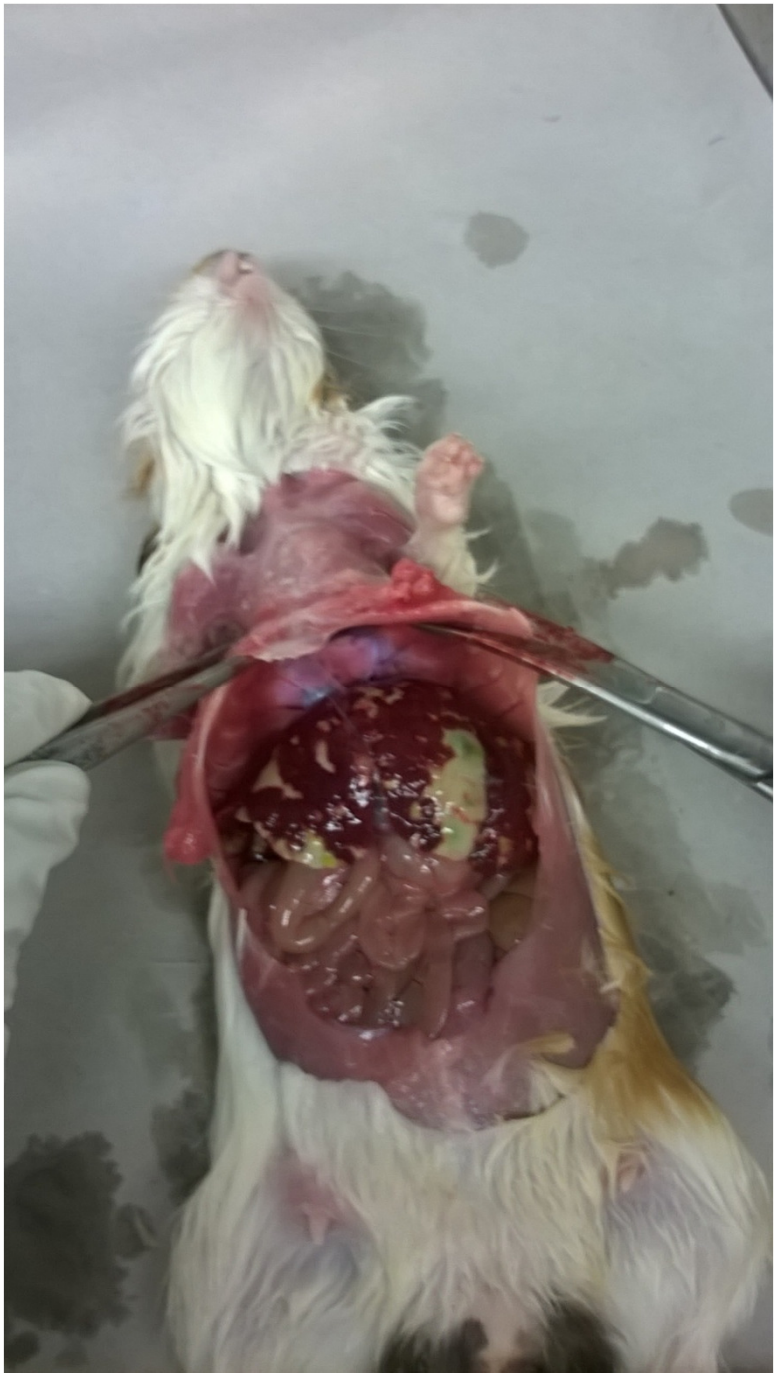
The animals which were recovering from the infection

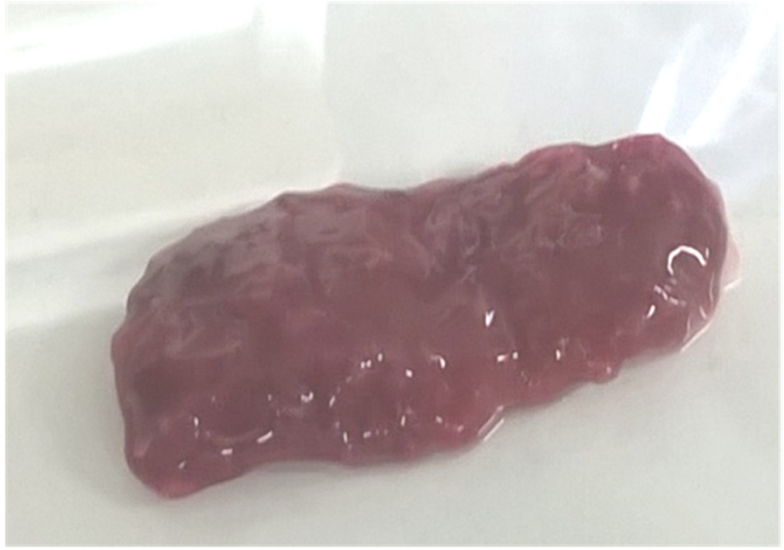
1. Bacilli was eliminated .
2. The antigens got cleared.
3. The granuloma got resolved finally.

The demonstration of antigen at the site of lesion may be potentially useful to discriminate between a persisting and a resolving tuberculous granuloma.

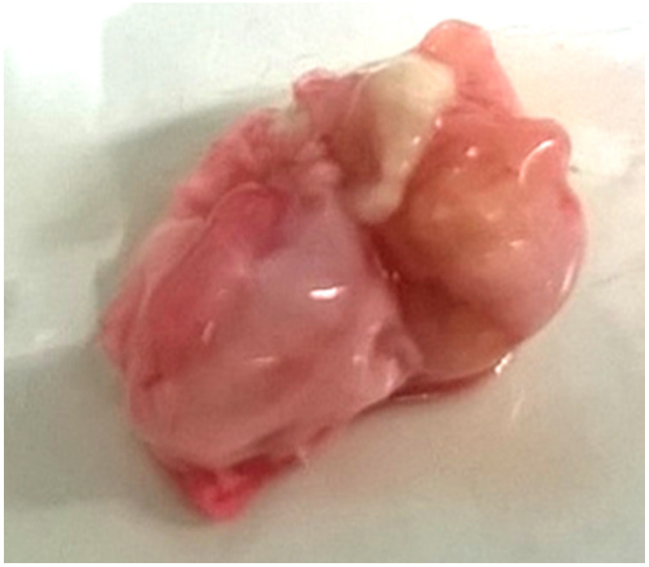
Caseous necrosis and Tubercles seen in liver and lungs



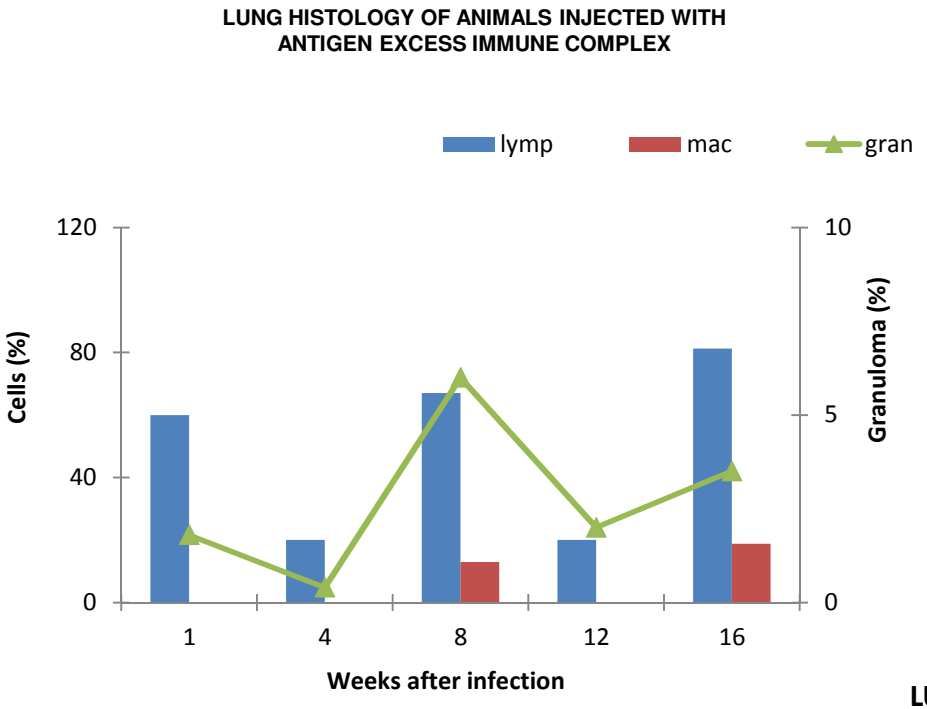


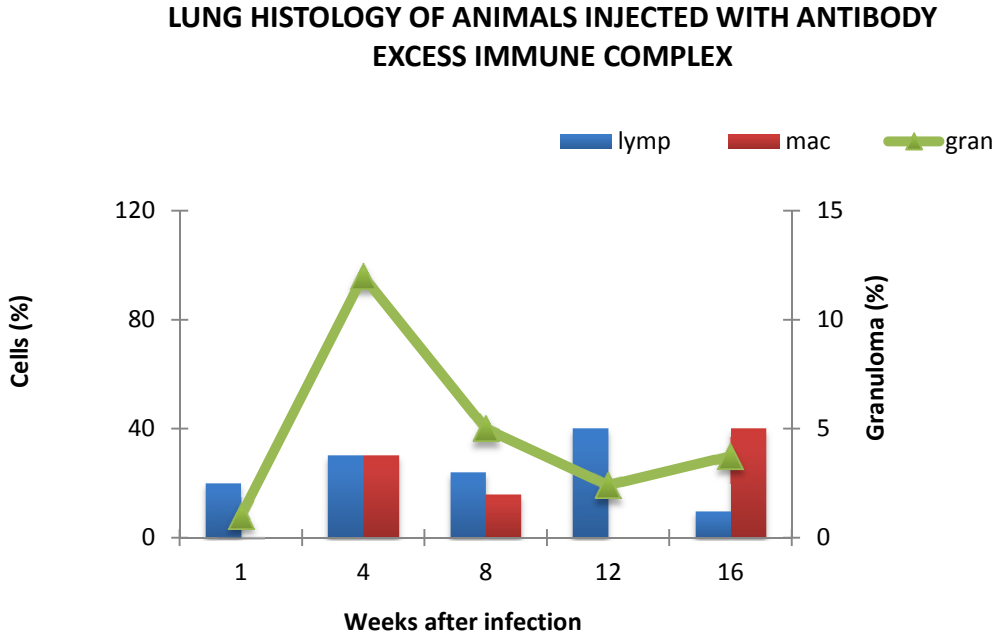


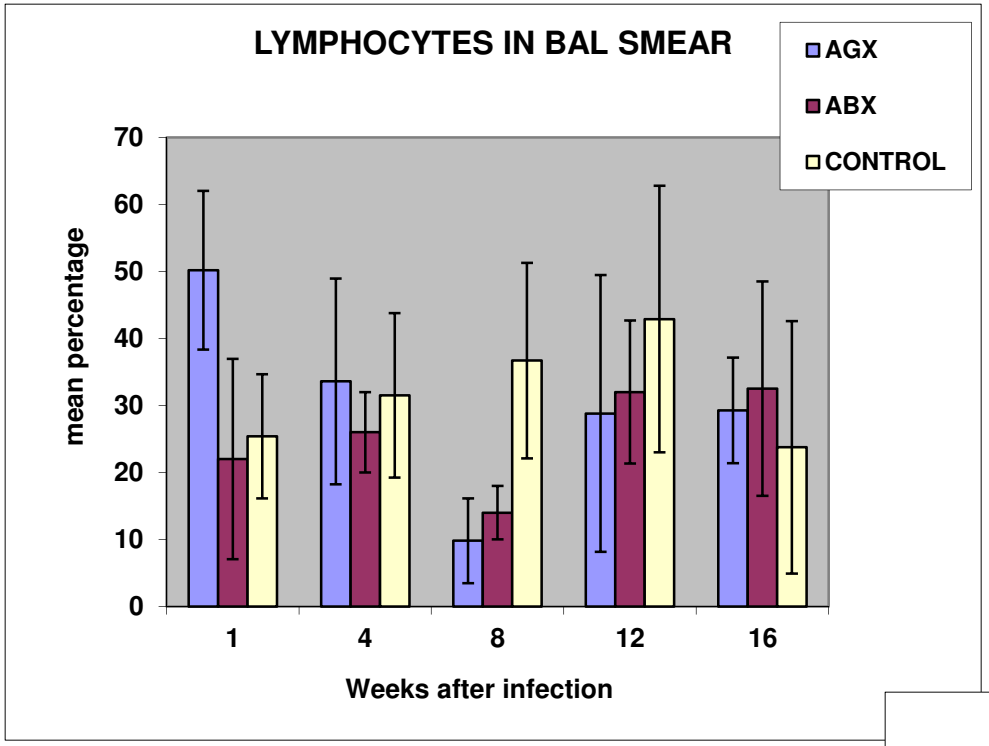
Enlarged Spleen with tubercles

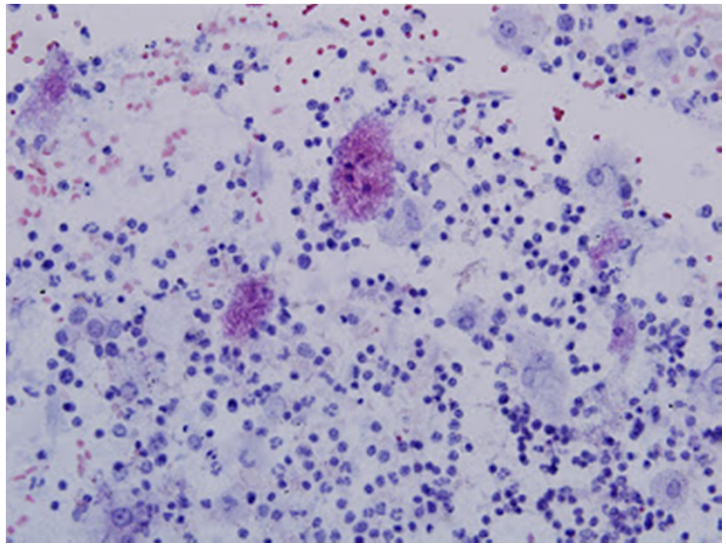


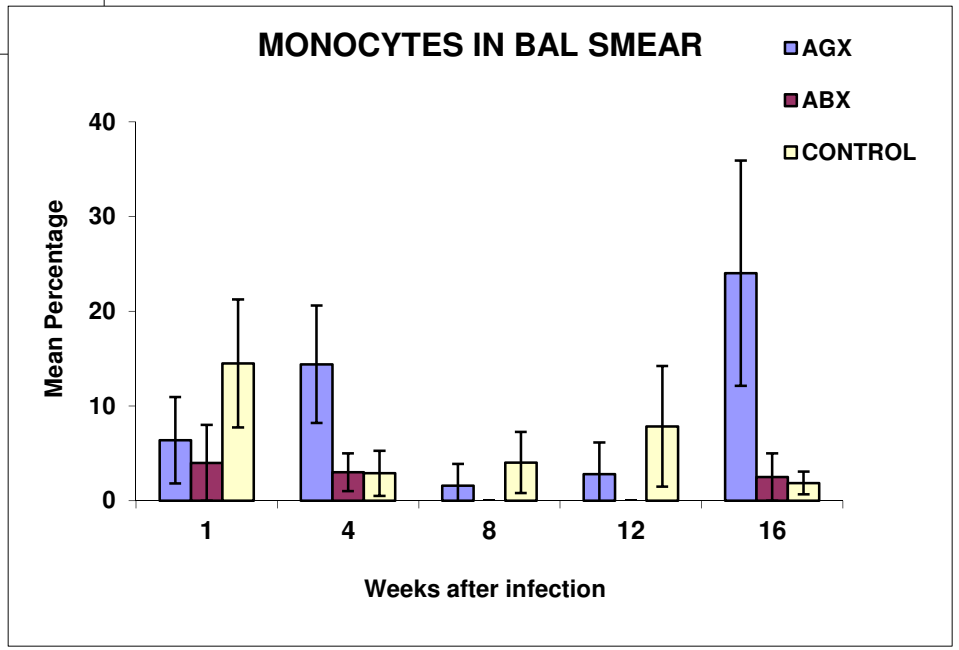
Enlarged lymph nodes


LUNG HISTOLOGY OF ANIMALS INJECTED WITH ANTIGEN EXCESS IMMUNE COMPLEX
lymp
mac
gran
120
80
40
0
10
5
0
Cells (%)
Granuloma (%)
1
4
8
12
16
Weeks after infection

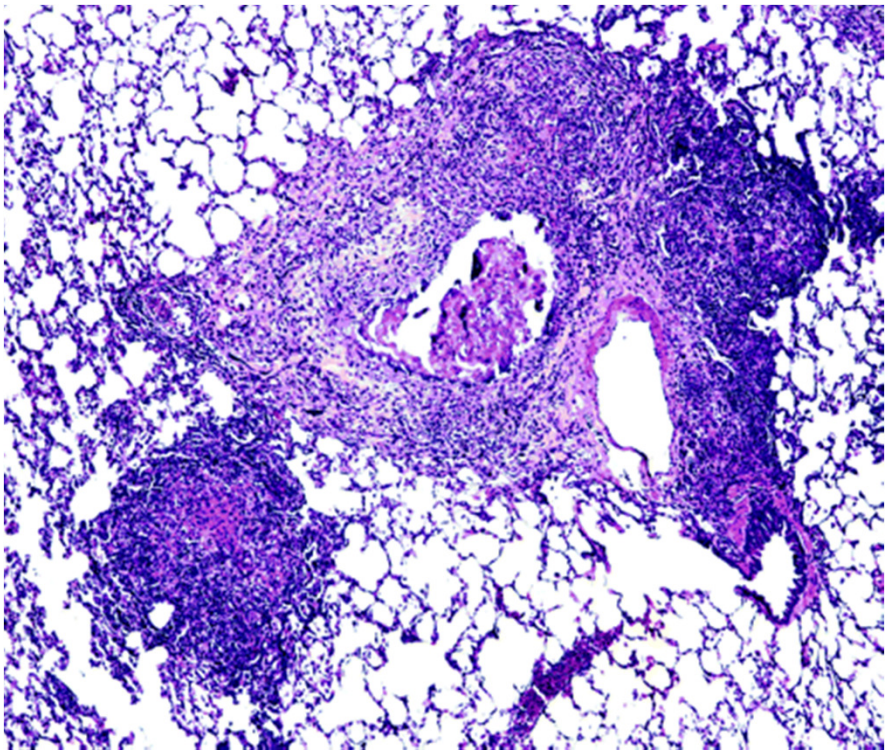

LUNG HISTOLOGY OF ANIMALS INJECTED WITH ANTIBODY EXCESS IMMUNE COMPLEX
lymp
mac
gran
120
80
40
0
15
10
5
0
Cells (%)
Granuloma (%)
1
4
8
12
16
Weeks after infection


LYMPHOCYTES IN BAL SMEAR
AGX
ABX
CONTROL
mean percentage
70
60
50
40
30
20
10
0
1
4
8
12
16
Weeks after infection

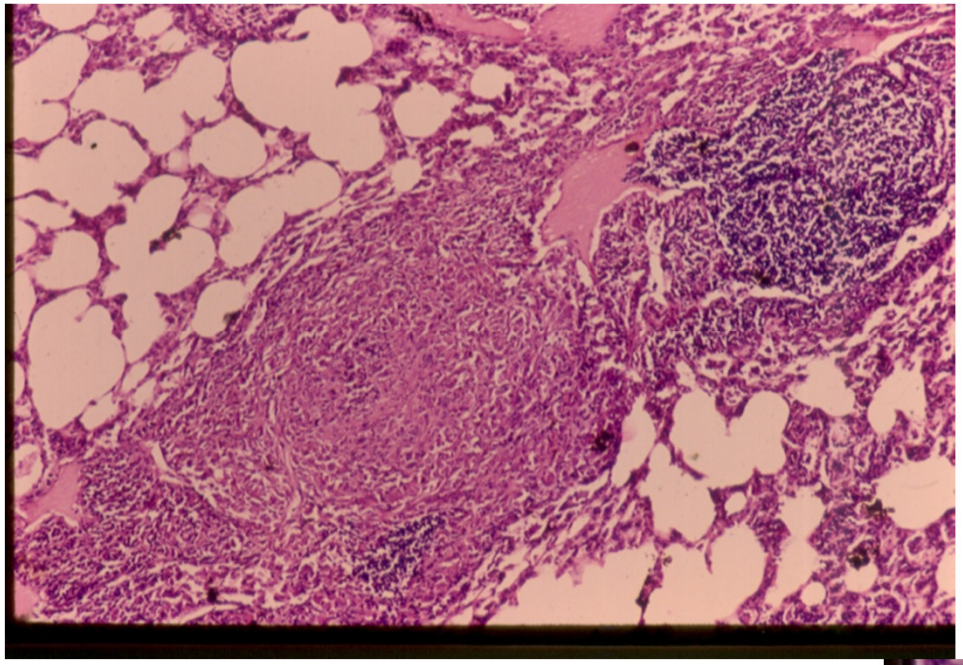



MONOCYTES IN BAL SMEAR
AGX
ABX
CONTROL
Mean Percentage
40
30
20
10
0
1
4
8
12
16
Weeks after infection

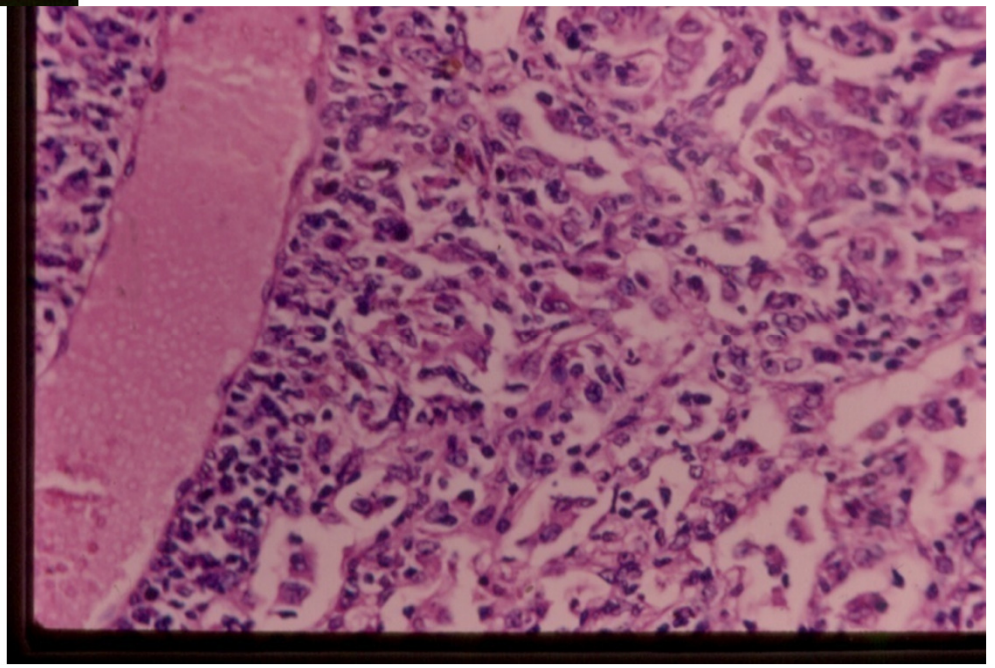
# GRANULOMATOUS RESPONSE TO TB IN HUMAN

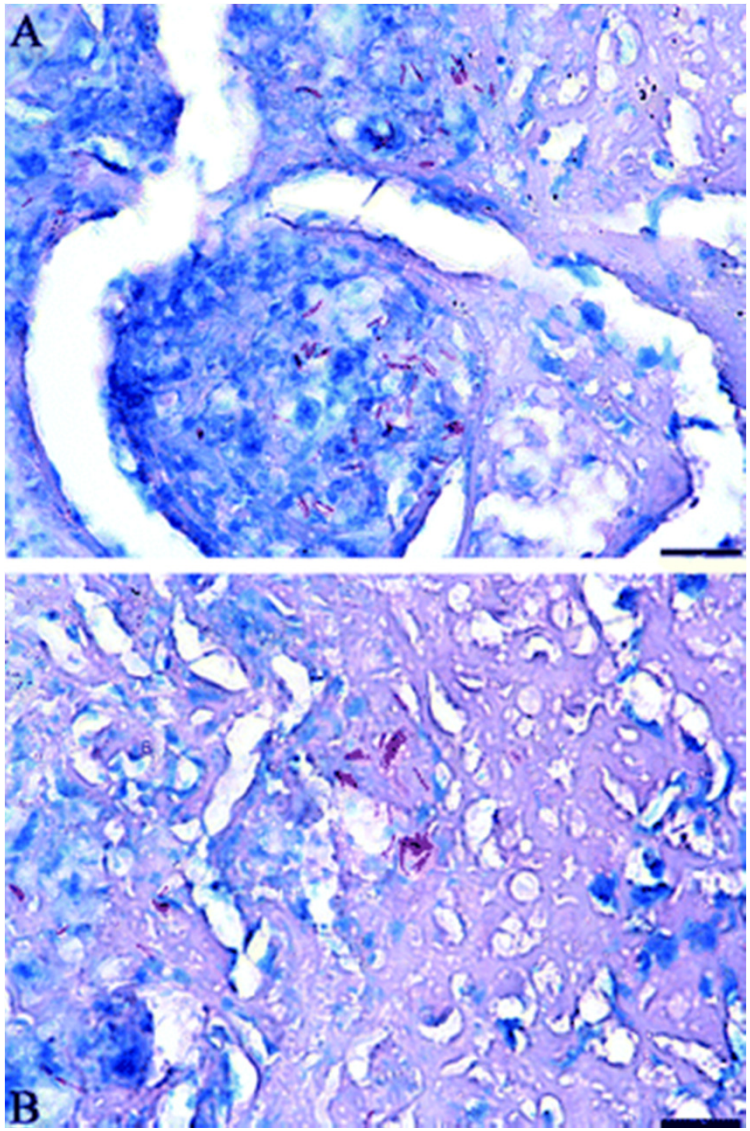


Histology of Lung (H&E staining)



# GRANULOMATOUS RESPONSE IN GUINEA PIGS



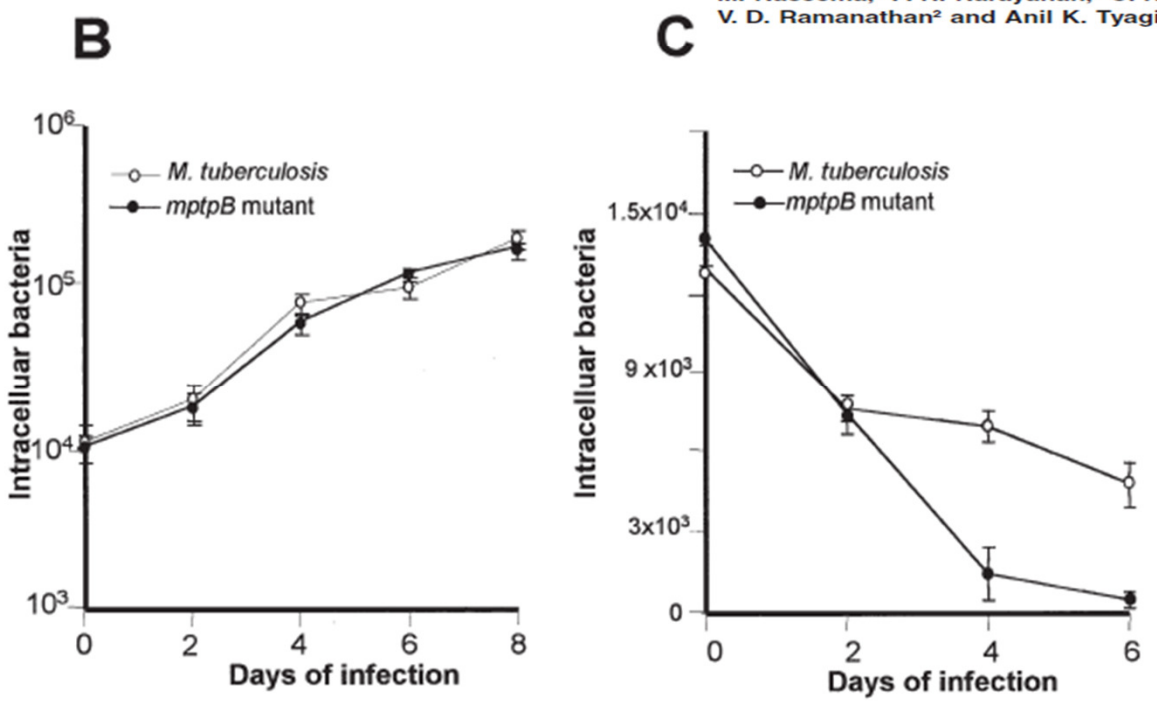


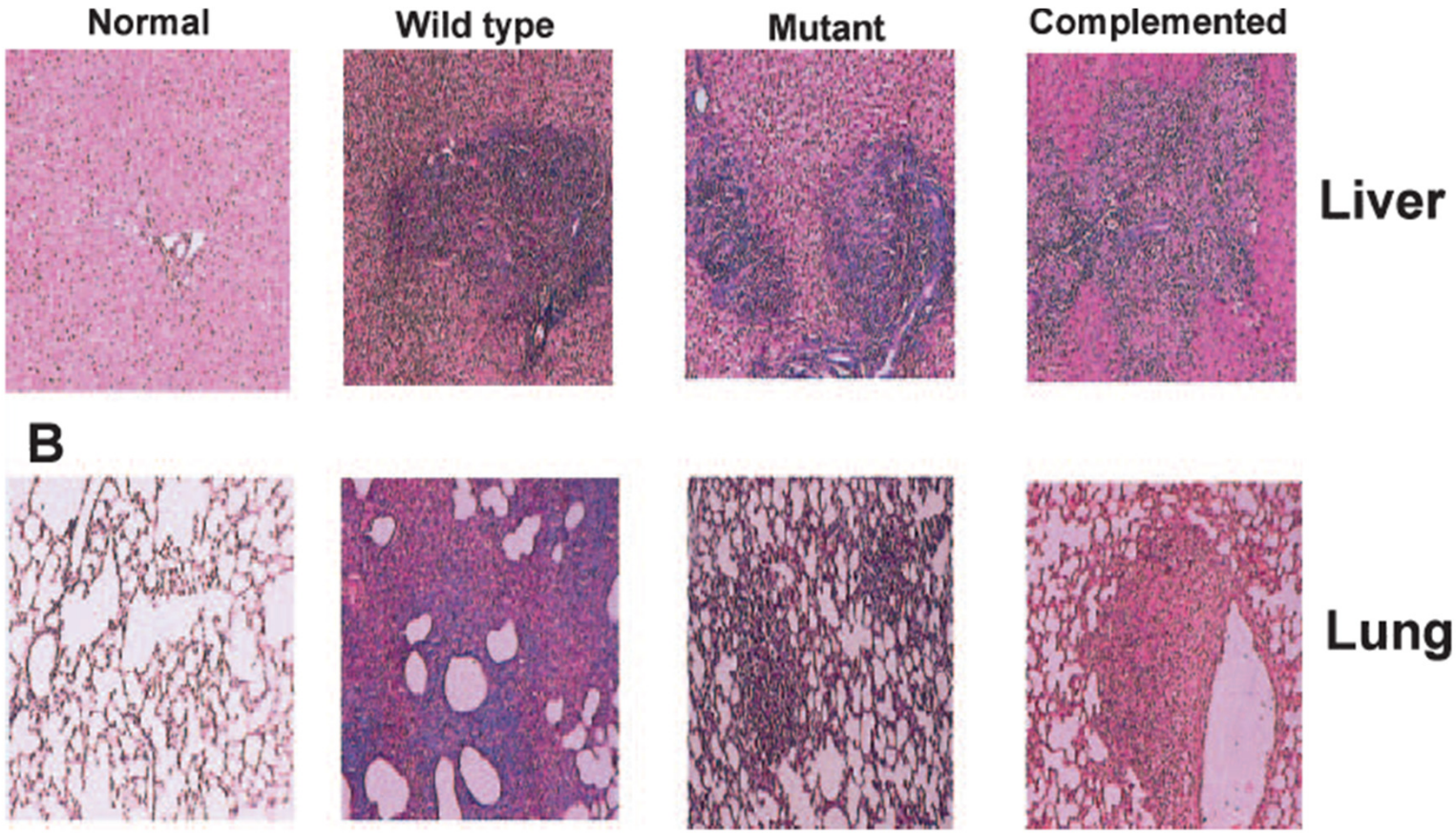

AFB staining TB Lung granuloma

# Disruption of *mptpB* impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs

Molecular Microbiology (2003) 50(3), 751–762

Ramandeep Singh,[1] Vivek Rao,[1] H. Shakila,[2]
Radhika Gupta,[1] Aparna Khera,[1] Neeraj Dhar,[1]
Amit Singh,[1] Anil Koul,[1,3] Yogendra Singh,[3]
M. Naseema,[2] P. R. Narayanan,[2] C. N. Paramasivan,[2]
V. D. Ramanathan[2] and Anil K. Tyagi[1]*




Normal
Wild type
Mutant
Complemented
Liver
B
Lung

❖**Infection of guinea pigs with the mutant strain resulted in a 70-fold reduction in the bacillary load of spleens in infected animals as compared with the bacillary load in animals infected with the parental strain.**

❖**Upon reintroduction of the *mptpB* gene into the mutant strain, the complemented strain was able to establish infection and survive in guinea pigs at rates comparable to the parental strain.**

❖**These observations demonstrate a role of MptpB in the pathogenesis of *M. tuberculosis*.**

ELSEVIER

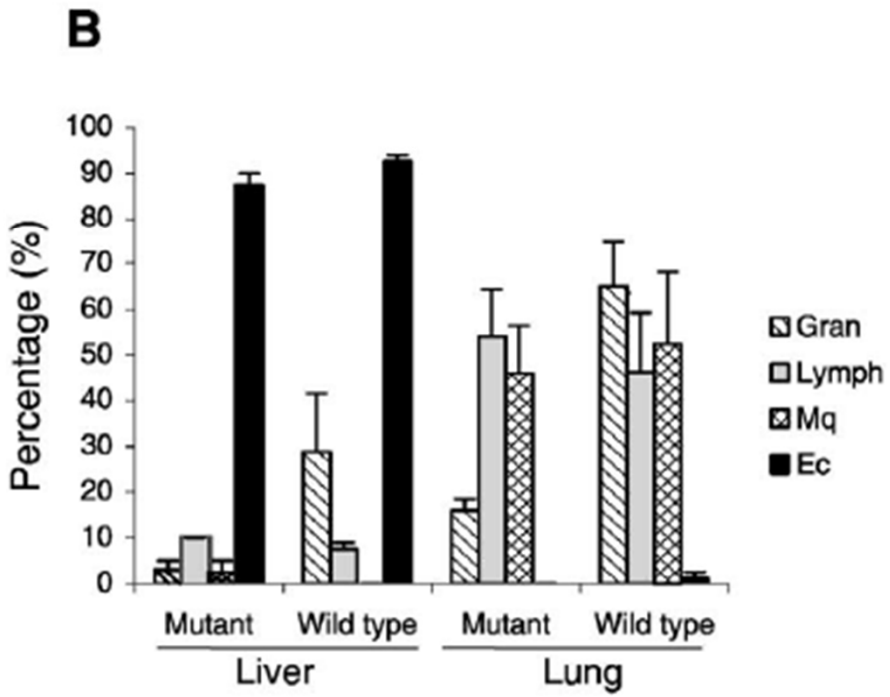
FEMS Microbiology Letters 231 (2004) 237–245

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

# Disruption of response regulator gene, *devR*, leads to attenuation in virulence of *Mycobacterium tuberculosis*

Vandana Malhotra [a], Deepak Sharma [a], V.D. Ramanathan [b], H. Shakila [b], Deepak K. Saini [a], Soumitesh Chakravorty [a], Taposh K. Das [c], Qing Li [d], Richard F. Silver [d], P.R. Narayanan [b], Jaya Sivaswami Tyagi [a,*]



**The attenuation in virulence of the devR mutant in guinea pigs together with DevR-DevS being a bonafide signal transduction system indicates that DevR plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within tissues.**

# Expression of mycobacterial cell division protein, FtsZ, and dormancy proteins, DevR and Acr, within lung granulomas throughout guinea pig infection

Deepak Sharma[1], Arpita Bose[1], H. Shakila[2], Taposh K. Das[3], Jaya Sivaswami Tyagi[1] & V.D. Ramanathan[2]

[1]Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India; [2]Department of Clinical Pathology, Tuberculosis Research Centre, Chennai, India; and [3]Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

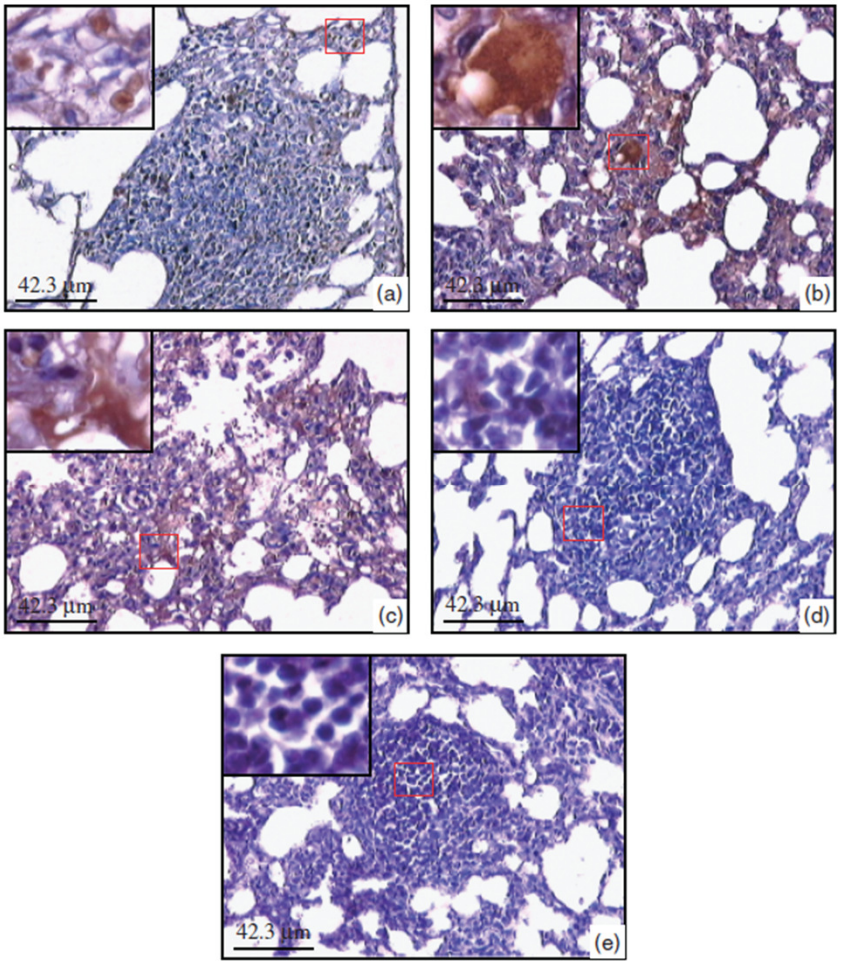



**Fig. 3.** Lung sections of guinea pigs infected with *Mycobacterium tuberculosis* H37Rv stained for FtsZ by immunohistochemistry (× 300 magnification). (a)–(d) refer to sections from 4, 20, 36 and 44 weeks post-infection, and (e) shows a section stained without primary antibody (anti-FtsZ). Brown colour shows positive staining. Red-coloured rectangles outline the areas shown in insets (× 1200 magnification).

FtsZ, Acr and DevR antigen staining combined with DNA analysis could be useful for the detection of intact (live) bacteria and for indicating the adequacy of chemotherapy in the absence of AFB staining in tissue sections.

SCANDINAVIAN JOURNAL OF
Immunology
AN INTERNATIONAL MONTHLY JOURNAL

# Increased Expression of *Mycobacterium tuberculosis* 19 kDa Lipoprotein Obliterates the Protective Efficacy of BCG by Polarizing Host Immune Responses to the Th2 Subtype

V. Rao[1]*, N. Dhar[2]*, H. Shakila†, R. Singh*, A. Khera*, R. Jain*, M. Naseema†, C. N. Paramasivan†, P. R. Narayanan†, V. D. Ramanathan† & A. K. Tyagi*

*Scandinavian Journal of Immunology* 61, 410–417 , 2005

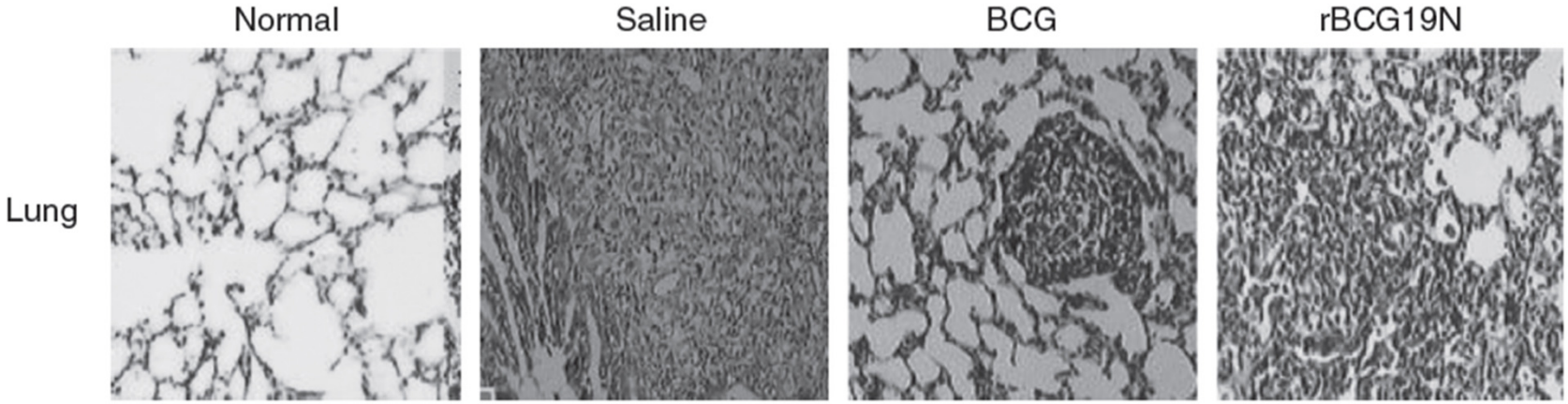
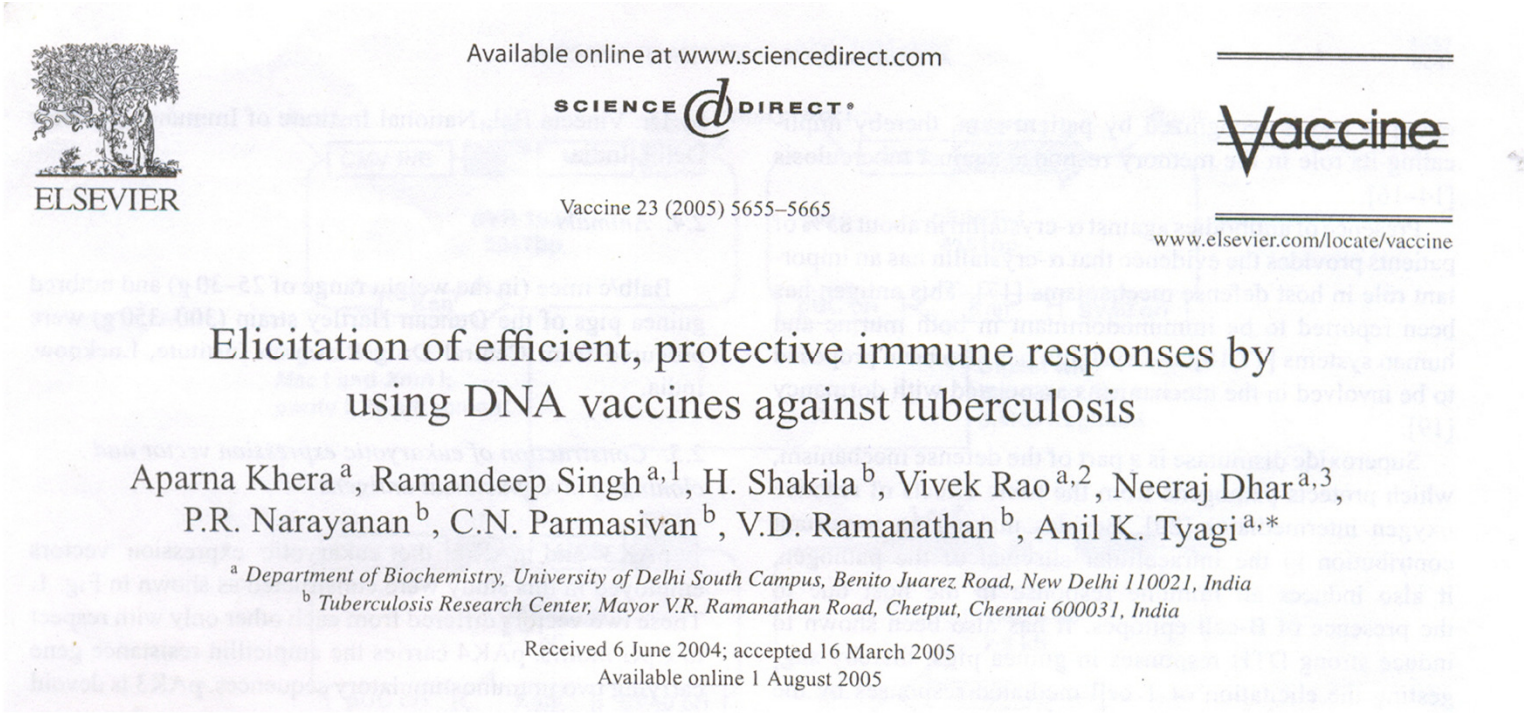



**Figure 4** Histopathological analysis of lung sections from immunized guinea pigs challenged with two doses (CD-1 and CD-2) of *Mycobacterium tuberculosis* $H_{37}Rv$. Sections of lung from animals in various groups were stained with haematoxylin and eosin as described in the *Materials and methods*. The sections were observed under a magnification of ×40 for the presence of granuloma. Tissue section from an unvaccinated and unchallenged animal (normal) was used as a reference for normal tissue histology.

**Over expression of 19kDa protein of BCG diminished the protective effect of BCG.**



Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vaccine 23 (2005) 5655–5665
Vaccine
www.elsevier.com/locate/vaccine

# Elicitation of efficient, protective immune responses by using DNA vaccines against tuberculosis

Aparna Khera [a], Ramandeep Singh [a,1], H. Shakila [b], Vivek Rao [a,2], Neeraj Dhar [a,3], P.R. Narayanan [b], C.N. Parmasivan [b], V.D. Ramanathan [b], Anil K. Tyagi [a,*]

[a] *Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India*
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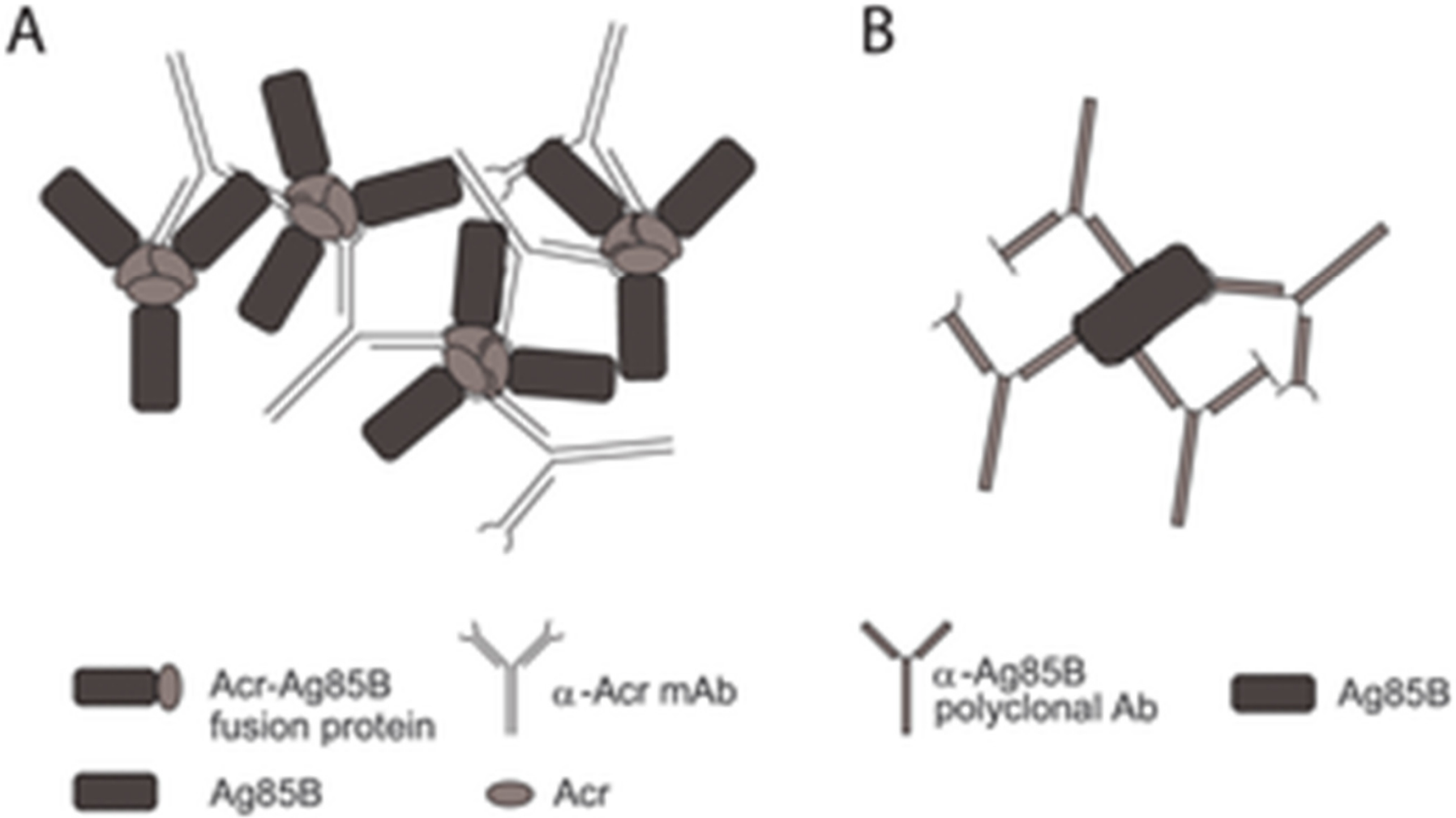
Received 6 June 2004; accepted 16 March 2005
Available online 1 August 2005

DNA vaccines containing CpG motifs **elicited the highest degree of immune response** in mice.

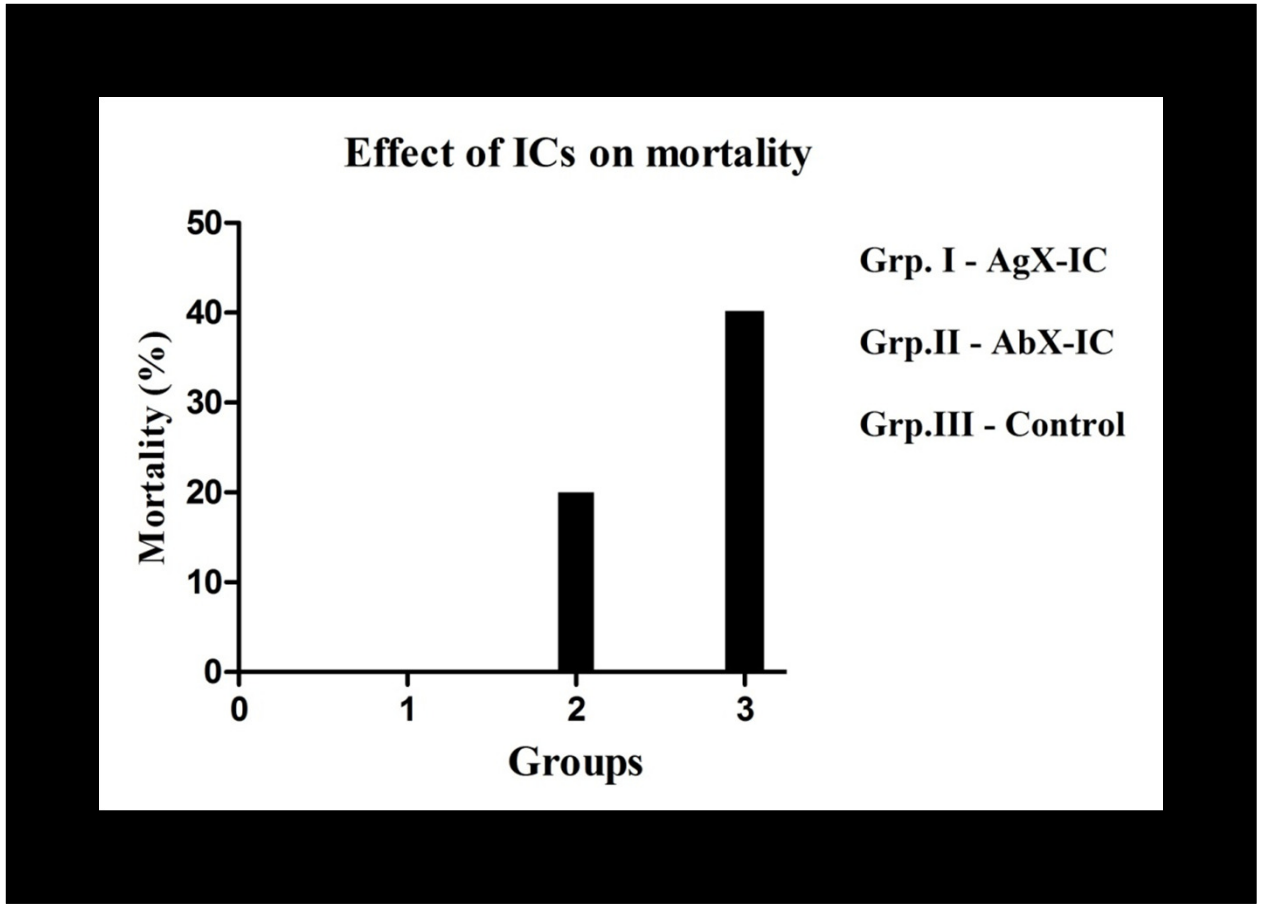
Among the three candidates, **SOD based DNA vaccine imparted an efficient protection** to guinea pigs against *M.tb*.

**Possibly, a cocktail of DNA vaccines expressing several protective antigens may provide better protection than a single antigen.**

# IMMUNE COMPLEXES AS VACCINES AGAINST TUBERCULOSIS



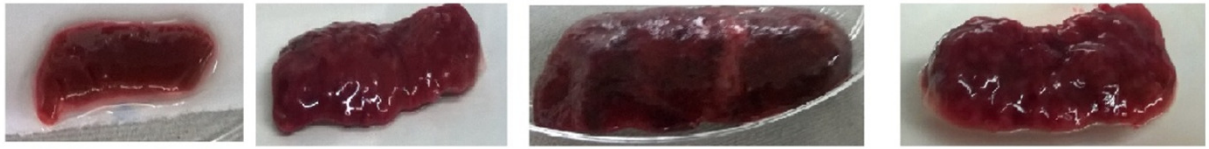
# PROTECTIVE EFFECT OF ANTIGEN EXCESS IMMUNE COMPLEX IN GUINEAPIGS INFECTED WITH *M.tb*




Group I with Agx-IC did not show any mortality when compared to Group II and Group III.

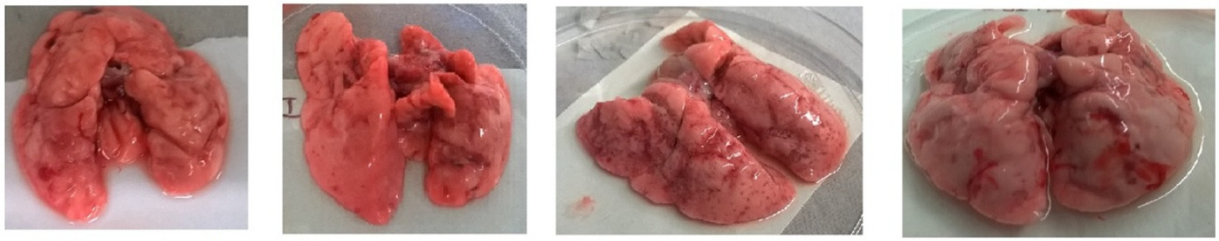
Pathophysiology of guinea pig Spleen (A) and Lung (B) infected with *M.tb* after immunizing with either AGX IC and ABX IC or control without any immune complexes.

**A. Spleen**



Naive animal | Grp. I - AgX-IC | Grp. II - AbX-IC | Grp. III - Control

**B. Lung**



Naive animal | Grp. I - AgX-IC | Grp. II - AbX-IC | Grp. III - Control

Conclusions from our work:

1. Immunization with Immune complexes improved the body weight.

2. The morbidity and mortality was less in animals immunized with ICs when compared to the controls.

3. The bacillary load was reduced.

4. The type of granuloma observed seem to be protective in nature.

5. The antibody titer in the serum were more.

**IMMUNE COMPLEXES HAVE A VACCINE AND ADJUVANT ACTIVITY**

# AEROSOL MODEL FOR TUBERCULOSIS

THANK YOU