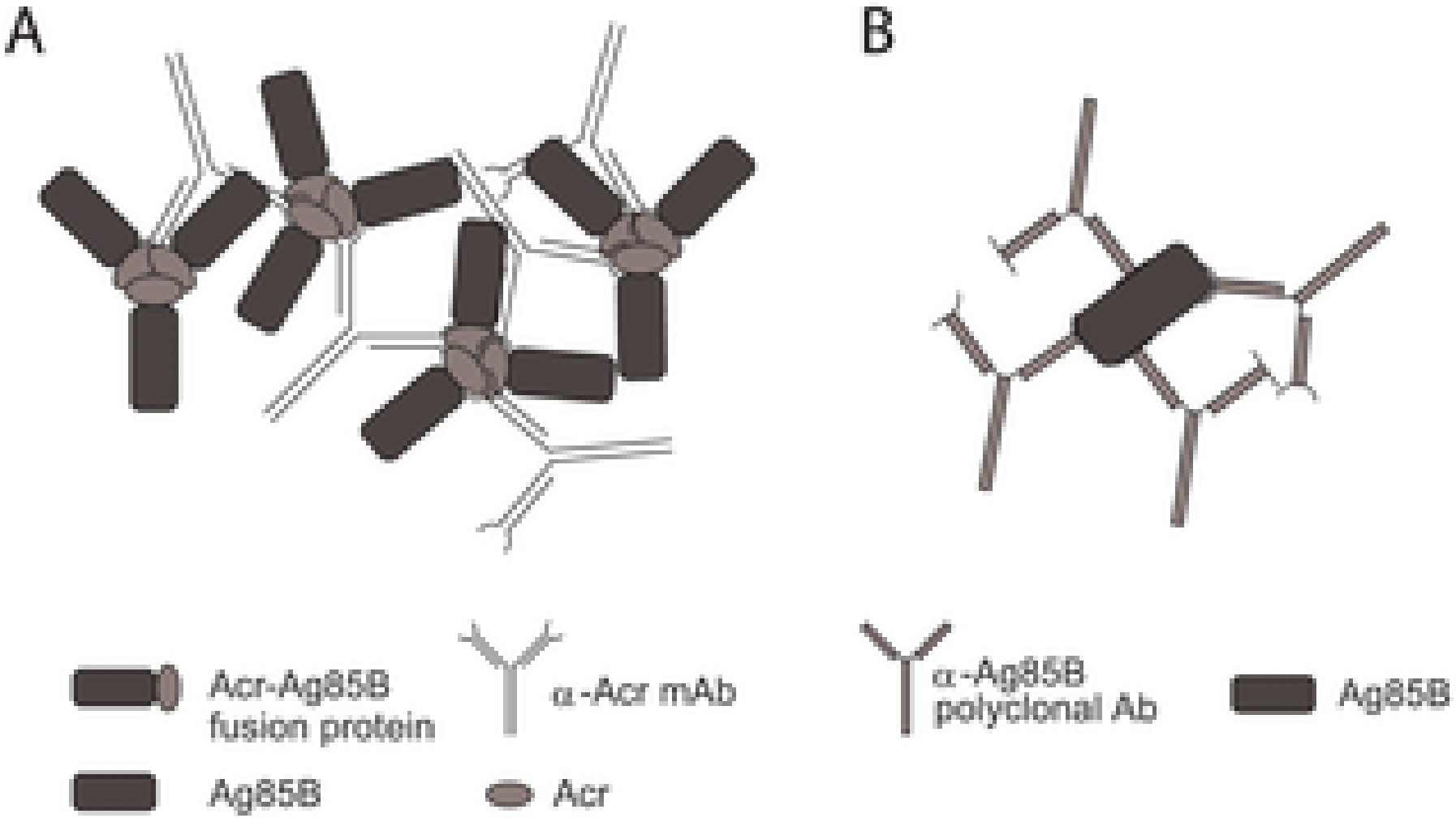
MADURAI KAMARAJ UNIVERSITY

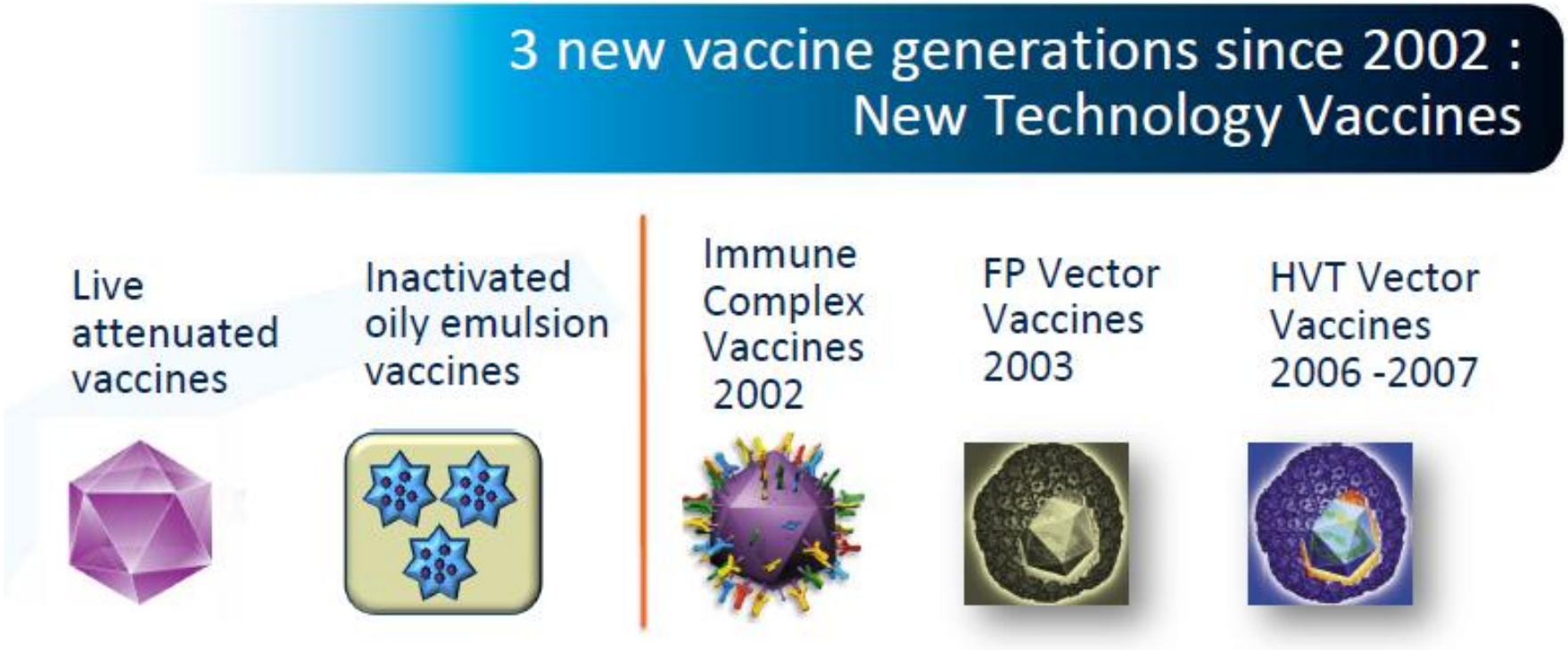
Immune Complex as Vaccine against Tuberculosis

H.Shakila,
Associate Professor,
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Madurai, Tamilnadu

IMMUNE COMPLEXES AS VACCINES AGAINST TUBERCULOSIS



ICs as Vaccine Candidate



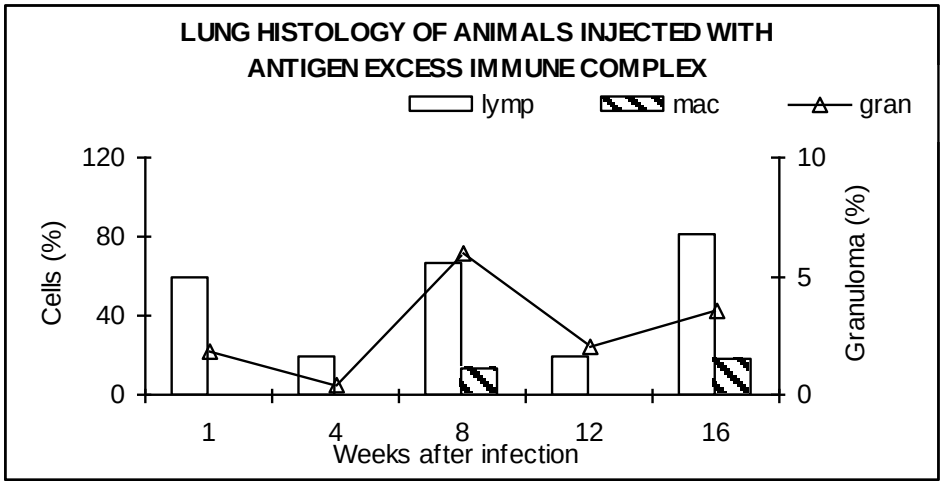
- Adapted as vaccine strategy for Chicken Infectious Anemia Disease (Karel *et al.*, 2011) and infectious bursal disease (Jeurissen *et al.*, 1998).
- Used to enhance antibody responses against neutralizing epitopes on HIV-1 envelope gp120 (Catarina *et al.*, 2009).
- Acts as molecular platform for adjuvant free vaccine delivery (Ilaria Pepponi *et al.*, 2013)

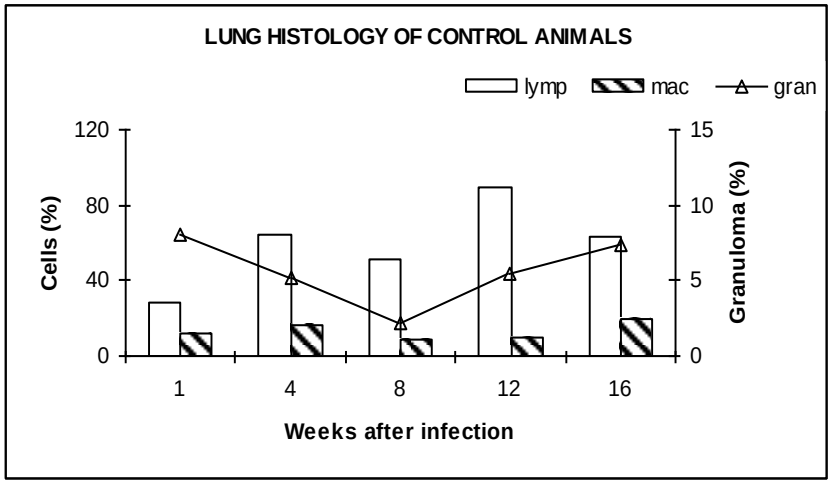
IC & TB

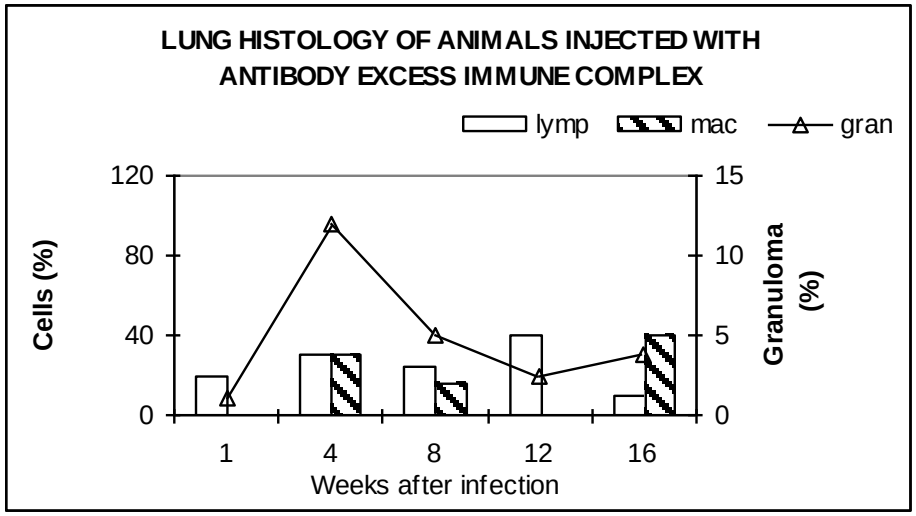
- Not all infected individuals develop TB especially the close contacts.
- It was found that values of CIC levels and occurrence are increased in most of the patients with active TB (Dubaniewicz and Magdalena 1999).
- It has been shown that ICs from patients with TB exhibit a profound effect on the granulocyte function, with activation of certain effector mechanisms and dampening of others (Senbagavalli *et al.,* 2012*).*
- It is hypothesized that ICs do have immunomodulatory role pertaining to TB.

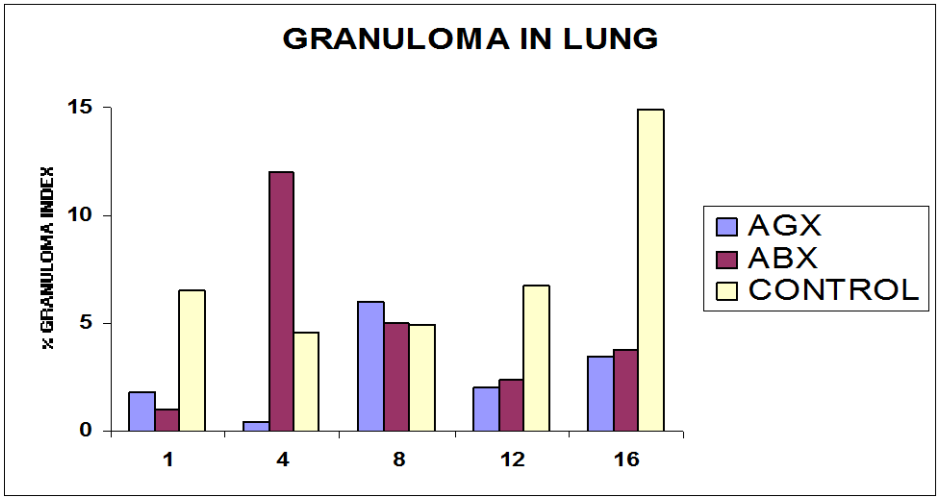
Studies using whole organisms and sonicated organisms of H37RV

(**Group A**) antigen excess immune complex preparation AGX=49:1 (where antigen was 49 parts and antibody was 1 part)	(**Group B**) antibody excess immune complex preparation ABX=1:49 (where antibody was 49 parts and antigen was 1 part)
Ag alone =49 volume of antigen +1 volume of normal saline	Ag alone = 1 volume of antigen+ 49 volume of normal saline
Ab alone = 1 volume of antibody + 49 volume of saline	Ab alone = 49 volume of antibody+1 volume of normal saline
Control= Normal saline (N/S)	Control= Normal saline (N/S)

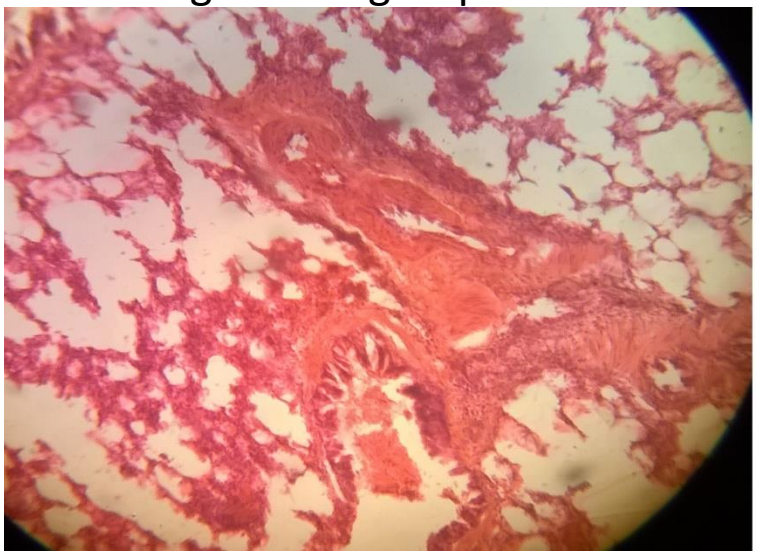

LUNG HISTOLOGY OF ANIMALS INJECTED WITH ANTIGEN EXCESS IMMUNE COMPLEX
lymp
mac
gran
Cells (%)
Granuloma (%)
120
80
40
0
10
5
0
1
4
8
12
16
Weeks after infection

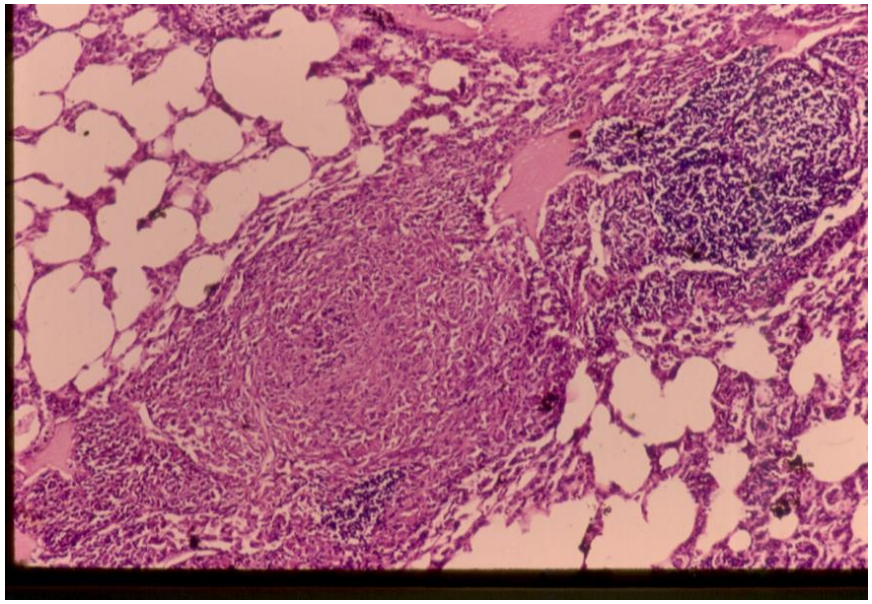

LUNG HISTOLOGY OF CONTROL ANIMALS
lymp
mac
gran
Cells (%)
Granuloma (%)
120
80
40
0
15
10
5
0
1
4
8
12
16
Weeks after infection


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


GRANULOMA IN LUNG
% GRANULOMA INDEX
15
10
5
0
1
4
8
12
16
AGX
ABX
CONTROL

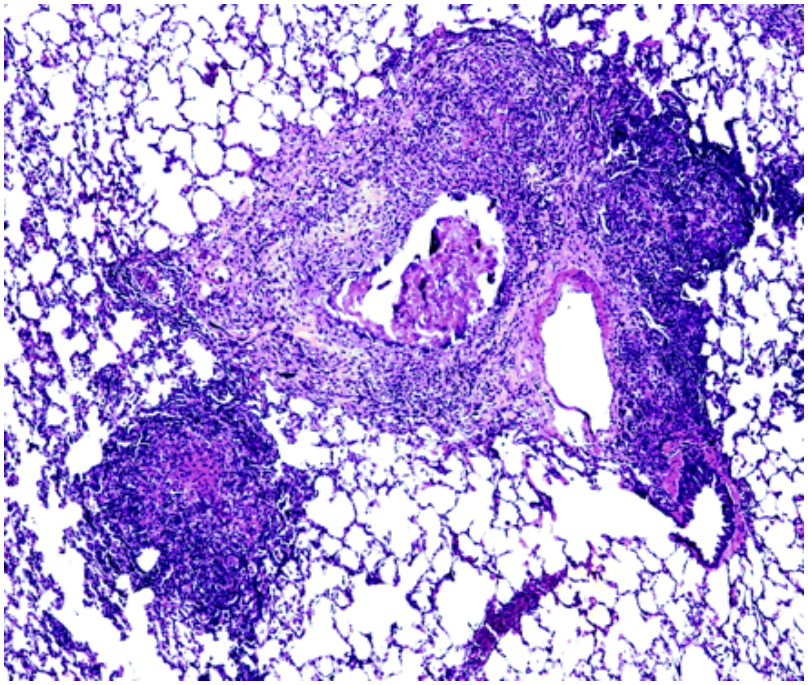
Lung of AGX group

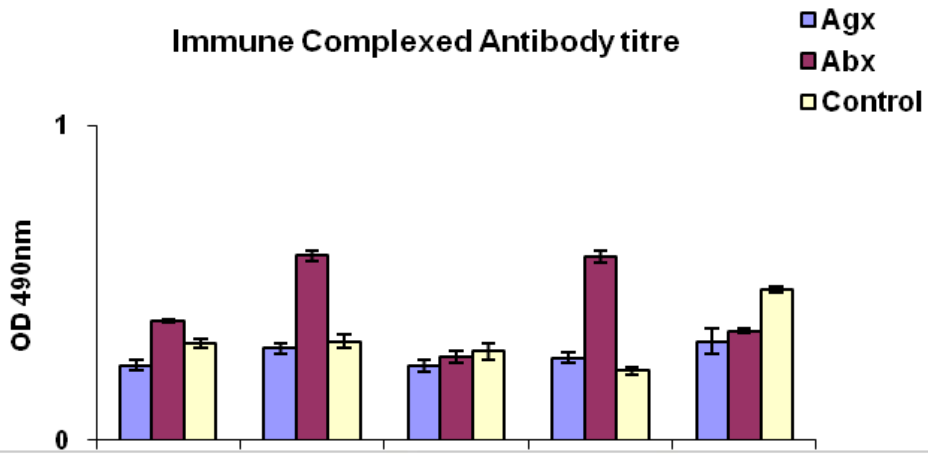


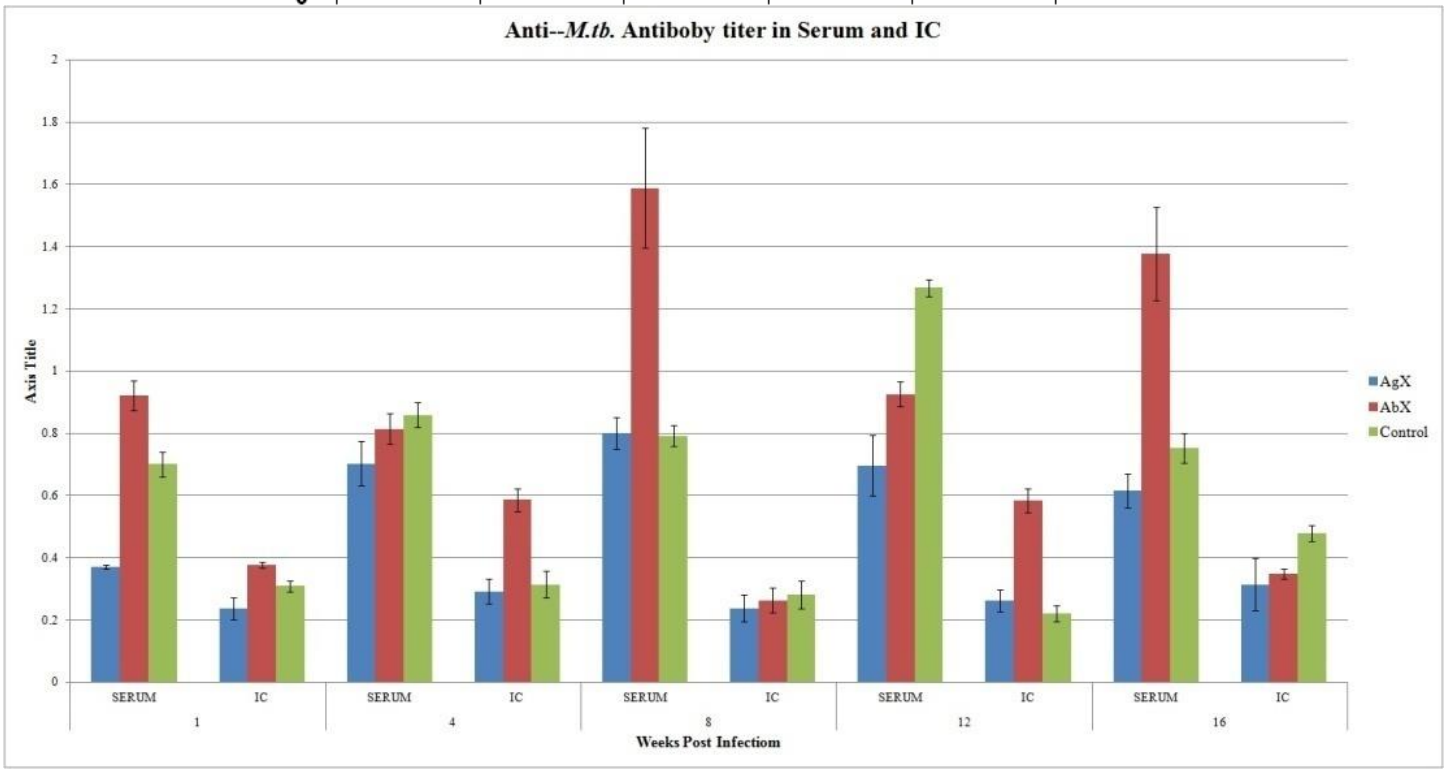


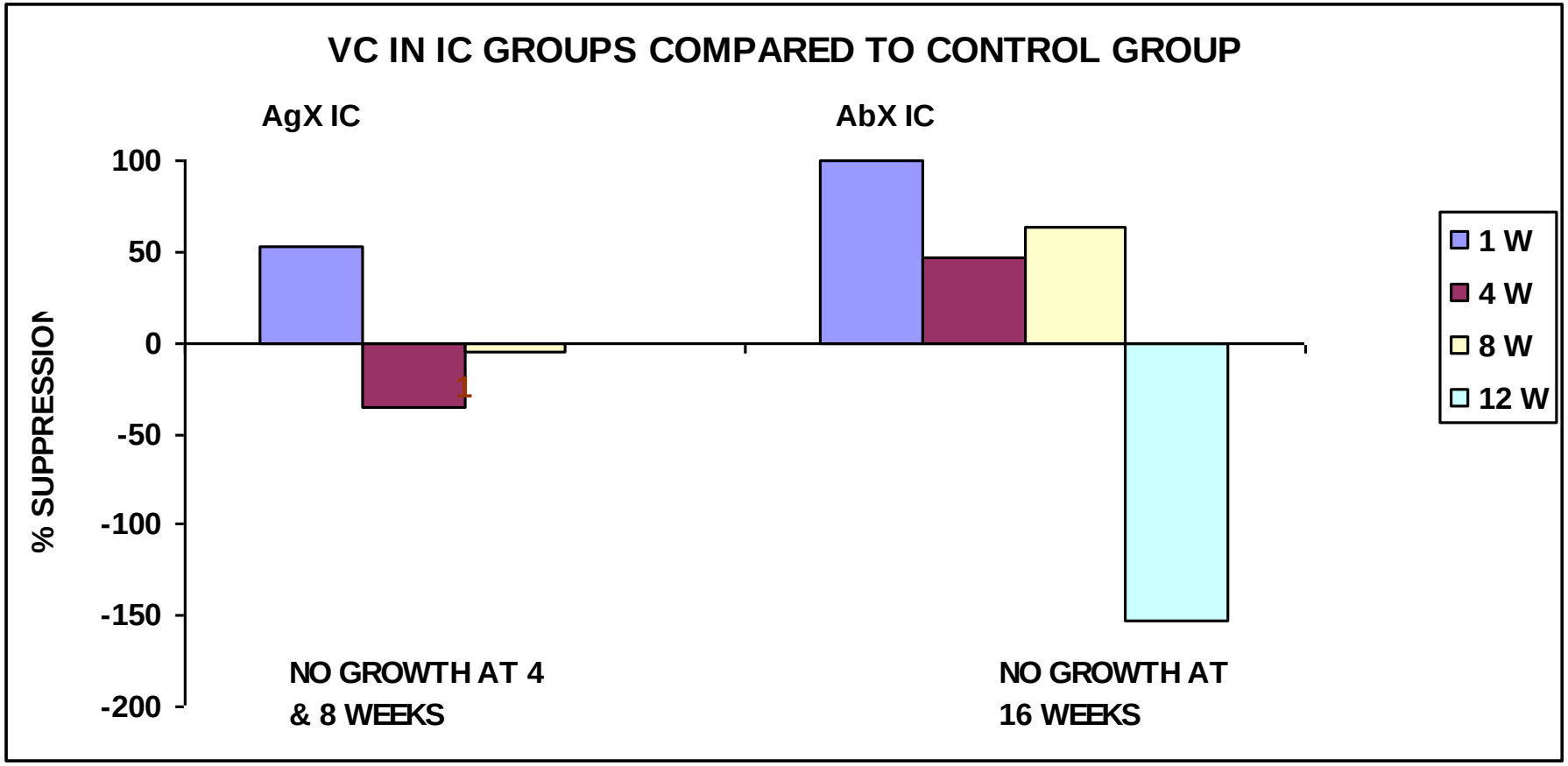
Lung of ABX group

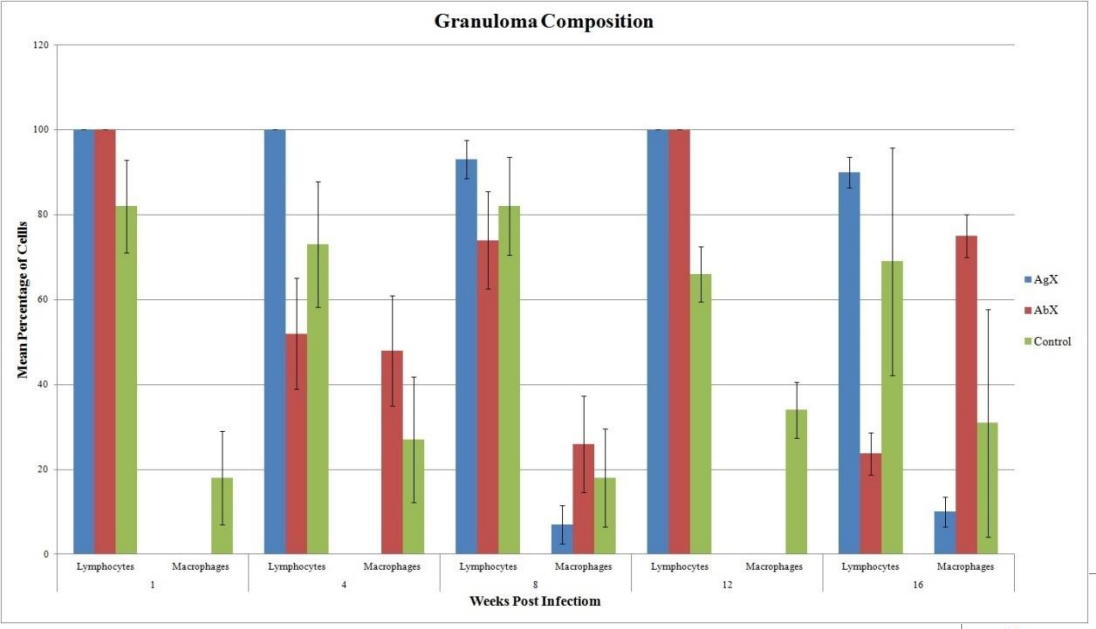
Lung of control group

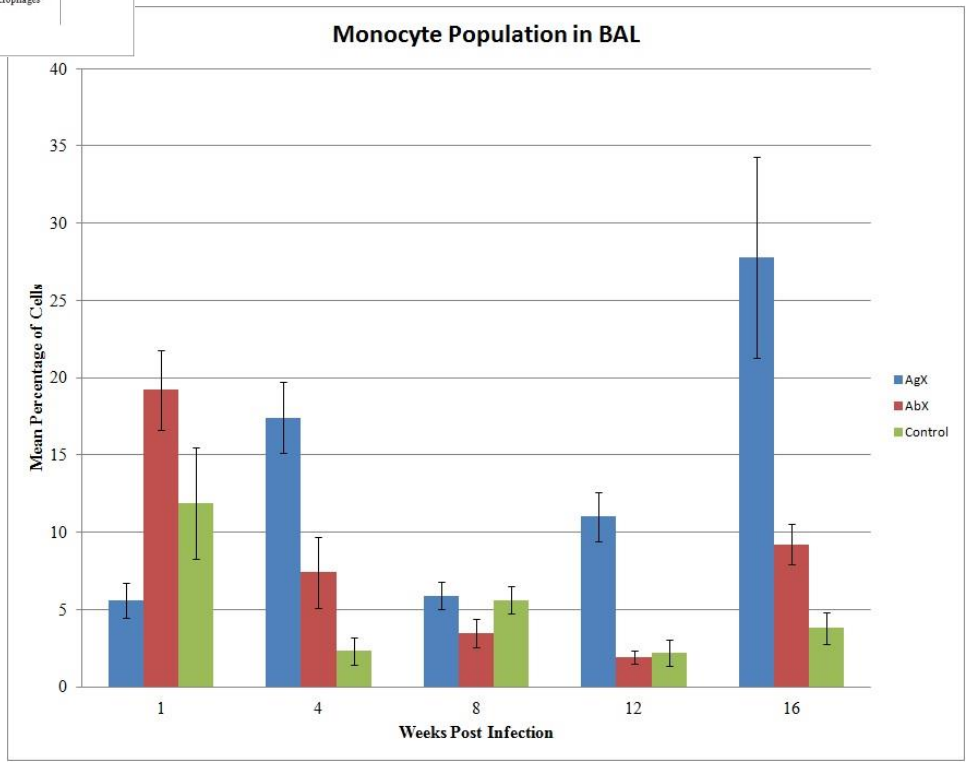



Immune Complexed Antibody titre
Agx
Abx
Control
OD 490nm
1
0


Anti--M.tb. Antiboby titer in Serum and IC
Axis Title
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SERUM
IC
1
4
8
12
16
Weeks Post Infectiom
AgX
AbX
Control


VC IN IC GROUPS COMPARED TO CONTROL GROUP
AgX IC
AbX IC
100
50
0
-50
-100
-150
-200
% SUPPRESSION
1 W
4 W
8 W
12 W
NO GROWTH AT 4
& 8 WEEKS
NO GROWTH AT
16 WEEKS


Granuloma Composition
Mean Percentage of Cells
120
100
80
60
40
20
0
Lymphocytes
Macrophages
1
4
8
12
16
Weeks Post Infectiom
AgX
AbX
Control


Monocyte Population in BAL
Mean Percentage of Cells
40
35
30
25
20
15
10
5
0
1
4
8
12
16
Weeks Post Infection
AgX
AbX
Control

Our Current Study with CFP as antigen and infection by aerosol inhalation

Preparation of Immune Complex

- Culture Filtrate Proteins and its specific antibody (BEI resources, NIH) was used.
- Immune complexes were prepared by incubating antigen and antibody together for 60mins at 37^0C.
- From the above mixture ICs were isolated by PEG precipitation method.

CFP as Antigen Source

- Culture filtra *erculosis* contains molecules whi nity to tuberculosis in animal mode
- The CF protein vere all identified as strong interfer fected mice and in tuberculosis pa dersen 1999).

TABLE 1. Pattern of reactivity of CFPs of *M. tuberculosis* H37Rv with known MAbs and polyclonal sera

Antibody[a]	Reported corresponding antigen	Reference[c]	Dilution of antibodies
IT-1 (F23-49-7)	16 kDa[b]	28	1:2,000
IT-3 (SA-12)	12-kDa GroES[c]	37	1:8,000
IT-4 (F24-2-3)	16-kDa α-crystallin[c]	28	1:2,000
IT-7 (F29-29-7)	40 kDa[c]	28	1:1,000
IT-10 (F29-47-3)	21 kDa[c]	28	1:1,000
IT-12 (HYT6)	19-kDa lipoprotein[c]	3	1:50
IT-17 (D2D)	28-kDa SOD[c]	65	1:8,000
IT-20 (WTB68-A1)	14-kDa α-crystallin[c]	10	1:250
IT-23 (WTB71-H3)	38-kDa PstS[c]	10	1:250
IT-40 (HAT1)	71-kDa DnaK[c]	3	1:50
IT-41 (HAT3)	71-kDa DnaK[c]	3	1:50
IT-42 (HBT1)	82 kDa[c]	35	1:50
IT-43 (HBT3)	56 kDa[c]	35	1:50
IT-44 (HBT7)	32 kDa[c]	35	1:50
IT-45 (HBT8)	96 kDa[c]	35	1:50
IT-46 (HBT10)	40 kDa[b]	35	1:50
IT-49 (HYT27)	32–33-kDa Ag85 complex[c]	50	1:50
IT-51 (HBT2)	17 kDa[c]	35	1:50
IT-52 (HBT4)	25-kDa MPT 51[c]	35	1:50
IT-53 (HBT5)	96 kDa[c]	35	1:50
IT-56 (CBA1)	65 kDa[d]	35	1:50
IT-57 (CBA4)	82 kDa[c]	35	1:50
IT-58 (CBA5)	47 kDa[c]	35	1:50
IT-59 (F67-1)	33 kDa[c]	28	1:100
IT-61 (F116-5)	30 kDa[c]	60	1:100
IT-67 (L24.b4)	24-kDa MPT 64[c]	40	1:50
IT-69 (HBT 11)	20 kDa[c]	35	1:6
F126-2	30 kDa[c]	60	1:100
A3h4	27 kDa[c]	45	1:50
HYB 76-8	6-kDa ESAT6[c]	27	1:100
Anti-MPT 32	50-kDa MPT 32[c]	39	1:100
Anti-MPT 46	10-kDa MPT 46[c]	39	1:100
Anti-MPT 53	15-kDa MPT 53[c]	39	1:100
Anti-MPT 57	12-kDa GroES[c]	39	1:100
Anti-MPT 63-K64	18-kDa MPT 63[c]	39	1:200

Sonnenberg & Belisle, 1997

Sensitization of Guinea Pigs with 10^3 *M. tuberculosis* H37RV-by aerosol inhalation method using inhalation exposure system to all the three groups-Group I, II & III.

↓ 30 days

Injection of two composition of Immune complex-**SC route** to Group I and Group II alone

Group I-Antigen excess immune complex
Group II-Antibody excess immune complex

↓ 7 days

Infection of Guinea pigs with 10^6 *M. tuberculosis* H37RV- by aerosol inhalation method using inhalation exposure system to all the three groups-Group I, II & III.

↓ 7 days

First Week – Blood Collection by retro orbital method from all the three groups-Group I, II & III.

↓ 60 days

8th Week-Blood Collection by cardiac puncture method and Harvesting the Tissue materials after sacrificing all the three groups-Group I, II & III.



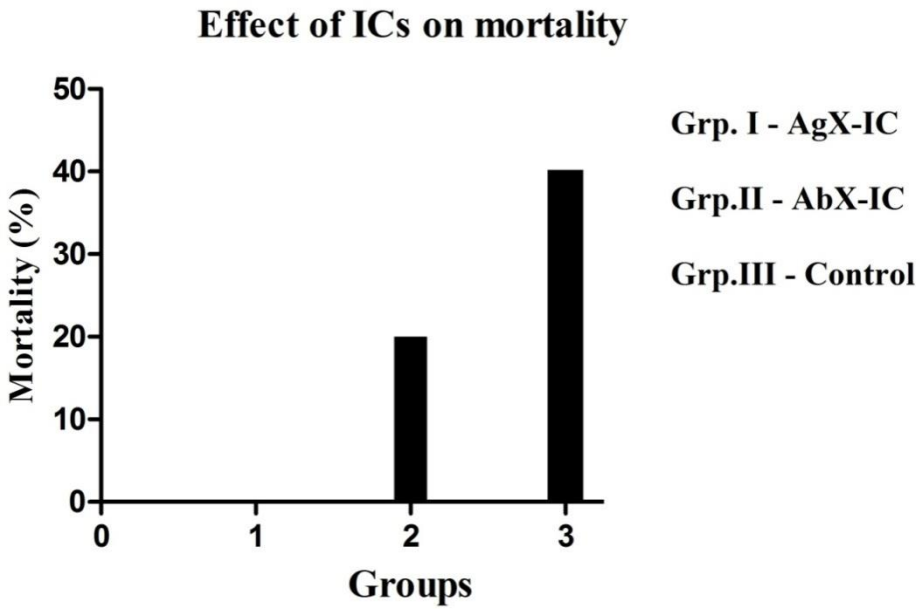




Experimental Set-Up

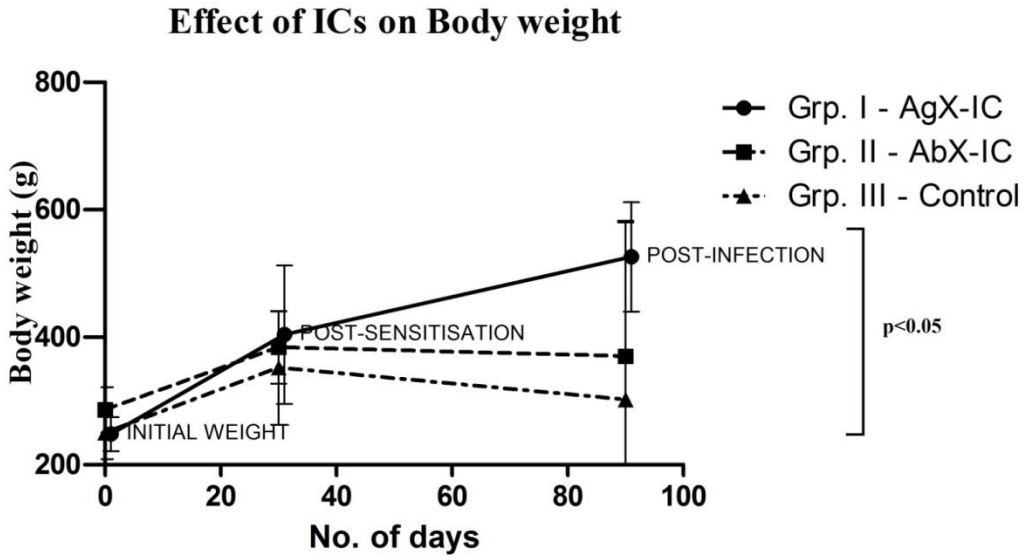


- **Animal:** *Cavia porcellus* (Guinea Pig-Dunkin Hartley)
- **Sex:** Male
- **Quantity:** 15 Nos. in three groups
- **Place of the Study:** BSL3 Laboratory for Animal Experiments, National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra, India.
  - ❖ Group I-Immunized with Antigen Excess IC (AgX-IC)
  - ❖ Group II-Immunized with Antibody Excess IC (AbX-IC)
  - ❖ Group III-Control
  - ❖ Naïve animal was also included.
- All the groups were maintained in separate cages in isolators with proper feed and controlled temperature and humidity.
- Infection was carried out via intra-nasal route using aerosol inhalation exposure system.

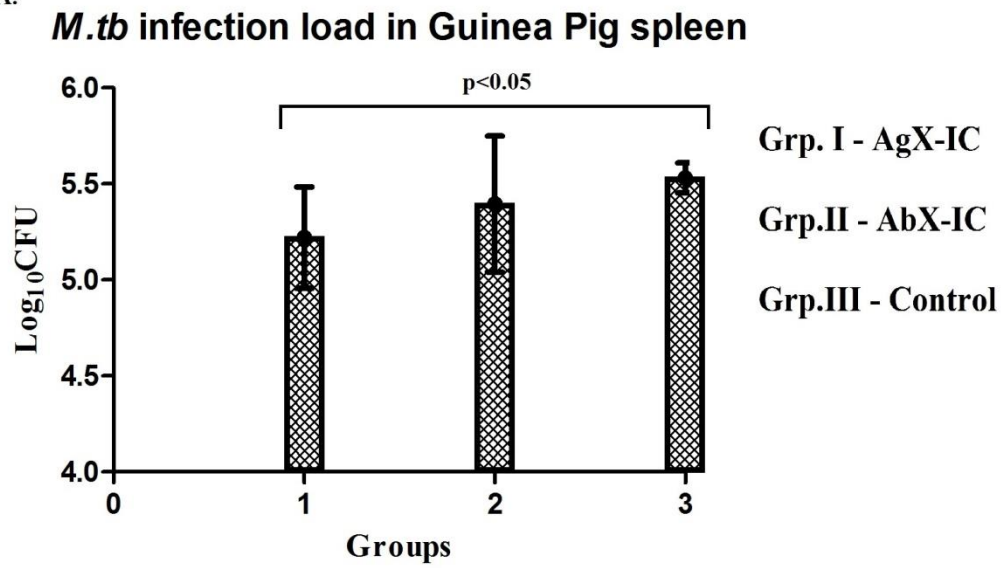


Group I with Agx-IC did not show any mortality while there was 20% and 40 % of mortality with Abx-IC group and control group respectively.

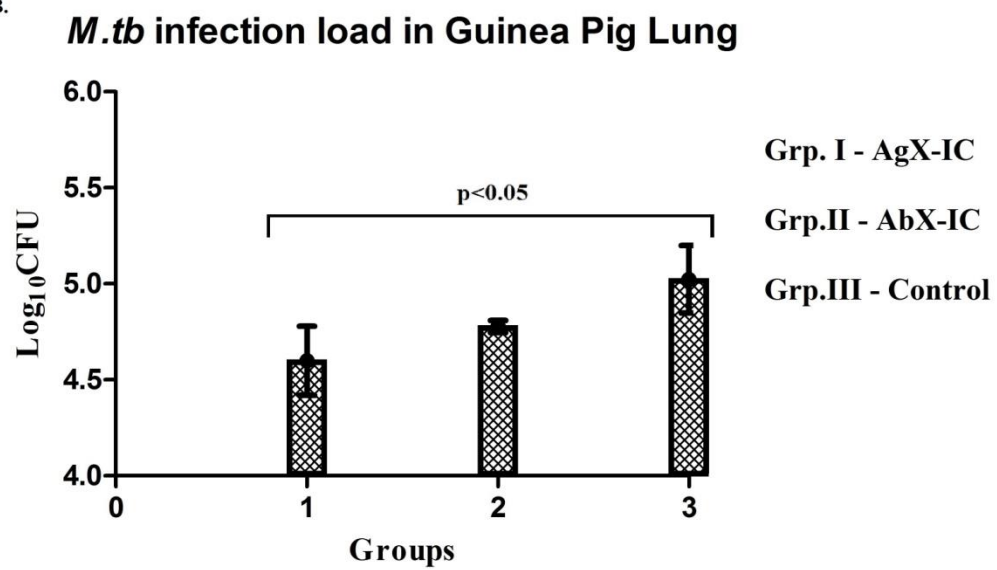
IC complex with antigen excess showing sustained increase in body weight of guinea pigs.



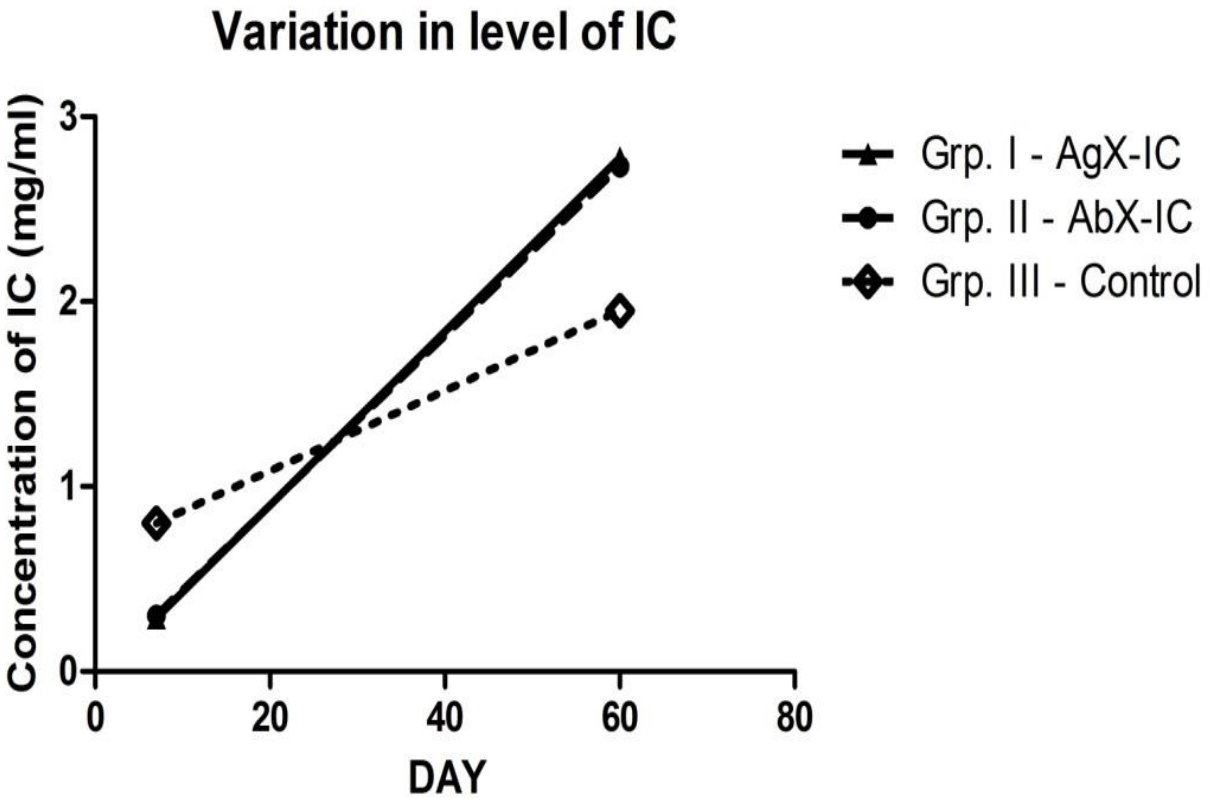
A.



B.



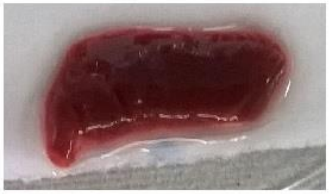
Colony Forming Units of *M.tb* in spleen (A) and lungs (B) of guinea pigs: IC immunized group I and II shows decrease in infection load than control group. The values are expressed as $\log_{10}$ with statistical significance: $p<0.05$.



CIC level in guinea pigs serum showing significant difference between IC immunized test group and non-immunized control group ($p<0.05$).

Pathophysiology of Spleen and Lungs Infected with *M.tb*

A. Spleen



Naive animal



Grp. I - AgX-IC

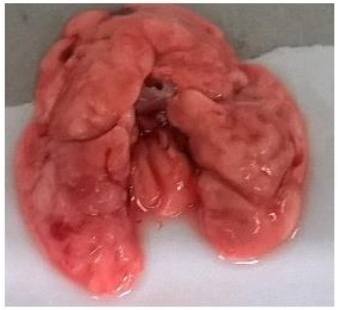


Grp. II - AbX-IC



Grp. III - Control

B. Lung



Naive animal



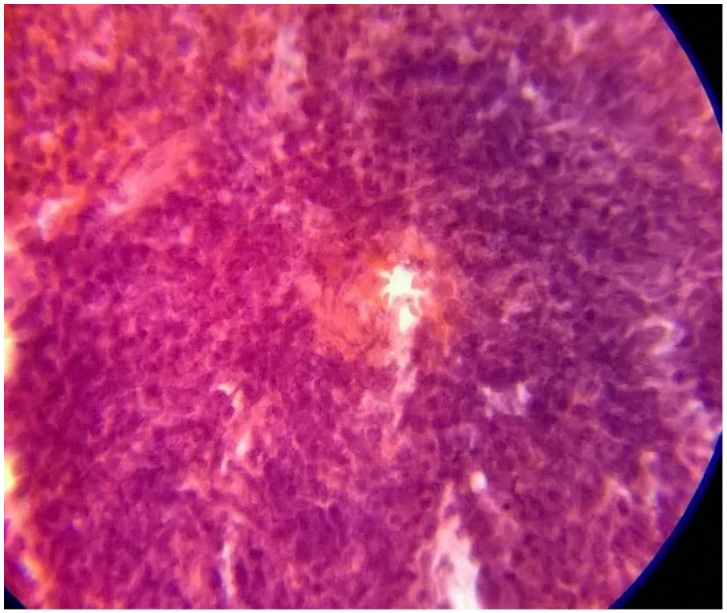
Grp. I - AgX-IC



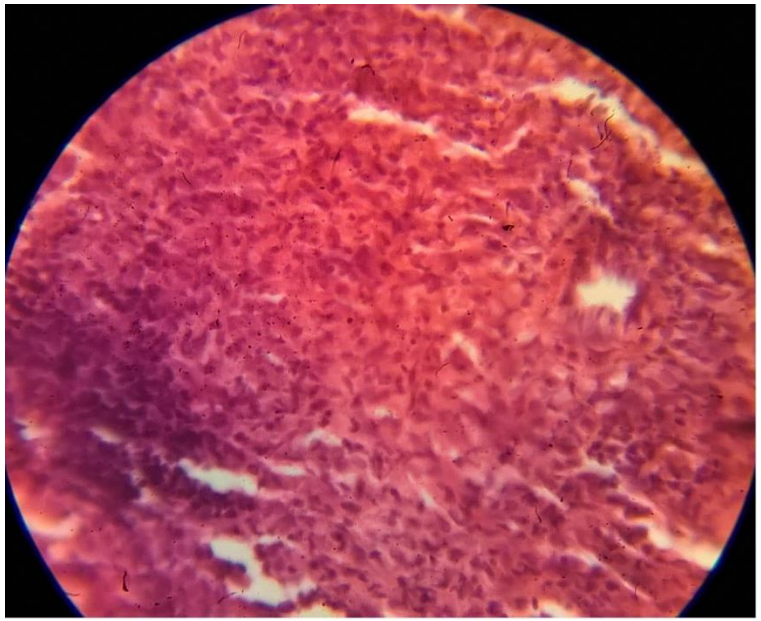
Grp. II - AbX-IC



Grp. III - Control



AGX spleen



Control spleen

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


THANK YOU