Immune Complex as Vaccine against Tuberculosis

H. Shakila,
Associate Professor,
Madurai Kamaraj University,
Madurai, Tamilnadu
IMMUNE COMPLEXES AS VACCINES AGAINST TUBERCULOSIS

A

\[\text{Acr-Ag85B fusion protein} \quad \alpha\text{-Acr mAb} \quad \alpha\text{-Ag85B polyclonal Ab} \quad \text{Ag85B} \]

B

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Vaccine Congress 2015
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

<table>
<thead>
<tr>
<th><strong>Group A</strong> antigen excess immune complex preparation AGX=49:1 (where antigen was 49 parts and antibody was 1 part)</th>
<th><strong>Group B</strong> antibody excess immune complex preparation ABX=1:49 (where antibody was 49 parts and antigen was 1 part)</th>
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<tbody>
<tr>
<td>Ag alone = 49 volume of antigen + 1 volume of normal saline</td>
<td>Ag alone = 1 volume of antigen + 49 volume of normal saline</td>
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<tr>
<td>Ab alone = 1 volume of antibody + 49 volume of saline</td>
<td>Ab alone = 49 volume of antibody + 1 volume of normal saline</td>
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<tr>
<td>Control = Normal saline (N/S)</td>
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LUNG HISTOLOGY OF ANIMALS INJECTED WITH ANTIGEN EXCESS IMMUNE COMPLEX

LUNG HISTOLOGY OF CONTROL ANIMALS

LUNG HISTOLOGY OF ANIMALS INJECTED WITH ANTIBODY EXCESS IMMUNE COMPLEX

GRANULOMA IN LUNG
VC IN IC GROUPS COMPARED TO CONTROL GROUP

-200
-150
-100
-50
0
50
100
1
2
% SUPPRESSION
1 W
4 W
8 W
12 W
NO GROWTH AT 4 & 8 WEEKS
AgX IC
AbX IC
NO GROWTH AT 16 WEEKS

NO GROWTH AT 4 & 8 WEEKS

NO GROWTH AT 16 WEEKS
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°C.
• From the above mixture ICs were isolated by PEG precipitation method.
CFP as Antigen Source

• Culture filtrate from Mycobacterium tuberculosis contains molecules which promote protective immunity to tuberculosis in animal models.

• The CF proteins CFP17, CFP21, CFP25, and CFP29 were all identified as strong interferon-γ inducers in M. tuberculosis-infected mice and in tuberculosis patients (Karin Weldingh and Peter Andersen 1999).
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.
IC complex with antigen excess showing sustained increase in body weight of guinea pigs.

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.
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**