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Proteoliposomes obtained from nonpathogenic mycobacteria as a protective vaccine candidates against Tuberculosis infection

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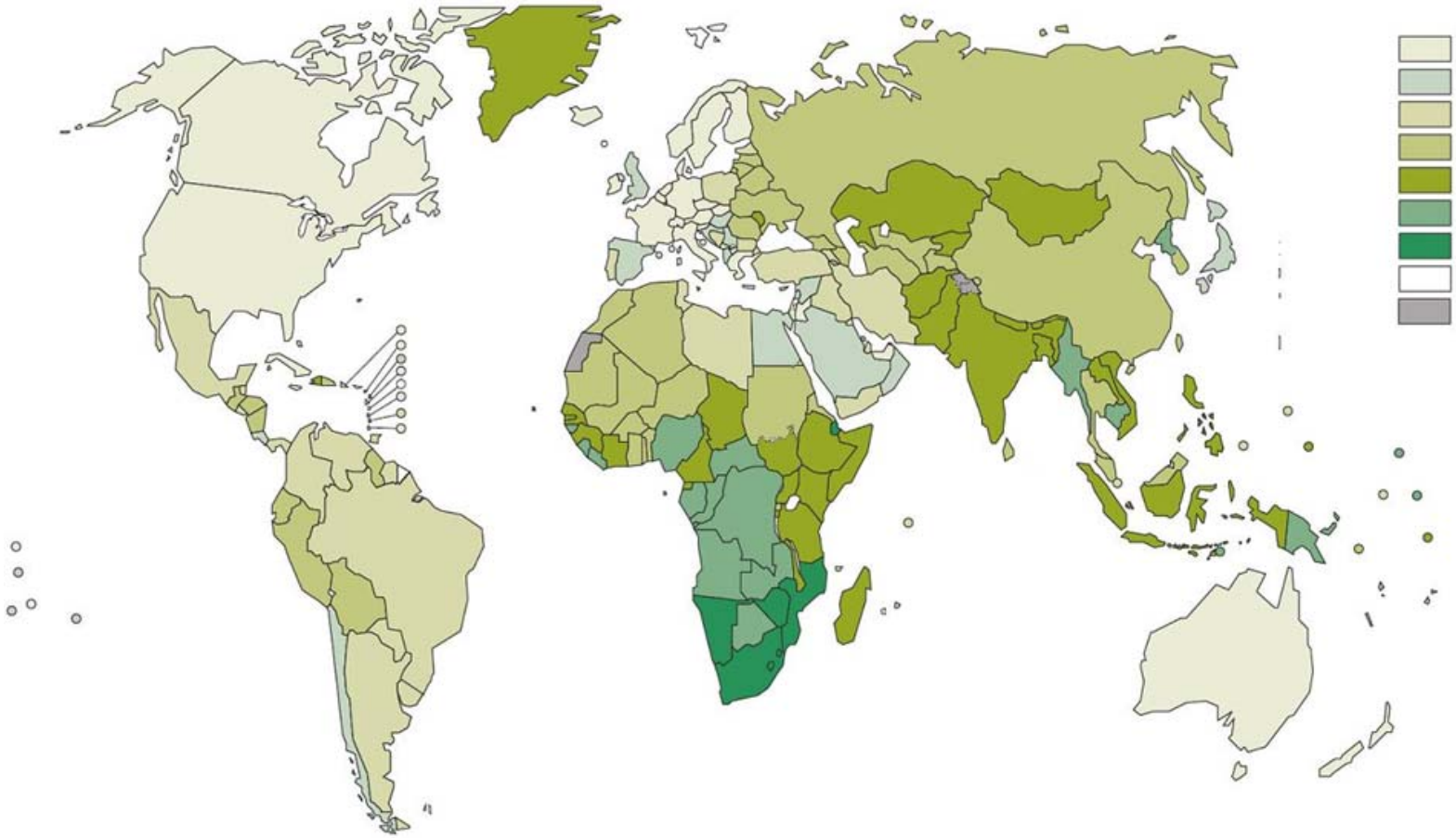
WHO
TB Report 2014

TB CONTINUES TO BE AN ALARMING DISEASE !!!

- In 2013, 6.1 million TB cases were reported to WHO. Of these, 5.7 million were people newly diagnosed and another 0.4 million were already on treatment.
- Of the estimated 9 million people who developed TB.
- An estimated 1.1 million (13%) of the 9 million people who developed TB were HIV-positive.
- There were an estimated 550 000 new cases among children.
- Every 15 seconds 1 person dies of tuberculosis.

Estimated TB Incidence by Country - 2013

WHO
TB Report 2014



Vaccines against Tuberculosis: BCG

- BCG, the only TB vaccine currently available

- BCG provides protection against disseminated TB in young children

- BCG does not protect against pulmonary TB, even when this is the most frequently and the responsable of disease transmisión

- Limited time of protection (10-20 years after vaccination)

There is an urgent need
to develop a new vaccines against TB

The development pipeline for new TB vaccines

WHO
TB Report 2014

Phase I

AdAg85A
McMaster, CanSino

MTBVAC
TBVI, Zaragoza, Biofabri

ID93+GLA-SE
Infectious Disease Research Institute (IDRI), Aeras

Crucell Ad35/MVA85A
Crucell, Oxford, Aeras

DAR 901
Dartmouth, Aeras

TB/FLU-04L Research
Research Institute for Biological Safety Problems, Research Institute on Influenza

Phase II

VPM 1002
Max Planck, VPM, TBVI, Serum Institute

H1+IC31
SSI, TBVI, EDCTP, Intercell

RUTI
Archivel Farma, S.L.

H56: IC31
SSI, Aeras, Intercell

H4: IC31
SSI, Sanofi-Pasteur, Aeras, Intercell

Crucell Ad35/ AERAS-402
Crucell, Aeras

Phase IIb

MVA85A
Oxford, Aeras

M72+AS01
GSK, Aeras

Phase III

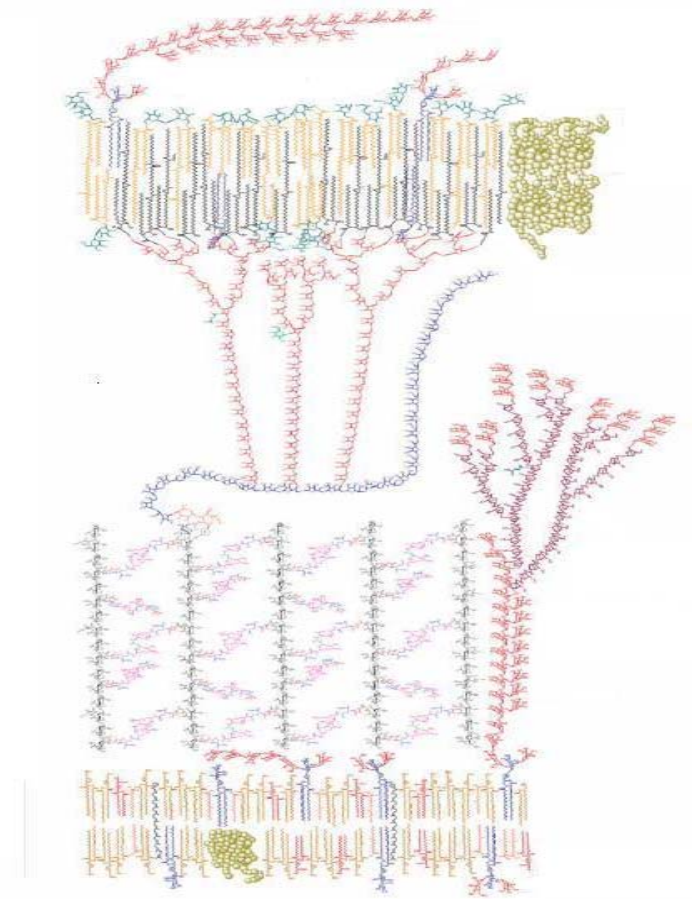
M. Vaccae
Anhui Zhifei Longcom

15 vaccine candidates

TB Vaccine Types

Viral-vectored: MVA85A, Crucell Ad35, AdAg85A, TB/FLU-04L
Protein/adjuvant: M72, H1+IC31, H4: IC31, H56: IC31, ID93+GLA-SE
rBCG: VPM 1002
Attenuated *M.Tb*: MTBVAC
Mycobacterial – Whole cell or extract: *M. Vaccae*, RUTI, DAR-901

Proteins and lipids of Mtb cell wall are immunogenic and associated with protection



Kaur *et al*., 2009

Hamasur *et al*., 2003. ***Mycobacterium tuberculosis*** arabinomannan-protein conjugates protect against tuberculosis.

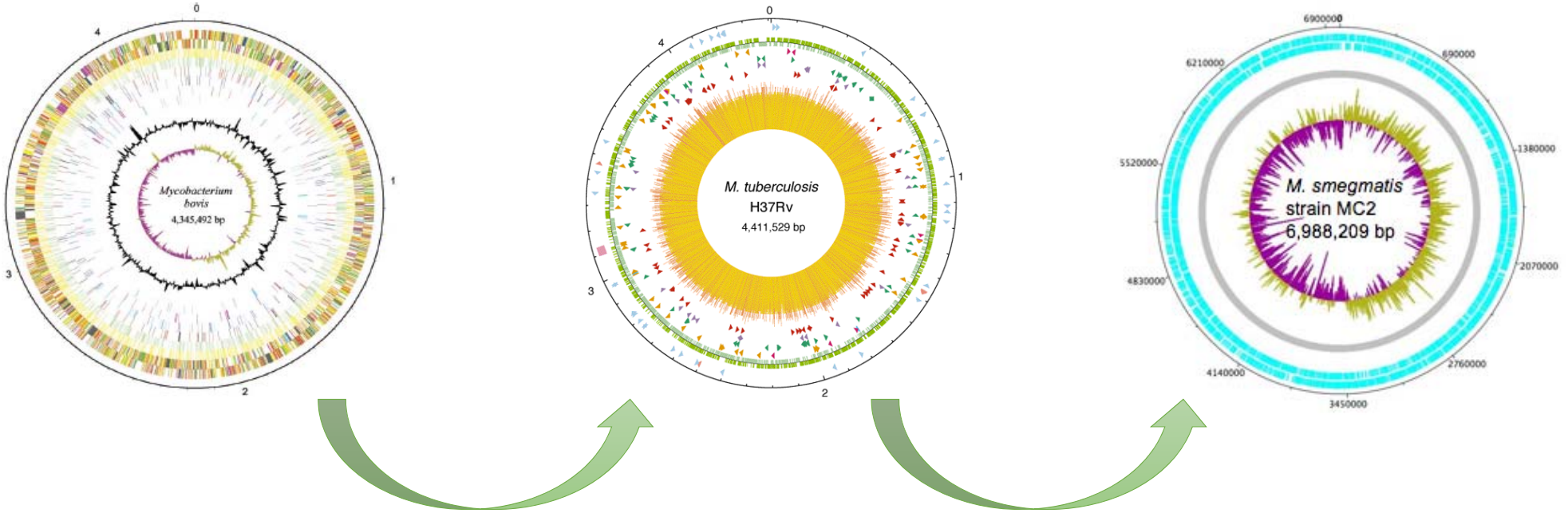
Hamasur *et al*., 2004. A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab') fragment prolong survival of mice infected with *Mycobacterium tuberculosis*.

Gilleron *et al*., 2004. Diacylated sulfoglycolipids are novel mycobacterial antigens stimulating CD1-restricted T cells during infection with *Mycobacterium tuberculosis.*

Jeon *et al*., 2007. Protection of Mice against *Mycobacterium tuberculosis* infection by immunization with Aqueous Fraction of Triton X-100-Soluble Cell Wall Proteins.

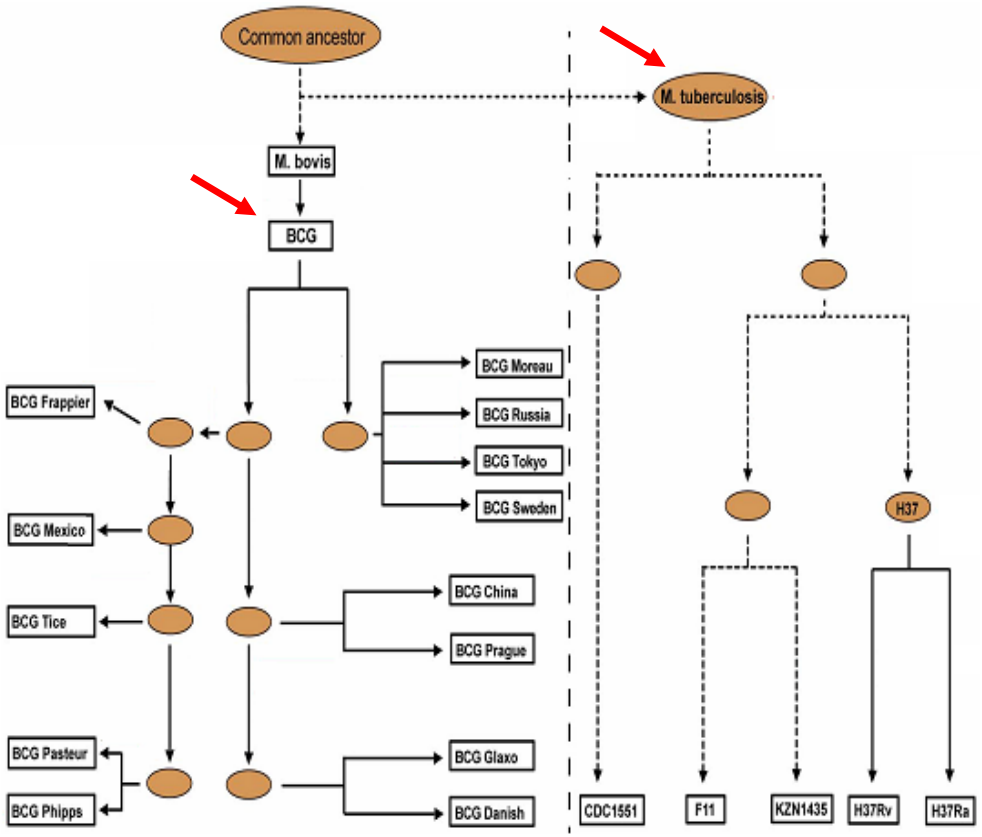
Vilaplana *et al*., 2011. Prophylactic Effect of a Therapeutic Vaccine against TB Based on Fragments of *Mycobacterium tuberculosis.*

BCG and *M. smegmatis* are non-pathogenic mycobacteria with high levels of genomic and antigenic homology with *M. tuberculosis*

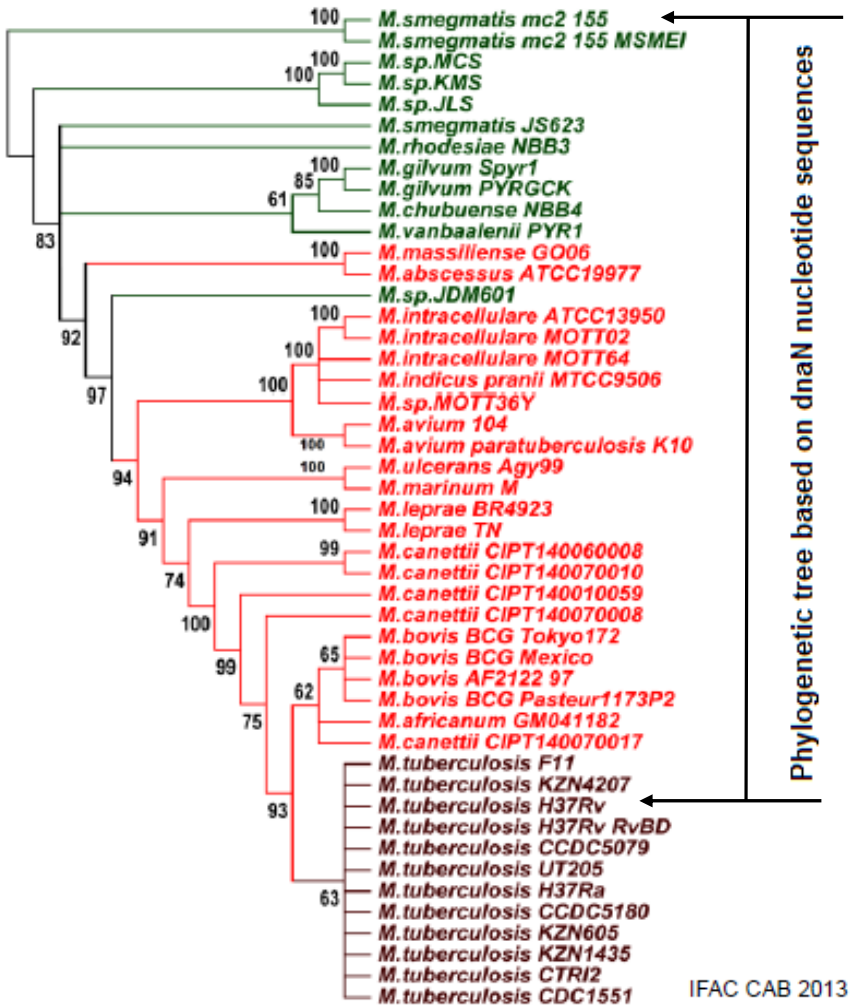


High similarity at DNA level (99.95%)

12 out of the 19 *M. tuberculosis* virulence genes described share closely related homologues in *M. smegmatis*



Garnier *et al.,* 2003; Gomes *et al.,* 2001; Zhang *et al*., 2013



IFAC CAB 2013
December 16-18, 2013.

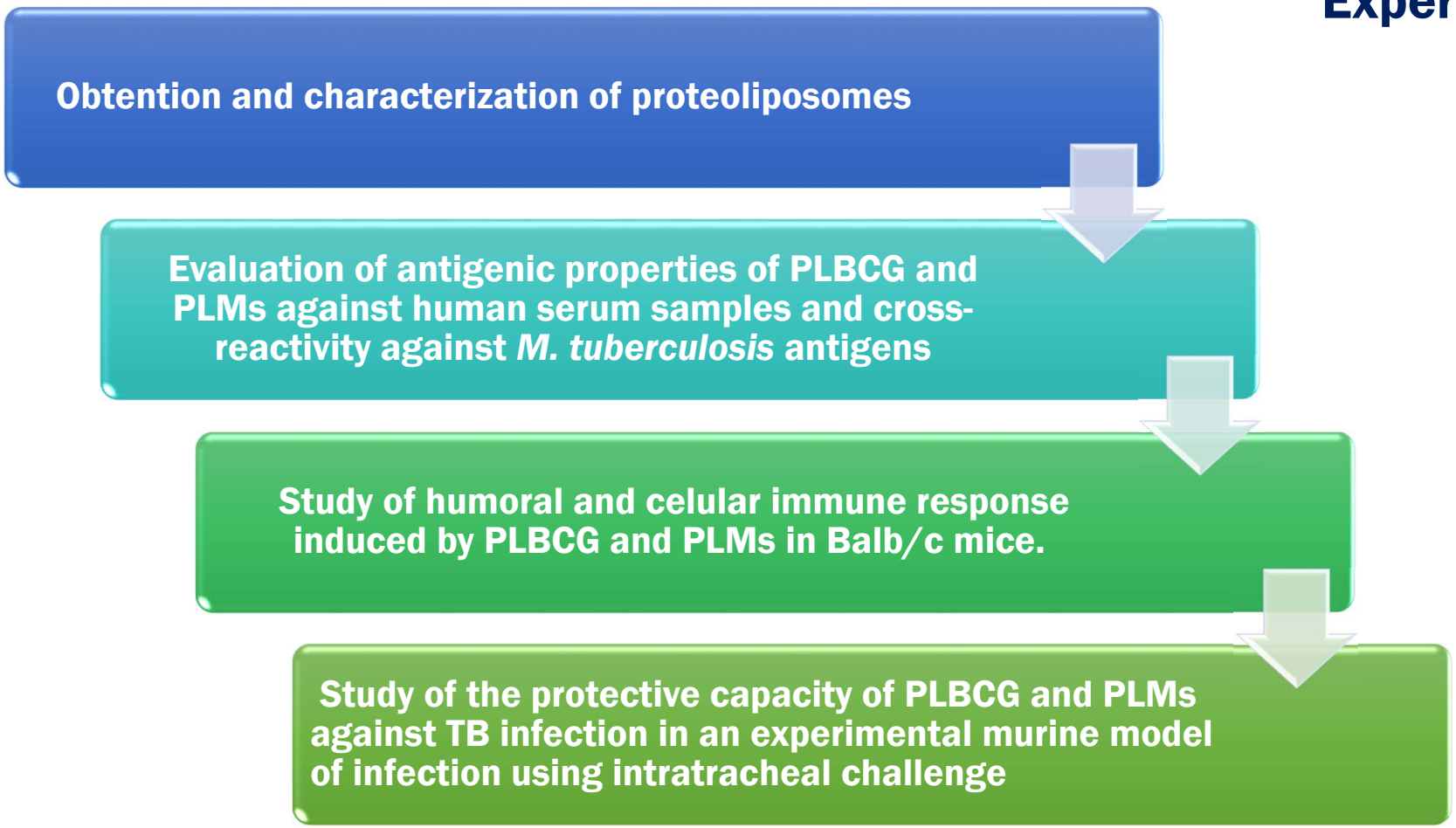
One potential strategy to explore is the use of proteoliposomes (*cell membrane extract*) from non pathogenic mycobacteria as new vaccine candidates against TB

Advantages

- ✓ Technology available.
- ✓ Work with non pathogenic bacteria.
- ✓ Possible inclusion of lipids, glycolipids and lipoproteins associated with immunogenicity and protection.
- ✓ Possible presence of conserved proteins of mycobacteria, some of them associated with latency and *in vivo* expression.

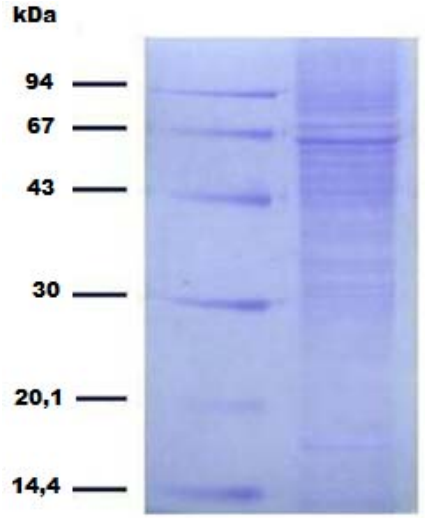
Although there are several studies that report the immunogenic properties of proteoliposomes, at present there are few proteoliposome based vaccines and none against *M. tuberculosis*.

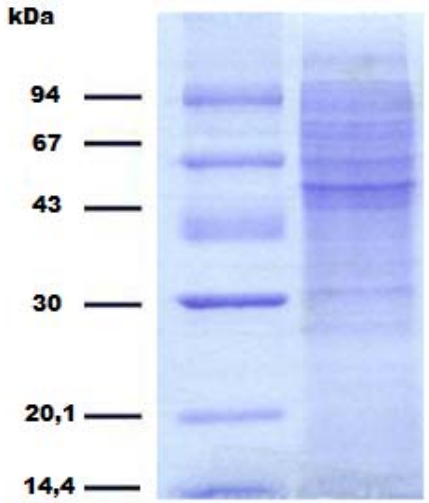
Experimental design



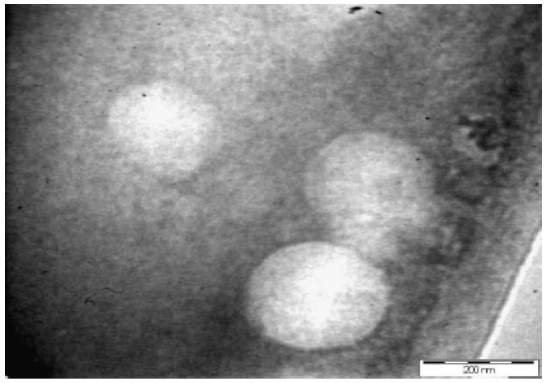
Proteoliposomes characterization

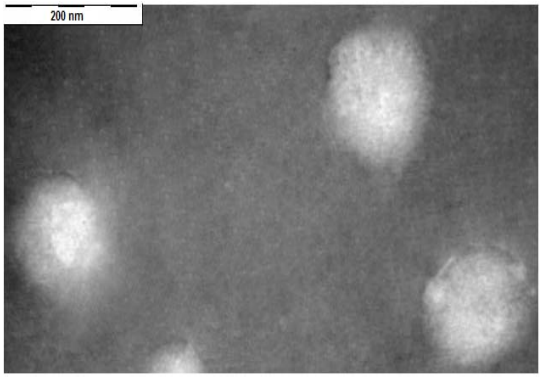
A – PLBCG
B - PLMs





A

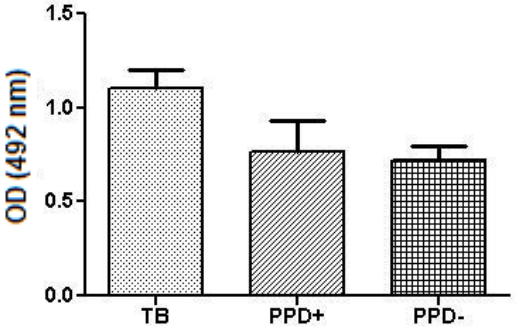




B

Proteoliposomes showed antigenic properties in human probably due to the presence of *M. tuberculosis* epitopes

A – PLBCG
B - PLMs



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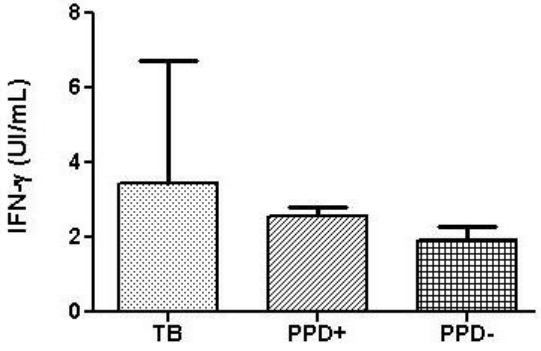

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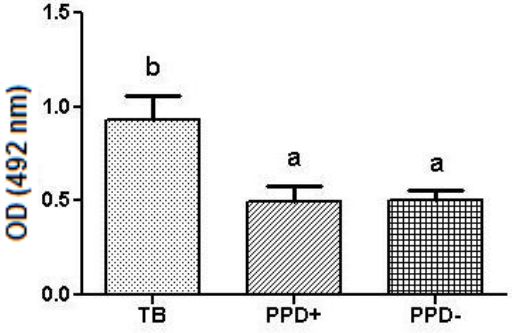
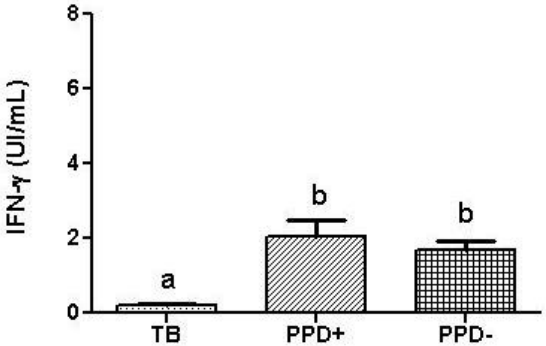
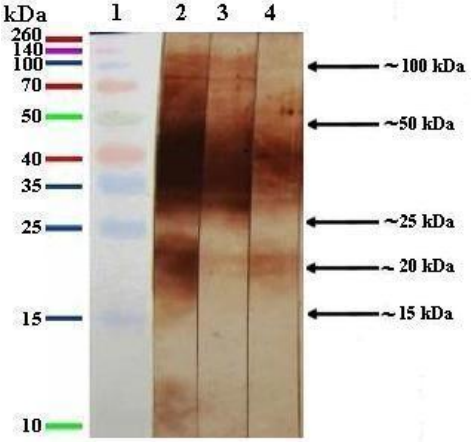


B



B

1- MOLECULAR WEIGHT
2- ACTIVE PULMONAR TB
3- HEALTH HUMANS PPD-
4- HEALTH HUMANS PPD+



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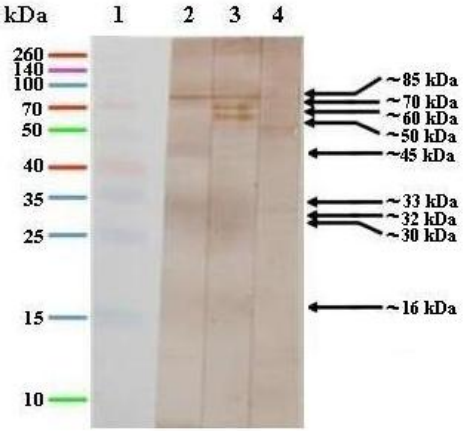
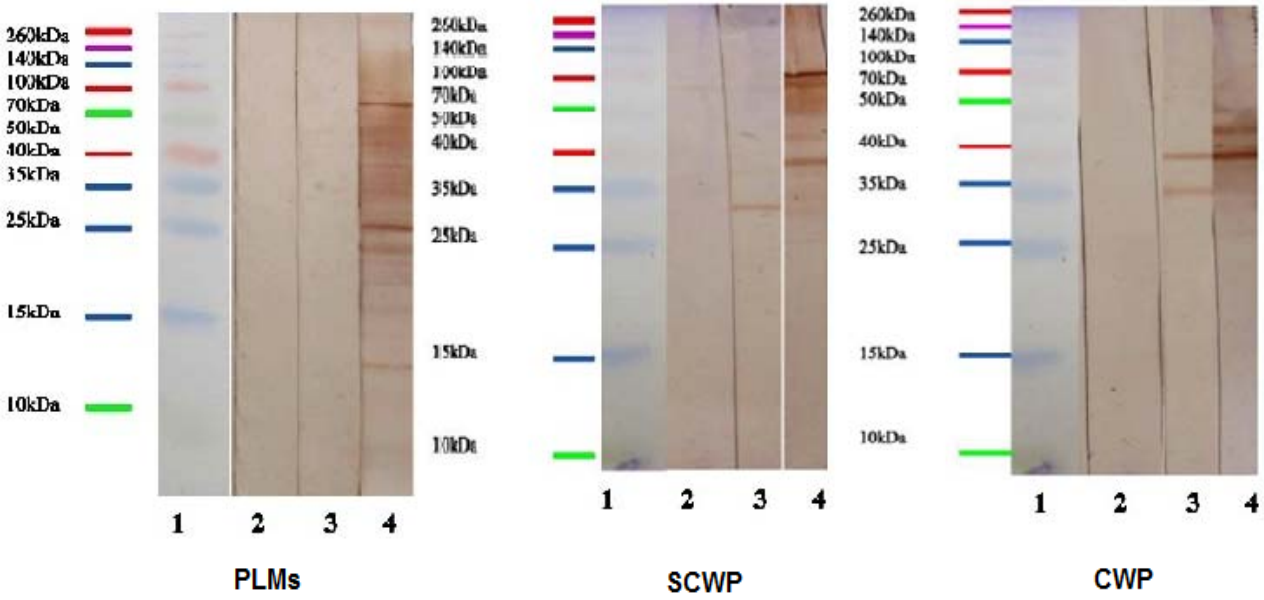


B

Proteoliposomes showed cross-reactivity against *M. tuberculosis* antigens

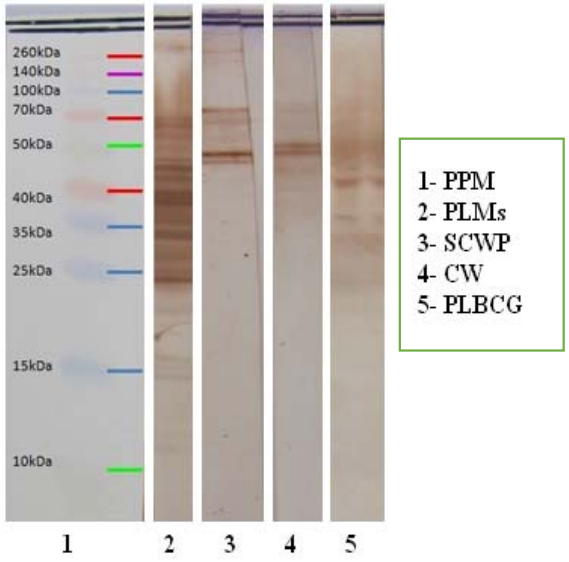
A – PLBCG
B - PLMs



1- MOLECULAR WEIGHT
2- SALINE SOLUTION
3- BCG
4- PLBCG+AI

1: MWP **2**: PBS **3**: BCG **4**: PLCG

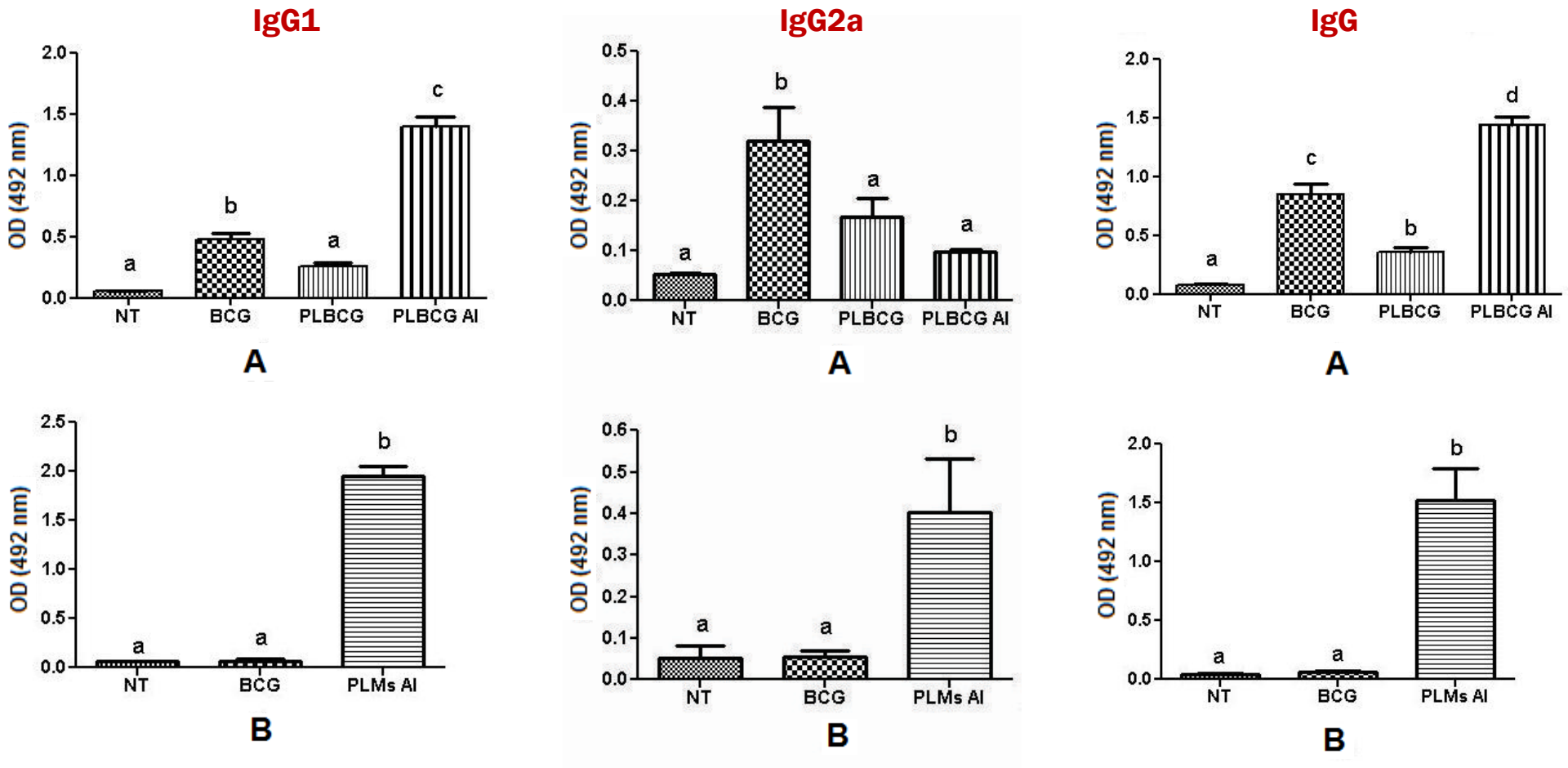
A



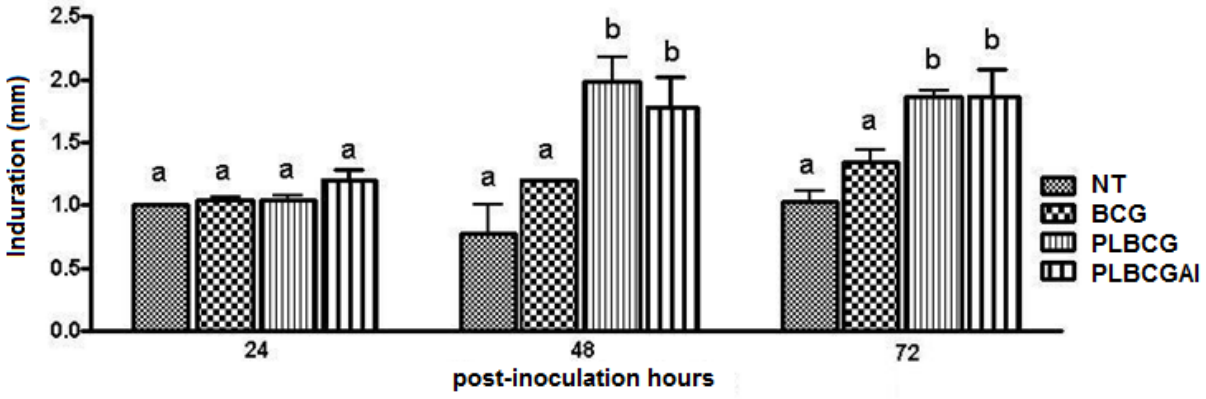
B

Proteoliposome from nonpathogenic bacteria induced humoral immune response in Balb/c mice, mostly combined with alum adjuvant

A – PLBCG
B - PLMs



Proteoliposomes induced delayed hypersensitivity reponse in Balb/c mice



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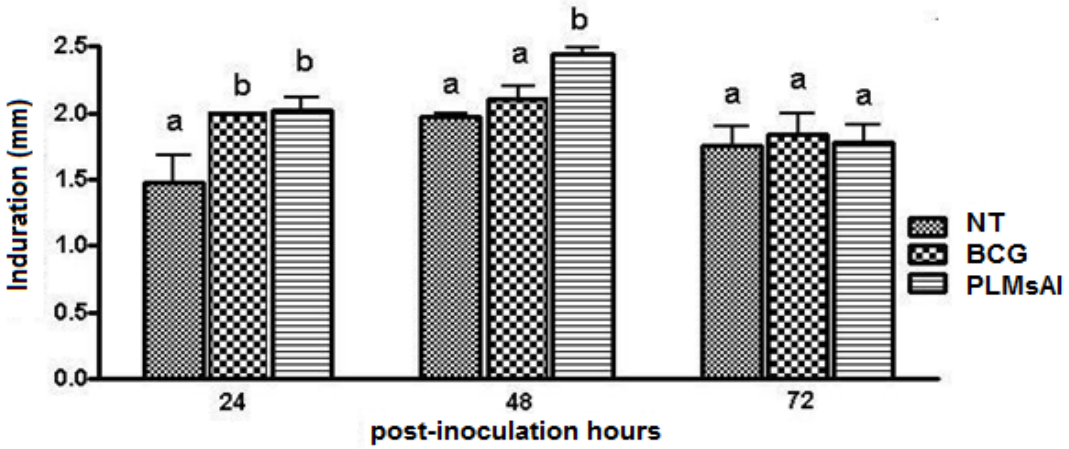


B

A – PLBCG
B - PLMs

Challenge experiment

Experimental model of intratracheal infection with Mtb
(Dr. Rogelio Hernandez-Pando, Mex)
Hernandez-Pando, *et al* 1996

1st dose (SC)

2nd dose (SC)

Challenge with Mtb H37Rv (IT) $2{,}5 \times 10^5$ CFU

21 days

2 months

2 months

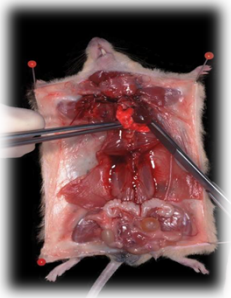
Groups

PBS
BCG (10^6 CFU)
PLMs (50 µg)
PLMs + Al (50 µg PL + 1mg Alu)
PLBCG (50 µg)
PLBCG +Al (50 µg PL + 1mg Alu)
BCG (1st dose) / PLMs +Al (2nd dose)
BCG (1st dose) / PLBCG +Al (2nd dose)

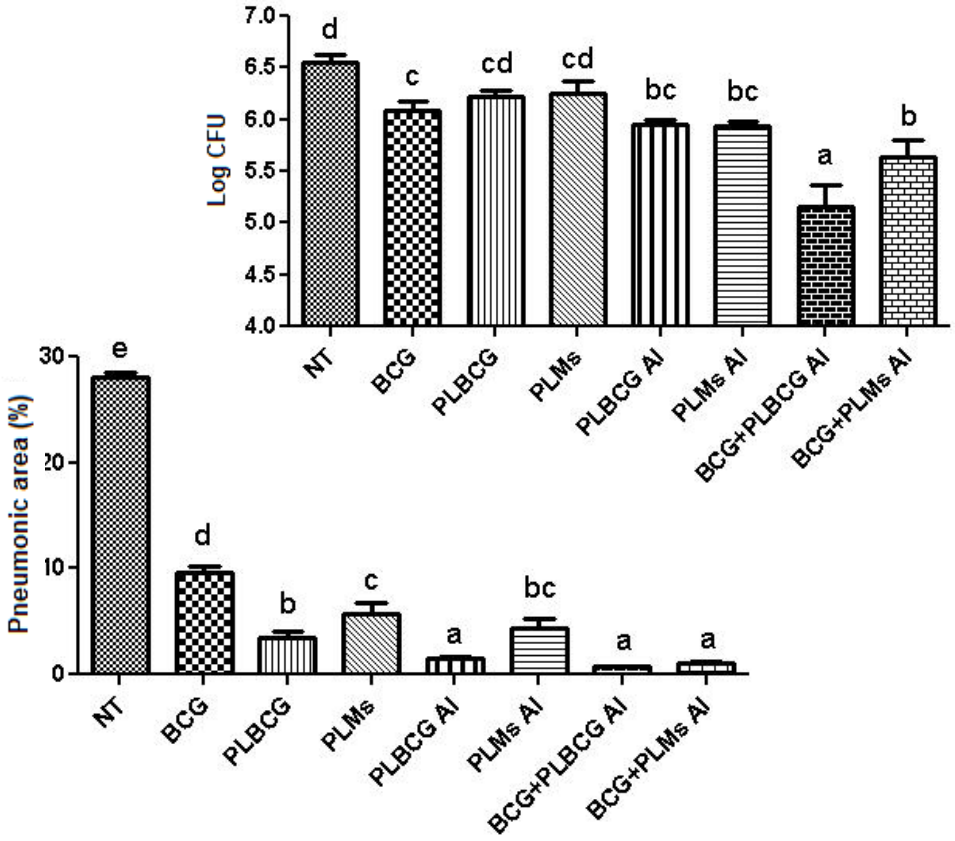
Lung samples extraction

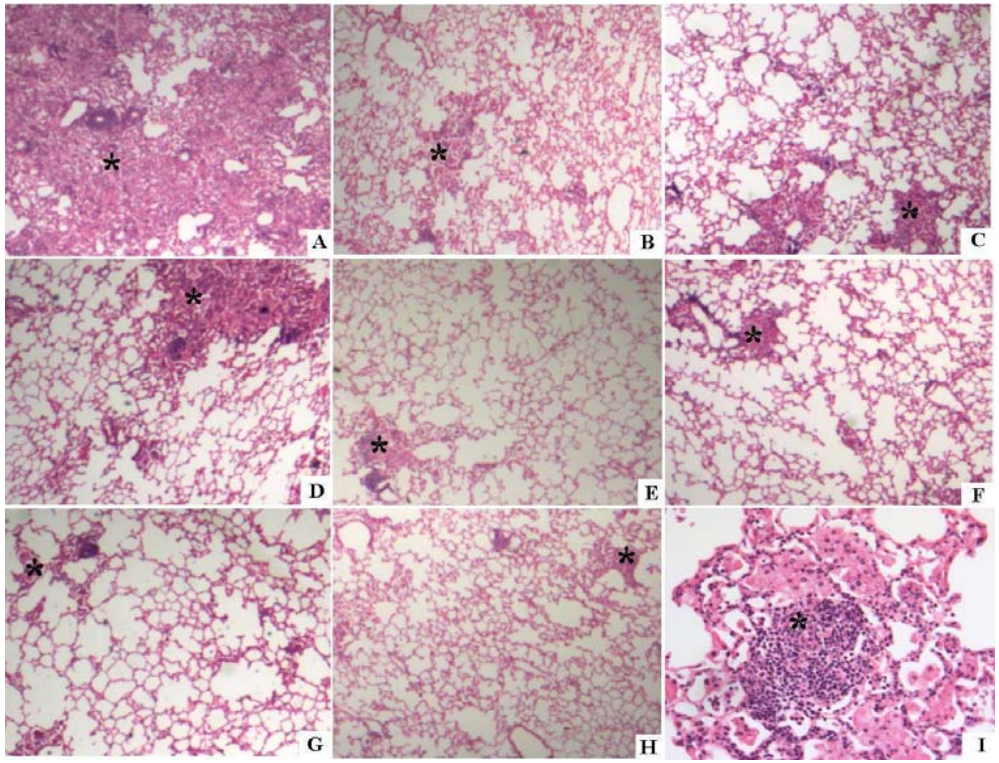
Bacterial load

Pneumonic área
(morphometric and
histopatological study)



Proteoliposomes from BCG and M. smegmatis protected significantly against experimental infection with MtbH37Rv than BCG





A: NT (10 x), asterisk shows extensive area of general pneumonia; **B**: BCG (10 x), **C**: PLBCG (10 x); **D**: PLMs (10 x); **E**: PLBCG AI (10 x); **F**: PLMs AI (10 x); **G**: BCG+PLBCG AI (10 x); **H**: BCG+PLMs AI, (10 x), asterisk show small pneumonic área; **I**: BCG+PLBCG AI (20 x); asterisk show a granuloma surrounded by a characteristic alveolar structure

FINAL REMARKS

- ✓ Proteoliposome derivated from *M. bovis* BCG and *M. smegmatis* are protective against TB infection in mice when were used with adjuvant as a prophylactic vaccine candidate in a prime-boost strategy.

- ✓ Both proteoliposomes could be used as a reinforce of BCG vaccination

ONGOING STUDIES

- ✓ Study of protective efficacy against hypervirulent *M. tuberculosis* strains

- ✓ Challenge experiment for study the protective capacity of PLMs and PLBCG in guinea pig model

Thanks to…

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