Protective capacity of proteoliposomes from *Mycobacterium smegmatis* and BCG in a mouse model of tuberculosis

ASEAN-EU STI Days 2014
Health: towards the Development of Improved TB Vaccines
Bangkok, Thailand
20 – 22 January 2014
Malaysia’s Vulnerability: Surrounded by High TB Burden Countries

- Myanmar
- Thailand
- Cambodia
- Viet Nam
- Philippines
- Indonesia
- Sabah
- Sarawak
- Johor
- KL
- Pinang
### Top 5 communicable diseases in Malaysia
(source: Health Informatics Centre, MOH)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence Rate (per 100,000)</th>
<th>Mortality Rate (per 100,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue</td>
<td>85.78</td>
<td>0.02</td>
</tr>
<tr>
<td>TB</td>
<td>62.26</td>
<td>5.53</td>
</tr>
<tr>
<td>Food poisoning</td>
<td>53.19</td>
<td>0.00</td>
</tr>
<tr>
<td>HFMD</td>
<td>46.21</td>
<td>0.00</td>
</tr>
<tr>
<td>HIV</td>
<td>16.84</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Why Proteoliposomes?

Proteoliposomes (PL) – detergent extracts of outer membrane of bacteria; contains PAMPs (pathogen-associated molecular patterns)

- Cell wall proteins of *M. tuberculosis* are protective

- There is great conservation in the cell wall proteins of mycobacteria
  (Tyagi & Sharma, Trends Microbiol, 10:68-8, 2002;
Mycobacterial lipids are immunogenic

- Lipids are important components of cell wall and are immunogenic and associated with protection

A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab')_2 fragment prolong survival of mice infected with Mycobacterium tuberculosis

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Diacylated Sulfoglycolipids Are Novel Mycobacterial Antigens Stimulating CD1-restricted T Cells during Infection with Mycobacterium tuberculosis

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Gamma globulins are reactive with mycobacterial lipids

IMMUNOLOGICAL ASPECTS

Prophylactic effect of administration of human gamma globulins in a mouse model of tuberculosis

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Human gamma globulins recognize BCG lipid components

Ivlg-Stained Western Blot

1. High MW ladder
2. BCG wcl sample 1
3. BCG wcl sample 2
4. Delipidated BCG wcl sample 1
5. Delipidated BCG wcl sample 2
Cell wall components of mycobacteria are immune stimulators

Cell wall components of *Neisseria meningitidis* are used in vaccine preparation

**Advantages**

- Adjuvant effect
- Stimulation of TLR
- Induction of TH1 responses
- Evaluated in infectious disease, cancer and allergic vaccines

Therefore, PL from non-pathogenic mycobacteria (*M. smegmatis* and BCG) could combine adjuvant properties with the induction of protective responses against tuberculosis.
Advantages of Proteoliposomes

- Strong immunoadjuvant properties
- Presence of conserved proteins of mycobacteria, some of them associated with latency and *in vivo* expression
- Presence of lipids, glycolipids, lipoproteins, etc associated with immunogenicity and protection
- Work with non-pathogenic and non-live bacteria
- Technology for mass production available
- Possibility to incorporate epitope constructions obtained from recombinant strains
- Potential to be used as a new candidate vaccine or as booster to BCG or other novel vaccines
PLs of *M. smegmatis* and BCG are potential vaccine candidates based on bioinformatics studies

- Proteoliposomes from *M. smegmatis* and BCG are predicted to contain B and T epitopes from Mtb expressed during the infection “in vivo”

- The epitope combination could be recognized by specific human populations (HLA prediction)
Characterization of Proteoliposomes

Cumulants Results

- Diameter: 157.2 nm
- Polydispersity Index (P.I.): 0.226
- Diffusion Const.: 3.128e-008 cm²/sec
- Molecular Weight: 3.197e+006 kDa

Measurement Condition

- Temperature: 25.0 °C
- Diluent Name: WATER
- Refractive Index: 1.3272
- Viscosity: 0.8878 cP
- Scattering Intensity: 10184 cps
Fig. 1. Characterisation of proteoliposomes. (A) Transmission electron microscopy showing the average particle size of the proteoliposomes at around 200 nm (size bar of 200 nm shown at bottom right-hand corner). (B) Size chromatography confirming the homogeneous size of the proteoliposome particles consistent with the observations made under TEM, and (C) SDS-PAGE of proteoliposomes showing a variety of proteins predominantly within the 25–67 kDa size range.
SDS-PAGE of (A) PL-Ms and (B) PL-BCG show the presence of a variety of proteins predominantly within the 25–67 kDa size range, probably comprising secreted proteins from the cell wall and outer membrane.
Characterization of Proteoliposomes

- SDS-PAGE profile shows the presence of multiple bands predominantly in the range of 25-67 kDa

- Exclusion chromatography showed a single sharp peak indicating the homogeneous size of the particles

- TEM revealed that the PL-Ms spherical particles averaged around 200 nm in diameter

- Similar results were obtained with proteoliposomes of *M. smegmatis* & BCG
Stimulation of human PBMC with PLs

Reactivity of human sera with PLs

IFN-γ production

Total IgG
Summary

- PL-Ms and PL-BCG are antigenic in humans.
- The recognition of PL-Ms and PL-BCG by specific antibodies in TB patients suggest the presence, in the PL formulations, of Mtb epitopes expressed during infection in vivo.
- PL-Ms and PL-BCG seem to be able to stimulate cell-mediated response.
Evaluation of humoral immunogenicity and cross reactivity in mice immunized with PL-Ms + adjuvant (Al - alum; M - montanide)

Evaluation of humoral immunogenicity and cross reactivity in mice immunized with PL-BCG+ adjuvant (Al - alum; M - montanide)

Antigen →

260kDa 140kDa 100kDa 70kDa 50kDa 40kDa 35kDa 25kDa 15kDa 10kDa
PLMs SCWP CW PLBCG
PL-Ms and PL-BCG are immunogenic and induce cross reactive responses against Mtb antigens which are influenced by the adjuvant used.


Total IgG response against (A) PL-Ms and (B) total lipids from Ms by BALB/c mice.

* $p < 0.01$ vs. PBS and BCG and ** $p < 0.01$ vs. all groups.
Immunogenicity studies (ELISA)

A: coating Ag – PL-Ms

B: coating Ag – total lipids from Ms

IgG1 and IgG2a response against (A) PL-MS and (B) total lipids from MS of BALB/c immunized with either PBS; BCG; PL-MS+IFA or PL-MS+Alum, respectively

(A) *p < 0.01 vs. PBS and BCG and **p < 0.01 vs. all groups.
(B) *p < 0.05 vs. PBS and BCG and **p < 0.05 vs. all groups.
Cross reactivity against BCG and Mtb antigens (SCWP and WCL) of BALB/c mice immunized with either PBS; BCG; PL-Ms+IFA or PL-Ms+Alum.

*p < 0.01 vs. PBS and BCG and **p < 0.01 vs. all groups.
Cross reactivity against WCL from Mtb. IgG1 and IgG2a response against WCL from Mtb in BALB/c mice immunized with either PBS; BCG; PLMS–IFA or PLMS–Alum.

\*p < 0.01 vs. PBS and BCG and \**p < 0.01 vs. all groups.
DTH response against Mtb WCL by mice immunized with PL-Ms

DTH response against WCL in BALB/c mice immunized with either PBS; BCG; PL-Ms+IFA or PL-Ms+Alum.

*p < 0.01 vs. PBS and **p < 0.01 vs. all groups.
Humoral immunity induced by PL-BCG.
Total IgG (A), IgG1 (B) and IgG2a (C) response against PL-BCG of Balb/c mice (n=5 per group) that received PBS, BCG, PL-BCG or PL-BCG+Al. Results are shown as mean ± SD of two independent experiments. One way ANOVA and Tukey multiple comparison tests were used to analyse the data. Different letters means statistical difference between groups (p<0.05).
Reactivity of total IgG from mice immunized with PL-BCG against MTB antigens. CWF: cell wall fraction, SCWP: soluble cell wall proteins, LAM: lipoarabinomannan, PPD: purified protein derivative. *p < 0.05 vs. NC group

Sera from mice immunized with PL-BCG reacted strongly with all MTB antigens
DTH test – against Mtb WCL

Induration (mm)

2.5
2.0
1.5
1.0
0.5
0.0

PBS
BCG
PL-BCG
PL-BCG + Alum

hours post-inoculation

24
48
72

* * *

We lead
Intratracheal challenge with H37Rv in mice

1. Immunize mice with 50µg of PL twice at 3-week interval in 100µl, sc (base of tail)
2. 2 months later: High dose (10^6 CFU of H37Rv) intratracheal challenge
3. 2 months later: Measure lung CFU & pneumonic area
**Challenge: BCG Prime, PL-Ms Boost**

* P < 0.05; ** p < 0.01 using One-Way ANOVA
Challenge: BCG Prime, PL-BCG Boost

* P < 0.05; ** p < 0.01 using One-Way ANOVA
Liposomes from *M. smegmatis*

Bacterial load in lungs of mice challenged with MTB H37Rv Two months after immunization with the liposomes.

Area of lung affected by pneumonia in mice challenged with MTB H37Rv. Two months of immunization with the liposomes.

* P < 0.05; ** p < 0.01 using One-Way ANOVA
Main Conclusions

PL-Ms and PL-BCG (& probably Lip of Ms) adjuvanted with alum

✓ seem to stimulate both humoral & cellular immune response in mice which are cross-reactive with BCG and Mtb antigens

✓ Provide better protection to Mtb challenge in a high-dose mouse model, thus may probably be useful as boosters to BCG-vaccinated individuals
Evaluation of proteoliposomes and liposomes from non pathogenic mycobacteria as vaccine candidates against *Mycobacterium tuberculosis*

(current funding: Ministry of Education, Malaysia)

**Ongoing / Planned work:**

1. Evaluation of the protection in the guinea pig model (possible collaboration with Dr. Rehm Bernd, New Zealand)
2. Challenge with Beijing and MDR strains (collaboration with Dr. Rogelio Hernandez-Pando, Mexico)
3. Assessment of therapeutic potential (collaboration with Dr. Rogelio Hernandez-Pando, Mexico)
4. Evaluation of the adjuvant capacity of the PLs and liposomes from non pathogenic mycobacteria (collaboration with Dr. Kris Huygen and Dr. Marta Romano, Belgium)
Use of human antibody libraries as platform for the discovery of candidates for new vaccines/immunotherapy/diagnostics for TB
(current funding: Ministry of Education, Malaysia)

Objective:
1. Development of antibody libraries from:
   • Resistant individuals to TB (HCW with more than 10 years of high exposure to TB; PPD+ and PPD-)
   • Susceptible individuals to TB (non-treated pulmonary and extrapulmonary TB patients)
2. Identify pattern of recognition associated with resistance/susceptibility

Expected outputs:
1. Identification of antigens/epitopes with potential for vaccine development
2. Identification of human monoclonal antibodies with potential application to immunotherapy of TB (MDR)
3. Discovering potential correlates of protection to be used in epidemiological studies and in clinical trials of vaccines
4. Identification of susceptibility signatures to be used in epidemiological studies and in clinical trials of vaccines
Potential Future Work / Collaborations

Use of human antibody libraries as platform for the discovery of candidates for new vaccines/immunotherapy/diagnostics for TB
(current funding: Ministry of Education, Malaysia)

Ongoing work:
1. Production of human antibody libraries from resistant individuals (accomplished)
2. Screening of the antibody library against relevant antigens from MTB (ongoing collaboration with Dr. Tom Ottenhoff, University of Leiden, The Netherlands)
3. Production of antibody library from TB patients (ongoing)

Expertise:
The group have the capability to:
• Screen the libraries against antigens supplied by different research groups
• Develop new human antibody libraries from samples provided by different research groups

Differential IgA variable chain gene usage between different groups - submitted

Also planning to work on TCR gene usage – construction of TCR library
Evaluation of pro-autophagic experimental vaccines against MTB (part of larger project on co-administration of BCG & pDNA)
(part of preliminary work funded by Ministry of Education, Malaysia)
(expression of Interest to TBVI, Dr. Marta Romano [PI])

Objective:
• Evaluation of the immunogenicity and protective capability of pro-autophagic experimental vaccines against MTB

Expected outputs:
1. Different vaccine candidates expressing pro-autophagic molecules with increased immunogenicity and protective capability against TB

Ongoing / Planned work:
1. Evaluation of the immunogenicity and protective capability of DNA vaccines containing pro-autophagic genes and epitopes/antigens from MTB (Drs. Kris Huygen, Marta Romano, Belgium, Gian Maria Fimia, Italy)

2. Evaluation of the immunogenicity and protective capability of *M. smegmatis* expressing pro-autophagic molecules and epitopes/antigens from MTB (ongoing) (Drs. Kris Huygen, Marta Romano, Belgium, Gian Maria Fimia, Italy)
Evaluation of vaccine/immunotherapeutic candidates in a high throughput model using zebrafish embryos

(Expression of Interest to TBVI, Dr Herman Spaink [PI])

Objective:

1. Development of a low cost, fast, high throughput model for screening of vaccine/immunotherapeutic candidates using zebrafish embryos
2. Predictive identification of vaccine/therapeutic candidates at early stage of development

Expected outputs:

Availability of a high throughput predictive system for vaccine/therapeutic candidates at early stage of development

Ongoing/Planned work:

1. High throughput screen for TB progression in zebrafish embryo (accomplished) (Prof. Herman Spaink and Annemarie Meijer, University of Leiden, The Netherlands)
2. Development of a system of evaluation of vaccine/immunotherapeutic candidates in a high throughput model using zebrafish embryos (planned) (Prof. Herman Spaink and Annemarie Meijer, Prof. Tom Ottenhoff, University of Leiden, The Netherlands, Prof. Kris Huygen and Marta Romano Belgium)
Use of Bioinformatics platform for identification of antigens/epitopes of vaccine and diagnostic interest

(previous funding: Ministry of Science, Technology & Innovation, Malaysia)

(current funding: Ministry of Education, Malaysia)

Objective:

– Development of bioinformatics algorithms for the identification of:
  • Antigens/epitopes (T and B) with suitable population coverage for vaccine/diagnostic development
  • Common antigens/epitopes (T and B) from pathogenic mycobacteria for vaccine/diagnostic development

– Identification of antigens/epitopes with vaccine/diagnostic interest

Expected outputs:

1. Identification of antigens/epitopes with potential interest for vaccine/diagnostic development

2. In silico evaluation of feasibility of prospective vaccine/diagnostic candidates
Use of Bioinformatics platform for identification of antigens/epitopes of vaccine and diagnostic interest

(previous funding: Ministry of Science, Technology & Innovation, Malaysia)
(current funding: Ministry of Education, Malaysia)

Ongoing work:

1. Evaluation of the recognition of identified antigens/epitopes by healthy individuals (non exposed and with long exposure to TB) and pulmonary TB patients (ongoing)

2. Use of identified antigens/epitopes in new experimental vaccines
   – Recombinant BCG and *M. smegmatis*.
   – *Lactococcus lactis* (Prof. Miguel Angel Alvarez Asturias, Spain and Prof. Rehm Bernd, New Zealand)
   – DNA vaccines (Kris Huygen and Marta Romano Belgium)
Acknowledgements

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2. Ministry of Education LRGS Grant [203.PSK.6722001] Malaysia,
3. Ministry of Science and Technology, Cuba.
Updates on TB Vaccine Development

THE ART AND SCIENCE OF TUBERCULOSIS VACCINE DEVELOPMENT

Preface

DZULKIFLI ABDUL RAZAK
CONCEPCION CAMPA HUERGO

tbvaccine.usm.my
Acknowledgements

This non-profit project is made possible by the great contributions from scientists and artists from around the world to address the scourge of TB. We are indeed privileged to be working with world renowned personalities in the arts and sciences, and are humbled by their dedication, generosity and patience. Their precious time and effort in ensuring the success of this project signify their readiness to contribute to a major problem of the world that primarily affects the underprivileged in underdeveloped nations. We hope their sincere contribution will make a difference to how we view and combat this disease. We are also aware that many others are willing to contribute to this project but are unable to do so due to time constraints. We hope this project will pave the way for future ‘editions’ of the book which will have additional contributions from other scientists and artists. It is also our intention to make each edition of the book freely available online.

We would like to recognize the support provided by Universiti...
Map Overlay

Oct 10, 2010 - May 17, 2011
Comparing to: Site

6,349 visits came from 486 cities
The field of tuberculosis (TB) vaccine development is a dynamic area of vaccinology. Recently, several vaccine candidates have entered clinical trials for the first time—almost 90 years since the introduction of BCG—the first live vaccine against the disease. The pathogenesis and immune response against Mycobacterium tuberculosis, the causative agent for the disease, is still not fully understood and this is reflected in the wide array of experimental strategies that are used for the development of new generation TB vaccines. Research and development on TB vaccines span from basic research up to clinical studies.

The objective of this book is to depict this diverse and, sometimes, controversial approaches in TB vaccine development. This book is not intended to provide a comprehensive review of all the efforts that are being pursued by various researchers working in this field but to give readers an insight into some of the multiple challenges of the field—covering areas in epidemiology, immunology, bioinformatics, technology platforms as well as ethical, regulatory and clinical aspects, among others.

The authors of the different chapters are renowned experts in tuberculosis vaccine development and in other relevant fields. As such, the editors have not attempted to unify their arguments and hypotheses on their respective subjects. Rather, the editors have left the personal views contained in the respective chapters to reflect the way this challenging field is evolving. In essence the “art” of scientific investigations are left to the imagination and skills of each author.

The first edition of the book—comprising 28 chapters published in 2010—received tremendous response from researchers around the globe. We are extremely encouraged that this second edition—which comprises 51 chapters—includes new and updated contributions from additional experts in the area. Like the first edition of the book, we have been very fortunate to be able to include quotes and images from eminent artists to show the human and social dimension of tuberculosis.

This updated and expanded second edition is an example of the sincere and altruistic collaboration of scientists and artists from different parts of the world to help disseminate the importance of our collective efforts in combating this scourge.

Norazmi Mohd Nor, Professor of Molecular Immunology at the School of Health Sciences, Universiti Sains Malaysia. Has been working on tuberculosis vaccine development since 2003 in collaboration with his co-editors. He started his work in this area using the recombinant BCG platform and is now exploring other potential strategies for boosting BCG, particularly aimed at latent infection. (contact: norazmi@tousm.my)

Armando Acosta and Maria Elena Sarmiento professors of Immunology and Physiology respectively, working at Finlay Institute. They have been working on tuberculosis vaccine development since 1991. They started their work in exploring the role of specific antibodies in the protection against mycobacteria. (contact: aracosta2005@yahoo.es)
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**The Editors**

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Thank you

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