UBC – Experimental Medicine
MEDI 501

Cellular Mycobacteriology

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http://www.id.med.ubc.ca/
Faculty/Faculty_Hmama.htm

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Cellular Mycobacteriology

1- Mtb features
2- TB disease
3- MDR- & XDR-TB
4- Mtb phagosome
Tuberculosis (TB)

TB is a pulmonary disease that results in ~4,500 deaths every day...most of them are vaccinated!

TB is the second leading cause of death globally from an infectious disease.
Mtb features

Mycobacterium tuberculosis (Mtb) is the causative agent of most cases of pulmonary tuberculosis (TB).

Discovered in 1882 by Robert Koch
The genome of Mtb has been sequenced in 1998 and shown to be ~ 4 million base pairs in size.

It comprises 3959 protein-coding genes.

52% of these genes can be assigned a function.

376 putative proteins are unique to Mtb!
Mtb features

The principal host cell for Mtb is the alveolar macrophage
Mtb features

Mtb is a slow growing pathogen.

It can survive inside the macrophage.

Persists as dormant bacteria in a host cell membrane-derived Vacuole or “Phagosome”.

Mtb
Pulmonary TB is an airborne disease
TB disease

Pulmonary TB is an airborne disease

A person may contract pulmonary tuberculosis from inhaling droplets from a cough or sneeze by an infected person.

Granuloma in lung tissue

[Image of a person's respiratory system and a chest X-ray with an arrow pointing to a granuloma]
Immune Response to Mtb

Minimum Infective Dose: 3-10 bacteria.

DosR
Dormancy gene program

Rpf
Resuscitation gene program

5 to 30 yr
Immune Response to Mtb

Apoptotic Vesicles Crossprime CD8 T Cells and Protect against Tuberculosis

Florian Winau, Stephan Weber, Subash Sad, Juana de Diego, Silvia Locatelli Hoops, Bernadette Breiden, Konrad Sandhoff, Volker Brinkmann, Stefan H.E. Kaufmann, Ulrich E. Schaible

Mac Ag-cross priming of DC
Immune Response to Mtb

Mouse model

Robert J. North and Yu-Jin Jung

Mtb infection in the lungs of B6 WT mice and in B6 gene-deleted mice
MDR-TB is defined as resistance to at least isoniazid and rifampicin

First line TB drugs: Standard TB treatment

Two months of ethambutol, pyrazinamide, isoniazid and rifampicin

Then 4 months of isoniazid and rifampicin alone.

MDR-TB is defined as resistance to at least isoniazid and rifampicin
Why TB treatment needs 6 month combination therapy with four different drugs?

http://en.wikipedia.org/wiki/Tuberculosis_treatment
XDR-TB is defined as resistance to any fluoroquinolone, and at least one of the aminoglycosides
Major problems for the success of antibiotherapy in developing countries:

1. Difficulties to access health services for most TB patients.

2. Non adherence of the patients to long term treatment
The worst has happened

The discovery makes India the third country in which a completely drug-resistant form of the disease has emerged, following cases documented in Italy in 2007 and Iran in 2009.

“Resistance is man-made, caused by exposure to the wrong treatment, the wrong regimen, the wrong treatment duration.”
Preventive vaccination is the best cost-effective strategy to stop the spread of TB, especially in developing countries.

M. Bovis strain by Calmette and Guerin ...1919
Preventive vaccination

BCG represents an attenuated strain of virulent *M. bovis*.

RD1 deletion happened in the lab, following many passages of *M. bovis*...from 1908 to 1919
Intracellular survival of Mtb is central to TB pathogenesis.

- **Major Feature of TB Pathogenesis**

  Mtb resist to intracellular killing and persists in a host cell membrane-derived Vacuole or “Phagosome”
The Mycobacterial Phagosome

A turning point for cellular microbiology


Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival

J. A. Armstrong and P. D’Arcy Hart

National Institute for Medical Research, Mill Hill, London, NW7 1AA, England

Macrophage with ferritin-labeled lysosomes were infected with (1) intact Mtb or (2) coated Mtb with specific antibodies.

P: phagosome, L: lysosomes, ETZ: electron transparent zone

Similar non fusion has been reported for Chlamydia and toxoplasma gondii
The Mycobacterial Phagosome

Phagolysosome fusion

- Late endosomes/Lysosomes
- Efficient Ag presentation
- MHC class II
- Mature lysosome
- Digested bacterium
- Bacterium
- Plasma membrane
- Bloodstream
- Macrophage

Efficient Ag presentation

Dept. Biol. Penn State ©2003
The Mycobacterial Phagosome

Mtb arrest phagolysosome fusion
Today, many investigators are focusing their efforts on the identification of mycobacterial factors that arrest phagosome maturation.

Mycobacterial factors that arrest phagosome maturation represent attractive drug targets.

KO of these mycobacterial factors would convert virulent Mtb into protective vaccines.
The Mycobacterial Phagosome

Work with Mtb (class III pathogen) requires BSL-3 laboratories

BCG is used as alternative surrogate pathogen for in vitro studies

Although BCG does not cause diseases in human, it mimics many feature of Mtb in vitro:

**Arrest of phagosome maturation**
Walburger A et al, Science. 2004
Hmama Z et al J Cell Sci. 2004
Vergne I et al, PNAS. 2005
Deghmane AE, J Cell Sci. 2007
Sun J, J Leuk Biol., 2007

**Inhibition of MHC class II-directed Ag presentation**
Gagliardi MC et al, Vaccine. 2004
Sendide K et al, Infect Immun. 2004
Sendide K, J Immunol. 2007
Phagolysosome fusion results from a process of phagosome remodelling (or maturation) through a series of independent events that culminates in phagosome acidification and complete fusion with lysosomes.
Mycobacterial phagosome: 2000 Model

Phagosome

Early endosomes
Rab5

Late endosomes
Rab7

Lysosomes
LAMP-1

Dead

Live bacterium

Rab5

Maturation arrest

EEA1

H^+-ATPase
pH: 4.5

Cathepsin D
pH: 6.4

pH: 4.5

Mycobacterial phagosome: 2000 Model
Hypothesis:
Mtb exports proteins and glycolipids into host cytosol to interact with and inhibit macrophage regulators of phagosome maturation
Lipoamide dehydrogenase mediates retention of coronin-1 on BCG vacuoles, leading to arrest in phagosome maturation

Diversity of host proteins secreted within the phagosome
Mycobacterial phagosome: Current Model

Cell membrane

Cor 1A

Phagosome

PLCγ1

Vps33-P

Vps33

Rab7-GTP

Rab7-GDP

Rab5-GDP

Rab5-GTP

EEA1

Rac1-GTP

Rac1-GDP

Coro 1A

Rac1

Lpd

ManLAM

PI[4,5]P2

IP3

[Ca2+]

Lysosomes

CaMKII

CaM

CaMKII-P

Vps34

p150

Snapsin

SNARE dependent membrane fusion

Lysosomes

NOX assembly/activation

Rac1

SNARE dependent membrane fusion

Lysosomes

Rac1

V-ATPase

Subunit H

PknG

Ndk

PtpA

Lpd

SapM
How Mycobacterium blocks phagosome acidification?

Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton–ATPase

S Sturgill-Kozycki, PH Schlesinger, P Chakraborty, PL Haddix, HL Collins, AK Fok, RD Allen, SL Gluck, J Heuser, DG Russell

The pH of IgG-beads (○), zymosan (△), and Leishmania phagosomes (□) rapidly decreased below 5.5 within the first 30 min after uptake. In contrast, the pH of M. avium phagosomes (○) decreased to about 6.3 before equilibrating to 6.5. The pH of the phagosome

![Graph showing pH changes over time](image)

1: IgG-beads
2: Leishmania
3: Mycobacterium

Fig. 4. Protein immunoblot analysis of isolated M. avium-, Leishmania-, and IgG-bead-containing vacuoles revealing the absence of proton-ATPase subunits from M. avium vacuoles. Gel panels were probed with (A) rat monoclonal antibody (mAb) to LAMP-1, (B) a mouse mAb to the 31-kD E subunit, (C) an affinity-purified rabbit polyclonal antibody to the 56-kD B subunit, and (D) mouse mAb to the 110-kD accessory protein.
The Mycobacterial Phagosome

How Mycobacterium blocks phagosome acidification?

V-ATPase acidifies intracellular organelles by coupling the energy of ATP hydrolysis to proton transport across the plasma membrane.

V-ATPase acts as proton pump at the phagosomal surface.

Does Mtb inactivates V-ATPase?

V-ATPase schematic
**The Mycobacterial Phagosome**

*Mycobacterium tuberculosis* protein tyrosine phosphatase (PtpA) excludes host vacuolar-H\(^{+}\)-ATPase to inhibit phagosome acidification

Dennis Wong, Horacio Bach, Jim Sun, Zakaria Hmama, and Yossef Av-Gay

Department of Medicine, Division of Infectious Diseases, University of British Columbia, Vancouver, BC, Canada V5Z 3J5

Edited by Carl F. Nathan, Weill Medical Collage of Cornell University, New York, NY, and approved October 21, 2011 (received for review June 8, 2011)

**PtpA interacts with the V-ATPase subunit H in vitro**

<table>
<thead>
<tr>
<th>Lysate</th>
<th>THP-1</th>
<th>His-PtpA</th>
<th>Subunit H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysate</td>
<td>+</td>
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</tr>
<tr>
<td>His-PtpA</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Subunit H</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Mt� PtpA inhibits phagosome acidification in the macrophages.

![Graphs showing phagosome pH](image)
**The Mycobacterial Phagosome**

*Mycobacterium tuberculosis* protein tyrosine phosphatase (PtpA) excludes host vacuolar-H^+–ATPase to inhibit phagosome acidification

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Mtb PtpA disrupts the interaction between the class C VPS and V-ATPase complexes during infection.

Loss of PtpA binding to V-ATPase subunit H impairs Mtb survival within host macrophages.
A model for the specific exclusion of V-ATPase and the inhibition of mycobacterial phagosome acidification by PtpA.
The Mycobacterial Phagosome

Early Endosomal Antigen-1 (EEA1) plays an essential role during phagosome maturation

Binds to PI3P through C-terminal domain (FYVE) and form a dimer.

N-terminal zinc finger domain binds to endosomes via RabGTPase.

Contains also a calmodulin binding domain.
The Mycobacterial Phagosome

Early Endosomal Antigen-1 (EEA1) plays an essential role during phagosome maturation

Latex beads model
The Mycobacterial Phagosome

Mtba phagosome

How Mtba excludes EEA1 from its phagosome?

EEA-1

Late Endosomes

The Mycobacterial Phagosome

Inhibition of Ca\(^{2+}\) Signaling by *Mycobacterium tuberculosis* Is Associated with Reduced Phagosome–Lysosome Fusion and Increased Survival within Human Macrophages

By Zulfiqar A. Malik,*‡ Gerene M. Denning,*§ and David J. Kusner*‡‡

Elevation of macrophage cytosolic Ca\(^{2+}\) results in increased phagosomal maturation.
The Mycobacterial Phagosome

Mycobacterium tuberculosis Phagosomes Exhibit Altered Calmodulin-Dependent Signal Transduction: Contribution to Inhibition of Phagosome-Lysosome Fusion and Intracellular Survival in Human Macrophages

Zulfiqar A. Malik, Shankar S. Iyer and David J. Kusner

J Immunol 2001; 166:3392-3401; 

Ca\textsuperscript{2+}-CAMKII Cascade

\[
\begin{align*}
\text{Ca}^{2+} & \rightarrow \text{Calmodulin} \\
\text{Ca}^{2+} & \rightarrow \text{Calmodulin} \\
\text{Ca}^{2+} & \rightarrow \text{Calmodulin} \\
\text{Ca}^{2+} & \rightarrow \text{Calmodulin} \\
\text{Ca}^{2+} & \rightarrow \text{Calmodulin} \\
\end{align*}
\]

Killed \textit{M. tuberculosis} (or live \textit{Mtb} + A23187)

- 1. Change in cytosolic Ca\textsuperscript{2+}
- 2. Translocation of CaM
- 3. Activation of CAMKII
- 4. Phagosome-Lysosome Fusion
- 5. Mycobacterial Killing

Live \textit{M. tuberculosis} (or killed \textit{Mtb} + MAPTAM)

- 1. Change in cytosolic Ca\textsuperscript{2+}
- 2. Translocation of CaM
- 3. Activation of CAMKII
- 4. Phagosome-Lysosome Fusion
- 5. Mycobacterial Killing

Dr David Kusner
Univ. Iowa
The Mycobacterial Phagosome

Tuberculosis Toxin Blocking Phagosome Maturation Inhibits a Novel Ca\(^{2+}\)/calmodulin–PI3K hVPS34 Cascade

Isabelle Vergne, Jennifer Chua, and Vojo Deretic

LAM inhibits [Ca\(^{2+}\)]\text{c} rise

Block of Ca\(^{2+}\)/calmodulin interaction inhibits phagosomal recruitment of EEA-1

W7 is a specific inhibitor of Ca\(^{2+}\)/calmodulin interaction
The Mycobacterial Phagosome

Tuberculosis Toxin Blocking Phagosome Maturation Inhibits a Novel Ca^{2+}/Calmodulin–PI3K hVPS34 Cascade

Isabelle Vergne, Jennifer Chua, and Vojo Deretic

Incubation of liposomes with macrophage cytosol (source of EEA1 & PI3K)

Incubation of calmodulin-coated beads with macrophage cytosol (source of PI3K)

PI3K and calmodulin regulate EEA1 recruitment

Interaction of PI3K with calmodulin is Ca^{2+}-dependent

Interaction of PI3K with calmodulin is Ca^{2+}-dependent
The study revealed a link between Ca\(^{2+}\) signaling, PI3K and EEA1 recruitment, and suggested that LAM interferes with EEA1-dependent phagosome maturation by blocking cytosolic Ca\(^{2+}\) rise.
The Mycobacterial Phagosome

Lipoarabinomannan (LAM) inhibits P-L fusion

LAM is a mycobacterial lipoglycan, which represents a major component of the cell wall.

A virulence factor that inactivate macrophages
The Mycobacterial Phagosome

Lipoarabinomannan (LAM) inhibits P-L fusion

Two type of LAM:

ManLAM: Mannose caped Lipoarabinomannan (pathogenic mycobacteria)

AraLAM: Arabinose caped Lipoarabinomannan (non-pathogenic mycobacteria)
Ex: M. smegmatis
PI3P is retained on phagosomes harboring dead mycobacteria but is eliminated from phagosomes with live bacilli.

Live bacteria secretes a lipid phosphatase, **SapM**, that hydrolyzes PI3P,
The Mycobacterial Phagosome

Mechanism of phagosomal exclusion of EEA-1 by Mtb

Phagocytosis

$[Ca^{2+}]$↑

CaM → Ca$^{2+}$-CaM → CaMKII → P-CaMKII

Vps34/p150

PI → PI[3]P → EEA1

LAM + SapM

Late Endosomes

EEA1 = EEA1

SapM

Rab5
Rab GTPases and mycobacterial phagosome

Rab5 and Rab7 GTPases contribute to vesicular trafficking.

They have a direct impact on phagosome maturation.
Rab5 and Rab7 GTPases contribute to vesicular trafficking. They have a direct impact on phagosome maturation.

**Rab GTPases recruitment and activation**

**Prenylation:** Rab \(\xrightarrow{\text{RGGTase}}\) Rab targeted to membrane of endosome

**Activation:** Rab-GDP \(\xrightarrow{\text{GEF}}\) Rab-GTP

- Rab5-GTP interacts with EEA1
- Rab7-GTP interacts with RILP

**Inactivation:** Rab-GTP \(\xrightarrow{\text{GAP}}\) Rab-GDP

**Recycling:** Rab-GDP \(\xrightarrow{\text{GDI}}\) Rab-GDI (GDP) (Cannot dock to membrane with GDI bound form)

Rab-GDI (GDP) \(\xrightarrow{\text{GDF}}\) Rab-GDP
The Mycobacterial Phagosome

Nucleoside diphosphate kinase of *Mycobacterium tuberculosis* acts as GTPase-activating protein for Rho-GTPases

Puneet Chopra\textsuperscript{a,b}, Harshavardhan Koduri\textsuperscript{a}, Ramandeep Singh\textsuperscript{b}, Anil Kou\textsuperscript{a,1}, Megha Ghildiyal\textsuperscript{a,c}, Kirti Sharma\textsuperscript{a}, Anil K. Tyagi\textsuperscript{b}, Yogendra Singh\textsuperscript{a,*}

\textsuperscript{a}Institute of Genomics and Integrative Biology, Mall Road, Delhi, India  
\textsuperscript{b}Department of Biochemistry, University of Delhi, South Campus, New Delhi, India  
\textsuperscript{c}Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India

NDK inactivates Rho GTPases?
The Mycobacterial Phagosome

Mycobacterial NDK expresses GAP activities in vitro

Does NDK GAP activity extend to Rab GTPases?

Does NDK contribute to the pathogenesis of mycobacterium?
The Mycobacterial Phagosome

Mycobacterial Nucleoside Diphosphate Kinase Blocks Phagosome Maturation in Murine Raw 264.7 Macrophages

Jim Sun¹, Xuetao Wang¹, Alice Lau¹, Ting-Yu Angela Liao¹, Cecilia Bucci², Zakaria Hmama¹*

January 2010 | Volume 5 | Issue 1 | e8769

Ndk binds to Rab5,

and catalyzes the switch GTP/GDP

Ndk-coated beads exclude EEA1 from their phagosomes

Jim Sun
The Mycobacterial Phagosome

Mycobacterial Nucleoside Diphosphate Kinase Blocks Phagosome Maturation in Murine Raw 264.7 Macrophages

Jim Sun¹, Xuetao Wang¹, Alice Lau¹, Ting-Yu Angela Liao¹, Cecilia Bucci², Zakaria Hmama¹*

January 2010 | Volume 5 | Issue 1 | e879

Ndk knock is associated with a phenotype of increased P-L fusion and reduced intracellular survival

![Bright Field and BCG/Dextran images](image)

![Bar chart showing CFU (Log10) for WT, S-Ndk, and AS-Ndk](chart)

BCG

LpdC

Ndk

WT S-Ndk AS-Ndk

55 kDa

17 kDa

0 h 24 h 48 h

*
The Mycobacterial Phagosome

Mechanism of phagosomal exclusion of EEA-1 by Mtb

Phagocytosis

\[ \text{Vps34/p150} \rightarrow \text{PI[3]P} \rightarrow \text{EEA1} \rightarrow \text{SapM} \rightarrow \text{Rab5-GTP} \rightarrow \text{Rab5-GDP} \rightarrow \text{EEA1} \]

\[ \text{[Ca}^{2+}] \uparrow \rightarrow \text{LAM} + \text{SapM} + \text{NDK} \]

Late Endosomes

\[ \text{LAM} \]

Image: Pathway illustration showing the interaction between phagocytosis and the Mycobacterial phagosome.
GAP in other organisms

Several pathogenic bacteria secrete proteins that act as GAP for members of Rho-GTPases, and eventually facilitate their pathogenesis:

Secreted cytotoxin, ExoS of Pseudomonas aeruginosa, disrupts the actin cytoskeleton

Yersinia pseudotuberculosis secretes a cytotoxic factor, YopE, which depolymerizes the actin stress fiber