Overview of Models of CNS TB and TB Meningitis

Tuberculosis Meningitis: Advancing Immunopathogenesis, Diagnosis, and Treatment
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Sanjay K. Jain, MD
Director, Center for Infection and Inflammation Imaging Research
Associate Professor of Pediatrics & International Health
Department of Pediatrics & Center for TB Research
Johns Hopkins University
High rates of TB meningitis in mostly HIV-negative young children in India

- 223 children ≤5 years (median age 31 months), with suspected TB were enrolled prospectively at BJGMC (Pune, India)
- 86% had received BCG, 57% were malnourished (WAZ ≤ 2 SD), and 10% were HIV-positive
- 12% (n = 26) had active TB (definite or probable) and extra-pulmonary disease was noted in 46% (n = 12), which was predominantly meningeal (75%; n = 9)
- 57% (4 of 7) of children with culture-confirmed TB, harbored drug-resistant (DR) strains of which 2 (50%) were multi-DR (MDR)
- Whole genome sequencing was performed on isolates from children with definite TB. *M. tuberculosis* strains causing TB meningitis clustered together, suggesting that *M. tuberculosis* possess virulence factors that promote the development of CNS disease.

Shaikh et al. Int J Tuberc Lung Dis. 2017
Acute diffuse tuberculous meningitis is characterized especially by its exudative inflammatory nature, and by the tendency to widespread necrosis of the inflammatory exudate and of contiguous meningeal tissues. Even in cases in which the duration has been sufficiently long to permit the appearance of a proliferative reaction leading to the formation of true tubercles or of a compact tissue composed of epithelioid cells and fibroblasts, one usually finds the remains of an earlier exudative-necrotic process. Since extensive exudative inflammatory reactions with prominent necrosis in tuberculosis are well known to be expressions of the allergic state, and since tuberculous meningitis develops only in persons carrying an older sensitizing infection somewhere...
Current Understanding of CNS Invasion by *Mycobacterium tuberculosis*

Tuberculomas or “Rich foci”

CEREBROSPINAL FLUID

MENINGES

BRAIN PARENCHYMA

Rupture of “Rich foci” leading to diffuse inflammatory meningitis

Animal models of CNS TB and TB meningitis

• Several animal models have employed direct intracisternal or intracerebral infection:
  – Tsenova et al. (rabbit)
  – van Well et al. (mouse)
  – Tucker et al. (baby rabbit)

• Useful for studying pathogenesis and antibiotic or host-directed treatments after established disease.

• Other models have utilized intravenous or aerosol infections to study the initial stage of invasion from the lung / bloodstream to the CNS:
  – Rich et al. (guinea pig, rabbit)
  – Be et al. (mouse, guinea pig – intravenous)
  – Skerry et al. (guinea pig – aerosol)
  – Zebrafish model

• Useful for studying early pathogenesis and studying microbial factors needed for translocation to the brain and for developing preventive strategies.

Bacterial traversal across the Blood-Brain Barrier (BBB)?

- *M. tuberculosis* can initiate CNS TB by crossing the BBB as free (extracellular) organisms or via infected leukocytes.

- Leukocyte trafficking could be restricted across the BBB, prior to the onset of TB meningitis.

- Data from Rich et al, and later confirmed by MacGregor et al, demonstrate that *free bacteria* can invade the CNS.

- Data utilizing CD18-/- (leukocyte adhesion deficient) mice suggest that free mycobacteria can traverse the BBB independent of leukocytes or macrophages.

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Microbial factors may be associated with TB meningitis

- Multiple reports have shown the association of distinct *M. tuberculosis* strains with extra-pulmonary and / or CNS dissemination
- Compartmentalization of strains in distinct physiological sites
- Association of certain lineages with dissemination and meningeal disease

Whole genome sequencing performed on 6 of the 7 isolates using the Genome Analyzer IIx (Illumina, USA). *M. tuberculosis* strains causing TB meningitis cluster together.

**Bacterial traversal across the Blood-Brain Barrier (BBB)?**

- *M. tuberculosis* can initiate CNS TB by crossing the BBB as free (extracellular) organisms or via infected leukocytes.

- Leukocyte trafficking is restricted across the BBB, prior to the onset of TB meningitis.

- Data from Rich et al, and later confirmed by MacGregor et al, demonstrate that free bacteria can invade the CNS.

- A study utilizing CD18-/- (leukocyte adhesion deficient) mice suggest that free mycobacteria can traverse the BBB independent of leukocytes or macrophages.

- Zebra fish model studies have shown traversal of bacteria within leukocytes.

References:

Intracranial injection of BCG in rats resulted in a rapid response which persisted for approximately two weeks, but disappeared by four weeks.

However, at 4 weeks, staining demonstrated the presence of BCG at the site of the original intracranial injection, suggesting that the inflammatory response had not cleared the bacteria completely.

No T-cell proliferation responses (to PPD) were noted from splenic or lymph nodes of animals injected with BCG intracranially.

Animals that were subsequently peripherally sensitized (subcutaneous injection of BCG) developed a strong delayed-type hypersensitivity response with extensive inflammatory lesions at the site of BCG injection in the CNS.

Delayed-type hypersensitivity responses could be detected for several months in the CNS in animals that underwent peripheral sensitization.

Invasion by *M. tuberculosis* may be an Active Process

- *M. tuberculosis* invade and traverse an *in vitro* blood-brain barrier
- Non-pathogenic mycobacteria do not stimulate internalization
- Bacterial internalization is partially dependent on actin polymerization

Are there specific *microbial* factors which promote invasion / survival in the host CNS?

**Aim:** To identify *M. tuberculosis* genes involved in CNS TB using a screen in an animal model

Nick Be
Mutant 1 is attenuated in the CNS
Several Mutants Identified as Attenuated Specifically in Guinea Pig CNS

- Selected mutants which were:
  - Significantly attenuated in the CNS, but **NOT** in the lungs

<table>
<thead>
<tr>
<th>Rv#</th>
<th>MT#</th>
<th>ORF Description</th>
<th>Fold Attenuation in Brain Relative to Lung</th>
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<td>MT0752 Possible Aldolase</td>
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<td>MT2456 Conserved Hypothetical Protein</td>
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<td>MT3178 Conserved Hypothetical Protein</td>
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<td>MT3247 PPE Family Protein</td>
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<td>MT3321 Iron-Regulated Dehydrogenase/Reductase</td>
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<td>MT3461 Conserved Hypothetical Protein</td>
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Also found in mouse model screen

PknD deficient *M. tuberculosis* Mutant is Attenuated for CNS Survival in the Mouse

![Bar chart showing Log_{10} CFU for CDC1551 and pknD Tn in various tissues and time points.](attachment:image.png)

PknD deficient *M. tuberculosis* Mutant has defective invasion into brain endothelia

- No difference in invasion was observed in HUVEC or A549
- Significant reduction in invasive capacity, observed in HBMEC (*p*=0.02)
- Defect was restored by genetic complementation with the native *pknD* gene
PknD deficient *M. tuberculosis* Mutant is NOT Attenuated in Macrophages
PknD sensor domain: A role in host-pathogen interaction?

- The PknD C-terminal sensor domain forms a symmetric β-propeller

- Cup of β-propeller exhibits polar and non-polar patches, contains residues unique to individual blades of the propeller
  - Indicates a functional binding surface

- Similar structures found in multiple proteins in other organisms which mediate adhesion

- **Could the pknD sensor domain assist in bacterial adherence to host cells in the CNS?**

PknD sensor domain localizes to the cell wall fraction *M. tuberculosis*
PknD sensor domain is sufficient to trigger association with brain endothelial cells

BSA-coated beads

pknD-coated beads

Red: coated beads
Green: actin

Mean Fluorescence (RFU)

p = 0.0001
PknD sensor domain is sufficient to trigger association with brain endothelial cells

Red: coated beads
Green: actin
PknD sensor domain is sufficient to trigger association with brain endothelial cells

Red: coated beads
Green: actin
PknD sensor domain is sufficient to trigger association with brain endothelial cells
PknD deficient *M. tuberculosis* Mutant exhibits reduced binding to laminin

Incubation of PknD sensor with brain endothelial lysates and immobilization on affinity column demonstrated interaction with laminin
Antibody specific for PknD reduced invasion of brain endothelia by *M. tuberculosis*
TB meningitis develops subsequent to hematogenous dissemination of bacteria.

The surface exposed PknD sensor is required for invading the BBB that protects the CNS from the systemic circulation.

We therefore hypothesized that antibody-mediated humoral immunity against PknD could be utilized as a strategy to protect against CNS TB in the guinea pig model.
BCG and TB

• BCG is the only licensed vaccine against TB and recommended (WHO policy) at birth to newborns in high TB burden countries.

• Protection by BCG vaccination is highly variable (0-80%), and its use is mainly guided by its ability to prevent TB meningitis and miliary TB in young children by decreasing the bacterial burden in lung tissues.

• Nonetheless, protection by BCG vaccination against TB meningitis and military TB is also inconsistent and only ~50%.

Colditz, G.A. et al. JAMA. 1994
Hypothesis: Vaccination with the *M. tuberculosis* PknD extra-cellular subunit protein will provide protection against CNS TB.

Aim: To use the guinea pig aerosol infection model to test the efficacy of the *M. tuberculosis* PknD sensor (extra-cellular subunit protein) against CNS TB and compare it with BCG.

Work led by Ciaran Skerry
PknD sensor as a Vaccine-target to prevent CNS TB: Methodology in the Guinea Pig model

Group

Untreated

BCG

PknD

Adjuvant

BCG Danish strain 1331 s.c 5x10^4 CFU

PknD 20ug+20ug DDA s.c

M. tuberculosis CDC1551 challenge via aerosol (day 1: ~3 log_{10})

Harvest organs

4 animals per group per time-point;
TCH plates also used for BCG infected animals

W-10 W-7 W-4 W0 W4 W6

3wks 3wks 4wks 4wks 2wks

4 animals per group per time-point; TCH plates also used for BCG infected animals
Vaccination with BCG limits *M. tuberculosis* burden at site of primary infection (Lungs)

Results are shown as mean CFU per lung (±SD). Animals vaccinated with BCG show significantly lower CFU than those treated with pknD or controls. (p = 0.01)

Vaccination with *M. tuberculosis* PknD protects against CNS TB

Results are shown as mean CFU per brain (±SD). The brains from PknD- and BCG-treated animals show significantly lower bacterial burden than those treated with PBS or adjuvant control. (p = 0.01)
Guinea pig survival

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<tr>
<th>Group</th>
<th>Deaths</th>
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<td>PBS</td>
<td>5/7 (71%)</td>
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<tr>
<td>DDA</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>BCG</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>PknD</td>
<td>1/5 (20%)</td>
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</table>
Lung pathology (computed tomography) in BCG, PknD and PBS vaccinated animals 6 weeks after aerosol challenge

Integrated over the whole lung (every slice), so more accurate than histopathology.
IgG levels in vaccinated guinea pigs

*M. tb.*-specific (bacterial lysates)

M. tb. PknD-specific

A

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
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<td>IgG</td>
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B

<table>
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<td>IgG</td>
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<td><strong>1.6</strong></td>
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Pre-incubation of *M. tuberculosis* with sera from PknD-vaccinated guinea pigs reduces invasion of brain endothelia

![Bar chart showing invasion of brain endothelia (% of control) for PBS, DDA, BCG, and pknD. The graph indicates significant differences with P-values of 0.003 and 0.002 for PBS and BCG, respectively.]

Summary

• Pathogenesis of TB meningitis remains poorly understood and this field of research remains under-studied.
• Animal models utilizing direct (intracisternal / intracerebral) or intravenous challenge have been described and could be useful to study the pathogenesis of TB meningitis as well as development of novel therapeutics – antibiotic, host-directed treatments and vaccines.
• *M. tuberculosis* PknD may be a key microbial factor with a role in CNS invasion.
• Vaccination with the *M. tuberculosis* PknD sensor domain can offer protection against CNS TB in the guinea pig model.
• Has a potentially a unique mechanism of protection (serological):
  – BCG does not make the extracellular portion of PknD
• Vaccination strategies against *M. tuberculosis* PknD could also protect against other forms of extra-pulmonary TB.
• Study limitations
  – Did not test protection in other extra-pulmonary sites
  – Protection could also be by mediated by additional mechanisms which were not fully evaluated
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