Use of Nonhuman Primates in TB vaccine research

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Mtb exposure leads to active or latent TB

Active Pulmonary TB

Reactivation TB (5-10% lifetime)

Latent Infection (90-95%)

Primary Active Disease (5-10%)

Asymptomatic

Mtb, SEM, CDC., 2008

Immunosuppression
Age

??
Mtbi exposure leads to active or latent TB

- Active Pulmonary TB
- Latent Infection (90-95%)
- Primary Active Disease (5-10% lifetime)
- Reconstitution TB (5-10% lifetime)

Mtbi, SEM, CDC., 2008

To 10% per year!!

Immunosuppression

Age ??

Asymptomatic
Tuberculosis is a global health crisis

WHO, Global Tuberculosis Report, 2017
Tuberculosis is the most common cause of morbidity and mortality in HIV+ individuals

Estimated HIV prevalence in new and relapse TB cases, 2016

WHO, Global Tuberculosis Report, 2017
BCG: Bacille Calmette Guerin

• First TB vaccine

• A live attenuated strain of Mycobacterium bovis that was cultured every 14 days for 230 passages (nearly 9 years!)

• First human test was in a neonate who lived in a house with a TB patient

• Vaccinated 20,000 neonates between 1921 and 1924

• Protects newborns from life threatening extrapulmonary TB (miliary TB) and TB meningitis
Neonates are still being vaccinated with BCG in high burden TB settings

WHO, Global Tuberculosis Report, 2017
Why is BCG insufficient as a vaccine?

• Limited and variable effect on reducing pulmonary TB in adults

• BCG is a live vaccine and can cause a systemic infection in immunocompromised individuals

• HIV+ infants are at an increased risk of developing disseminated BCG, although they should still be vaccinated (WHO, 2018)
TB Vaccine Pipeline 2017

13 Candidates
5/4/3/1

Phase 1
- MTBVAC
  Biofabri, TBVI, Zaragosa
- Ad5 Ag85A
  McMaster, CanSino
- ChAdOx1.85A/MVA85A
  Oxford, Birmingham
- MVA85A/MVA85A(ID, Aerosol)
  Oxford
- TB/FLU-04L
  RIBSP

Phase 2a
- RUTI
  Archivel Farma, S.L
- H1/H56: IC31
  SSI, Valneva, Aeras
- H4: IC31
  Sanofi Pasteur, SSI, Aeras
- ID93 + GLA-SE
  IDRI, Wellcome Trust

Phase 2b
- DAR-901
  Dartmouth
- VPM 1002
  SII, Max Planck, VPM, TBVI
- M72 + AS01E
  GSK, Aeras

Phase 3
- Vaccae™
  Anhui Zhifei Longcom

Voss et. al., F1000 Research, 2018

AERAS | Advancing Tuberculosis Vaccines for the World

Revised on February 2, 2017
Please note: Information is self-reported by vaccine sponsors
What are some animal models used to test TB vaccines?
Advantages:
1. Genetically defined
2. Rapid evaluation of interventions
3. Small size and low cost
4. Availability of immunological tools

Disadvantages:
1. TB granulomas and disease are different than humans
2. No disseminated disease
Guinea Pig

Advantages:
1. Susceptible to TB
2. Granulomas are more ’human-like’
3. Bigger than mice
4. Can still be used for vaccine efficacy and drug development

Disadvantages:
1. Lack of good immune reagents
2. No latent TB
3. More expensive than mice
Rabbit

Advantages:
1. Very susceptible to TB
2. Form ‘human-like’ necrotic granulomas
3. Can develop latent TB, depending on infecting strain
4. Can still be used for vaccine efficacy and drug development
5. Model for TB Meningitis

Disadvantages:
1. Lack of good immune reagents
2. More expensive than other small models
Nonhuman primates (NHPs)

Advantages:
1. Susceptible to TB
2. Form a spectrum of granulomas similar to humans
3. Can still be used to test interventions
4. Longitudinal tracking
5. Immune reagents are available
6. *Gold standard*

Disadvantages:
1. VERY expensive
2. Genetically outbred
Is TB disease in NHPs similar to humans?
Humans and NHPs develop similar granulomas

**Human**

- Caseous/necrotic

**Cynomolgus macaque**

- Caseous/necrotic
- Nonnecrotic
- Mineralized

*Lawn & Zumla, Lancet, 2011; Ulrichs et. al., J Path, 2006; Lin et. al., I and I, 2009*
Ways to measure TB disease in NHPs

- Clinical markers: ESR, BAL culture, Gastric aspirates, Cough
- Serial PET/CT imaging
- Measuring bacterial growth at necropsy
University of Pittsburgh
Regional Biocontainment Laboratory
BSL-3 PET/CT Imaging Suite

• Siemens MicroPET Focus 220
• Neurologica CereTom CT scanner
• Integrated animal handling system
• eVent Inspiration LS critical care ventilator (not shown)
• Spectra AG5 vital signs monitor (not shown)
• Bear Hugger warming device (not shown)
• Isoflurane anesthesia machine (not shown)
$^{18}$F-Fluorodeoxyglucose (FDG) PET/CT analyses to track lesions
Pre-necropsy PET/CT provides map of lesions (lesion size and SUV)

Image-guided necropsy yields individual lesions

CFU

[Rx] and/or imaging mass spectrometry

Histopathology

Immunology

Gene expression

Spectrum of TB progression in NHPs by PET/CT

Pre-necropsy images are taken at 5-6 months or at humane endpoint.*

Chinese cynomolgus macaques

rhesus macaques

Mauritian cynomolgus macaques

Good

“Typical”

Bad

*Good, Bad, “Typical”

CCM MCM Rhesus

TB progression varies widely between animals

All pre-Nx images obtained at 5-6 mo. p.i. unless humane endpoint reached (*)
Individual granulomas can be tracked (CONTROL)

- New Site
- Worse
- Improvement
- Stable

8 weeks
12 weeks
16 weeks
20 weeks
Individual granulomas can be tracked (NO control)

- New Site
- Worse
- Improvement
- Stable
- Initial Site
Bacterial Colony Forming Units (CFUs) decline as adaptive immunity develops.

Lin et. al., Nat Med, 2014
TB disease is not the same in all NHP populations
FDG avidity increases during infection and is different across NHP populations

Chinese cynomolgus macaques

Rhesus macaques

Mauritian cynomolgus macaques
Bacterial CFU at necropsy is higher in different NHP populations

Each symbol = one animal

Maiello et al., Infect and Imm, 2017
TB vaccines can be tested in NHPs
The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection

Philana Ling Lin,¹ Jes Dietrich,² Esterlina Tan,³ Rodolfo M. Abalos,³ Jasmin Burgos,³ Carolyn Bigbee,⁴ Matthew Bigbee,⁴ Leslie Milk,⁴ Hannah P. Gideon,⁴ Mark Rodgers,⁴ Catherine Cochran,⁴ Kristi M. Guinn,⁵ David R. Sherman,⁵ Edwin Klein,⁶ Christopher Janssen,⁶ JoAnne L. Flynn,⁴,⁷ and Peter Andersen²
Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine

Scott G Hansen¹,⁸, Daniel E Zak²,⁸, Guangwu Xu¹,⁸, Julia C Ford¹, Emily E Marshall¹, Daniel Malouli¹, Roxanne M Gilbridge¹, Colette M Hughes¹, Abigail B Ventura¹, Emily Ainslie¹, Kurt T Randall¹, Andrea N Selseth¹, Parker Rundstrom¹, Lauren Herlache¹, Matthew S Lewis¹, Haesun Park¹, Shannon L Planer¹, John M Turner¹, Miranda Fischer¹, Christina Armstrong¹, Robert C Zweig¹, Joseph Valvo², Jackie M Braun², Smitha Shankar², Lenette Lu³, Andrew W Sylwester¹, Alfred W Legasse¹, Martin Messerle⁴, Michael A Jarvis⁵, Lynn M Amon², Alan Aderem², Galit Alter³, Dominick J Laddy⁶, Michele Stone⁶, Aurelio Bonavia⁶, Thomas G Evans⁶, Michael K Axthelm¹, Klaus Früh¹, Paul T Edlefsen⁷ & Louis J Picker¹

Nature Medicine, 2018
But, we really need a TB vaccine for HIV+ individuals!
NHP co-infection models:

1. **Mtb** infection first; **SIV** infection second
   - Rhesus macaque – Latent Mtb CDC1551 followed by SIVmac239 *(Foreman et. al., PNAS, 2016)*

2. **SIV** infection first; **Mtb** infection second
Hypothesis: SIV infection disrupts the development of immune responses to an Mtb infection, which leads to rapid TB progression.

- Serial PET/CT imaging
- Defining T cell populations
- Characterize T cell function in PBMC and granulomas

Infect with ~5 CFU Mtb x 8

Infect with SIV, wait 6 months x 7

Infect with ~5 CFU Mtb
Mtb infection
ONLY

SIV (6 months) followed by Mtb
Mtb infection
ONLY

SIV (6 months)
followed by Mtb
Longitudinal PET/CT scanning does not differentiate between groups

- **Mtb only**
- **SIV/Mtb**
Granuloma counts reveal rapid dissemination in SIV/Mtb animals

- Mtb only
- SIV/Mtb
Granuloma counts reveal rapid dissemination in SIV/Mtb animals

4 weeks

8 weeks

Mtb Only

SIV + MtB

# Granulomas

p=0.6668

Mtb Only

SIV + MtB

# Granulomas

p=0.0491

Mtb only

SIV/Mtb
Balance between too much and too little of an immune response!
Conventional T cell responses to Mtb

SIV infection is known to lead to hyperactivation
Central memory CD4 and CD8 T cells have higher PD-1 expression in co-infected animals

![Graph showing PD-1 expression in CD4 and CD8 T cells]

- Percent CD4 CM T cells that are PD-1+: SIV/Mtb vs. Mtb only, p=0.0002
- Percent CD8 CM T cells that are PD-1+: SIV/Mtb vs. Mtb only, p=0.0004
Final Thoughts

- TB disease can be modeled in macaques infected with a low dose of *M. tuberculosis*.

- Macaques develop a spectrum of TB disease that is similar to what is observed in humans.

- SIV co-infection exacerbates TB disease.

- Future studies in SIV+ and SIV-naïve macaques can be used as a platform for testing TB vaccines.
Thank you!

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