Human immunogenetic risk factors for *M. tuberculosis* infection and TB disease

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Outline

1. Genetics
   Innate Immunogenetics & TB

2. Genomics
   Resistance to Mtb Infection
   A. Clinical epidemiology of resisters
   B. Monocyte transcriptional profiles
   C. HDAC & Mtb
   D. HDAC & HDT

3. Future: Host Directed Therapy Vaccines
Genetics: Why do Mtb and BCG affect individuals differently?

Pathogen
Virulence factors

Host
External Factors:
Exposure
Immunosuppression

Host
Genetics

Aims
1. Identify Variation
2. Pathogenesis
3. New Rx

Why are Major TB Susceptibility Genes Unknown?
Genetics & TB

I. Twin Studies

  # Tb cases/100 co-twins
  Monozygotic     31.4
  Dizygotic       14.9


II. Mendelian & BCG

  IRF8  Hambleton, Gros NEJM 2011

III. Genome-wide Linkage Studies

  11p14, 5p15 South Africa: Cobat JEM 2009 (LTBI)
  2q21, 5p13, 7p22 Uganda: Stein Plos1 2008 (LTBI & ATBI)

IV. Association Studies

  A. GWAS 18q11.2 Thye et al Nat Gen 2010; 11p3 Thye Nat Gen 2012
     Curtis et al ASAP1 Nat Gen 2015; HLA Class II Sveinbjornsson Nat Genetics 2016
  B. Candidate Gene
Pathogenesis

Lung 1° Infection
Alveolar Mφ
Spread to LN
Bacillemia

80% Reactivate

Lung Apex
“Rich Foci”
Meninges & Brain

10% Latent

Disseminated
Miliary

10% Pediatric
Primary
Progressive Dz

Lymph Node
Bone (Pott’s Disease)
Genitourinary

BCG

Droplet Inhalation
No Infection

TLR Immunogenetics & TB

April Randhawa

TLR1/6 Deficiency
↓ TLR2-mediated IL6

TOLLIP Deficiency

Javeed Shah

Promotes TH1 Response to BCG

↑ IL2
↑ IFNγ

↓ TCM to BCG
↓ CD4+IL2+

↑ Mtb Infection
↑ TB Disease

Randhawa et al  PloS Pathogens (2011)

Shah et al  AJRCCM (2017)
2. IDENTIFY HOST-DIRECTED THERAPY THAT REPROGRAMS MACROPHAGES TO BE RESISTANT

1. IDENTIFY GENETICALLY ENCODED MACROPHAGE RESISTANCE PATHWAYS

- INNATE VARIANTS
- Cytokines & Chemokines
- INFLAMMATION
- T
- Mφ or DC
- TLR
- Mtb
- MICROBIAL KILLING

ADAPTIVE
Hypothesis: Control of Mtb Infection is Regulated by Variation in the Macrophage Innate Immune Response

Chetan Seshadri, Monica Campo at UW
Cathy Stein, Henry Boom at CWRU
Harriett Mayanja-Kizza, Ezekiel Mupere at Makerere Univ

Kampala, Uganda
Household Contact Study

TB Index Cases = 975
Cult+, >18yo, HIV+/HIV-
2002-2012

Contacts (HHC) = 2585

Follow for 2 yrs:
Evaluate exposure hx, HIV, TST/IGRA, TB Dz

Among All contacts: ~9 RSTRs

2015 Recontact for 3 visits: QFT, QFT, QFT/TST
Is resistance to Mtb infection genetically regulated?

1. Genome-wide linkage scan: regions linked to RSTR phenotype: chr 2 and 5

Cathy Stein, CWRU

2. Macrophage transcriptional signature
Transcriptome Project

Experimental Design

<table>
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<tr>
<th>RSTR</th>
<th>LTBI</th>
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<td>18</td>
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Archived PBMC → CD14+ isolation → Monocytes → Infect live H37Rv (MOI 10:1) 6 hrs → RNA Extraction → QC → Microarray Analysis

Phenotypes

A. Resistant to infection (RSTR) (persistent TST negative x 2 yrs)
B. Susceptible to infection (LTBI)

Illumina Bead Array HT-12

- 48,000+ probes covering > 25,000 genes
- At least two probes per gene
- 20-90 beads per probe
- 100,000+ probes per bead
- Dynamic Range: 0 – $2^{16}$
Analyses

1. Single Gene & Clustering Analysis
   No significant difference between RSTR and LTBI
   However...

2. Network Analysis
   GSEA
Gene Set Enrichment Analysis: Pathways with Possible Drug Targets

RSTR: 27 gene sets significant at FDR < 0.20
  – Major themes
    • Macrophage function & Signaling
  – Top hit is sodium butyrate
  – 5\text{th} hit is HDAC gene set

LTBI: 25 gene sets significant at FDR < 0.20
  2 HDAC gene sets
Histone Deacetylases (HDACs)

**HDACi increase transcription:**
induce apoptosis, differentiation, immunologic program & more

**Mechanism:**
A. Histone modification  
B. Post-translational modification of non-histone proteins

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**HDACi Phenylbutyrate & TB**
Inhibits Mtb growth in broth culture  
Inhibits Mtb growth in MDMs  
Induces change in inflammatory gene program in macrophages  
Phase 2 trial in Bangladesh for Rx of pulmonary TB

Coussens et al PlosPath 2015, Mily et al PlosOne 2015
Questions
Which HDAC?
Improve potency?
Host or Mtb Target?
What Mechanism?
HDAC1 genetic variants are associated with resistance to Mtb infection in Uganda

Cathy Stein

<table>
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<tr>
<th>HDAC1 SNPs associated with RSTR phenotype (n=466)</th>
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<tr>
<td>SNP name</td>
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<tr>
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<tr>
<td>rs16834963</td>
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Histone Deacetylases (HDACs)

Family of 11 genes
Class I: HDACs 1,2,3,8
Class IIa: HDAC 4,5,7,9
Class IIb: HDAC 6,10
Class IV: HDAC11

Class I & 2a inhibitor (HDACS1-5,7-9)
Phenylbutyrate

HDAC3i
RGFP966
HDAC3i controls Mtb replication in MDMs, AMs, & Broth Cx Alone

Luminescent Mtb Erdman strain from J.Cox
HDACi Mechanism & Mtb

1. Phagocytosis & phagosome maturation/function
   - TLR, CLR
   - Phagolysosome
   - mRNA
   - HDAC
   - Promoter
   - gDNA

2. Signaling
   - Cytokines

3. Anti-Microbial Mechanisms
   - Mtb Killed
   - PBA
   - RGFP966

4. Mtb Replication
PBA, but not HDAC3i, differentially regulates proinflammatory cytokines in Mtb-infected macrophages

**IL6**

**IL1β**

**RGFP966**

**RGFP966**
HDAC3i + VitD3 Induces CAMP expression in MCSF derived MDMs
HDACi Mechanism & Mtb

1. Phagocytosis & phagosome maturation/function

2. Signaling

   - PBA \(\downarrow\) IL-6 \(\uparrow\) IL-1\(\beta\)
   - RGFP966: no effect

3. Anti-Microbial Mechanisms
   - \(\uparrow\) CAMP

4. Mtb Replication
   - \(\downarrow\) replication
Summary: RSTR vs LTBI

1. Epidemiologic variables do not distinguish RSTR from LTBI
2. Monocyte mRNA profiles with GSEA
   Several pathways specific to RSTR
   Top hit: Butyrate/HDAC
3. Multiple candidate HDACi with different mechanisms
   PBA & HDAC3i inhibits Mtb growth with ↑CAMP
   Dual effect on host and Mtb

Next steps:
Killing Mechanism
Macrophage studies with CRISPR/Cas9, HDACi
Macrophage studies with RSTRs & HDACi
HDAC genetics
Innate Immune Variation & Disease

- **TLR VARIANTS**
  - TLR1, 5, 6
  - TIRAP, TOLLIP

- **Cytokines & Chemokines**

- **INFLAMMATION**
  - TLR1/6 & TOLLIP & BCG Immunity
  - TOLLIP & TB Disease

- **Mφ or DC**
  - IL10/IL12

- **T**

- **TB or BCG**
  - TLR1/2/6

- **TB Clinical Phenotypes & mRNA profiling**

- **RSTR vs LTBI**
  - HDACs

- **MTB INFECTION → ? HDT Rx STRATEGIES**
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How Might Humans Resist Mtb Infection?

1. Classical: Class I or II restricted Heterologous Immunity (BCG, NTMs)
2. “Innate T-cells” (CD1, MR1, \(\gamma\delta\), HLA-E)

INNATE

1. Uptake
2. Phagosome & Intracellular Niche

ADAPTIVE

1. Natural Ab
2. T cell-dependent
3. T independent

EFFECTOR

RSTR

LTBI

Killing
PBA & HDT Properties

Not Favorable
Side effects
Dosing interval
High MIC

Favorable
FDA-approved
Dual effect on bacillus & macrophage
Induces CAMP
Induces IL-1β
Human dosing achieves concentrations above MIC