Insights into the host response and the potential for HDT from studies of HIV-TB associated TBM-IRIS

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Tuberculosis Meningitis: Advancing Immunopathogenesis, Diagnosis, and Treatment
0900-0930 Tuesday 23rd May 2017
NIAID, Rockville, MD
Acknowledgements

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Funding agencies
Wellcome Trust
National Institutes of Health
Research Councils (UK)
European Union
EDCTP
MRC (SA)
NRF (SA)
TB in Khayelitsha, South Africa

- Population: 391,749
- Antenatal HIV prevalence: ~ 30%
- TB incidence 917/100,000 (i.e. ~ 1 % per annum)
- ~60% TB is HIV associated
- ~ 4040 cases per year
- Vastly expanded antiretroviral access and coverage

Data courtesy of Judy Caldwell, Provincial government
The medical consequences of large scale antiretroviral roll out

1. Access, and adherence, to care
2. Drug interactions
3. Shared side effects
4. Antiretroviral and antibiotic resistance
5. Immune reconstitution inflammatory syndrome
6. Metabolic effects of antiretrovirals
7. Interaction with non-communicable disease

Secondary care

Graeme Meintjes
Wellcome Trust Fellow

Suzaan Marais
Wellcome Trust Fellow

Rachel Lai
MRC Career Dev Fellow
Patient diagnosed with TB and started on TB treatment

Improving on TB treatment then starts ART

Recurrence of TB symptoms and new or recurrent clinical manifestations of TB (Usually 1-4 weeks after starting ART)

Up to 40% of patients starting ART in sub-Saharan Africa are on TB treatment

Major risk factors:
- Low CD4 count
- Disseminated TB
- Short interval between TB treatment and ART

Paradoxical TB-IRIS is clinically highly heterogenous

Multiply elevated cytokines, a distinct pattern of matrix metalloproteinase, and innate immune activation characterizes TB-IRIS

MTB stimulated transcript abundance *in vitro* at either 6 or 24 hrs

MTB stimulated gene induction *in vitro* at either 6 or 24 hours

MTB stimulated cytokine secretion *in vitro*

Cytokine detection *in vivo*

Corticosteroid modulated *in vivo*

Transcriptomic profiling in human tuberculosis

Active tuberculosis has a transcriptomic signature dominated by a neutrophil-driven type 1 and type 2 interferon-inducible gene profile\textsuperscript{1}

The transcriptomic signature relates to disease extent and resolves during successful treatment\textsuperscript{1,2}

The differentiation of active from latent tuberculosis and other conditions may be aided by transcriptomic profiling\textsuperscript{3,4}

The diagnosis of tuberculosis in children may be aided by transcriptomic profiling\textsuperscript{5}

Differentially abundant transcripts in TB-IRIS are associated with innate signalling pathways\textsuperscript{6}

3. Bloom, CI et al. PLOS One (2013) \textbf{8}: e70630
Sample schedule

Antitubercular therapy

Antiretroviral therapy

Corticosteroid therapy

Sampling (week) 0 0.5 1 2

Differentially abundant transcripts in TB-IRIS are associated with innate signaling pathways

Week 0.5

Week 2

Top 5 Canonical Pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Role of JAK family kinases in IL-6-type cytokine signaling</td>
<td>1.14E-06</td>
</tr>
<tr>
<td>Acute phase response signaling</td>
<td>9.96E-06</td>
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<tr>
<td>Role of macrophages, fibroblasts &amp; endothelial cells in Rheumatoid Arthritis</td>
<td>8.23E-05</td>
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<tr>
<td>Role of JAK2 in hormone-like cytokine signaling</td>
<td>3.33E-04</td>
</tr>
<tr>
<td>Role of JAK1 &amp; JAK3 in cytokine signaling</td>
<td>1.17E-03</td>
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Top 5 Canonical Pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Toll-like-receptor signaling</td>
<td>1.29E-06</td>
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<tr>
<td>TREM1 signaling</td>
<td>2.55E-05</td>
</tr>
<tr>
<td>Role of pattern recognition receptors in recognition of bacteria &amp; viruses</td>
<td>2.62E-04</td>
</tr>
<tr>
<td>Role of macrophages, fibroblasts &amp; endothelial cells in Rheumatoid Arthritis</td>
<td>4.43E-04</td>
</tr>
<tr>
<td>Production of nitric oxide &amp; reactive oxygen species in macrophages</td>
<td>8.99E-04</td>
</tr>
</tbody>
</table>

Tracking the transcriptomic dysregulation that leads to TB-IRIS

**Top 5 Canonical Pathways (43 genes)**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>TREM1 signaling</td>
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<tr>
<td>Toll-like receptor signaling</td>
<td>2.01E-04</td>
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<tr>
<td>Endothelin-1 signaling</td>
<td>3.84E-04</td>
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<tr>
<td>Role of pattern recognition receptors in recognition of bacteria &amp; viruses</td>
<td>8.83E-04</td>
</tr>
<tr>
<td>IL-1 signaling</td>
<td>8.83E-04</td>
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</tbody>
</table>

**Week 2 (43 consistently overabundant transcripts)**

<table>
<thead>
<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>ACSL1</td>
<td>C19orf35</td>
<td>HECW2</td>
<td>RHOBTB1</td>
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<tr>
<td>ADCY3</td>
<td>CARD17</td>
<td>IFIT3</td>
<td>SERPING1</td>
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<tr>
<td>AGPAT9</td>
<td>CASP5</td>
<td>IFITM3</td>
<td>SIGLEC9</td>
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<tr>
<td>AIG1</td>
<td>CDK5RAP2</td>
<td>KCNJ15</td>
<td>SIPA1L2</td>
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<tr>
<td>ANXA3</td>
<td>DHR13</td>
<td>KLHL2</td>
<td>SMARCD3</td>
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<tr>
<td>APOB48R</td>
<td>DKFZp761E198</td>
<td>LOC100128460</td>
<td>TLR2</td>
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<tr>
<td>ASPRV1</td>
<td>DOK3</td>
<td>MANSC1</td>
<td>TLR5</td>
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<tr>
<td>B3GNT8</td>
<td>ECE1</td>
<td>MAPK14</td>
<td>TPST1</td>
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<td>BAMBI</td>
<td>GNB4</td>
<td>OAS1</td>
<td>TRIB1</td>
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<tr>
<td>BASP1</td>
<td>GPR160</td>
<td>PFKFB3</td>
<td>TUFT1</td>
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<tr>
<td>BCL2A1</td>
<td>GPR97</td>
<td>PSG9</td>
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</tbody>
</table>
Both canonical and non-canonical inflammasomes are activated in TB-IRIS

Caspase 1

Caspase 5

Caspase 3

IL-1β

IL-1α

The worst form of TB-IRIS is neurological IRIS

TBM was diagnosed in 120/211 patients (57%) with meningitis

Retrospective study
• 23 (12%) of 190 TB-IRIS patients had neurologic TB-IRIS
  8 meningitis, 7 tuberculoma, 5 both, 3 radiculomyelopathy
• 87% required hospital admission (median 12 days)
• 91% received corticosteroids (median 58 days)
• 6 month outcome: 70% alive, 13% dead, 17% LTFU

Prospective study
• 16/34 (47%) TBM patients developed TBM-IRIS despite steroids in 13/16
• Death occurred in 4 (25%) TBM-IRIS patients
• TBM-IRIS patients had higher CSF neutrophil counts
• *Mycobacterium tuberculosis* culture +ve CSF in 94% TBM-IRIS compared with 33% non-IRIS

Radiographic examples of neurological TB-IRIS

Marais S et al. (2013) Clin Infect Dis 56: 450
Implication of neutrophils in TBM-IRIS

CSF *M. tuberculosis* culture positivity strongly associates with TBM-IRIS

<table>
<thead>
<tr>
<th>Patients</th>
<th>TBM diagnosis</th>
<th>ART start</th>
<th>2 weeks post ART start</th>
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<td>16</td>
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IRIS 15/16
16 (15-20 days)

No IRIS 6/18
(14-32 days)

Relative risk of IRIS if culture positive = 9.3 95% CI 1.4-62.2
P=0.0004

Risk of death from TBM-IRIS ~ 25%

CSF mediators 2 weeks after starting ART (typical time of TBM-IRIS)

Blood transcriptomic signature at time of TBM diagnosis

Transcriptional signature at onset of ART

Transcriptional signature at time of TBM-IRIS onset


<table>
<thead>
<tr>
<th>Top 5 canonical pathways</th>
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<tbody>
<tr>
<td>Inflammasome activation</td>
<td>3.72E-05</td>
</tr>
<tr>
<td>Phagosome formation</td>
<td>6.08E-05</td>
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<td>Role of PRR in recognition of bacteria &amp; viruses</td>
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</table>

**Figure b)**

**Figure c)**

**Figure d)**

**Figure e)**

Tracking the transcriptomic perturbation associated with TBM-IRIS

22 common transcripts

21 Inflammasome transcripts

Conclusions

• Combined life-saving therapy for HIV-TB is frequently complicated by iatrogenic worsened immunopathology that can be fatal: TB-IRIS

• The cause of TB-IRIS appears to be a change in innate recognition of a pre-existing pathogen load with downstream inflammatory consequences

• The intense inflammation of TBM-IRIS is antigen load driven associated with inflammasome activation, neutrophil peptides, and elevation of MMP-9

Translational consequences

• Optimize antimicrobial penetration into CSF

• More rational, effective and safe host-directed therapies of inflammation in tuberculosis should be feasible including both biologics and small molecules
  • doxycycline
  • anti-TNF
  • anti-IL-6
  • PDE4 inhibition
  • CCR5 antagonism
  • inflammasome or IL-1 blockade
Reflections on the road map toward improving HDT

- Insufficient knowledge of pathogenesis

- No *in vitro* or *ex vivo* assays of efficacy to rank and prioritise candidates

- Do preclinical models adequately reflect complexity esp. of HIV-TBM?

- *De novo* approaches would appear less feasible than repurposing

- Drug interactions with rifamycins and ART liable to be significant

- Significant heterogeneity: therapy may ideally be ‘personalised’

- Piggyback approaches in PTB but avoid assumption that ‘one size fits all’
Thank you!

Zeke du Plessis