A TOLLIP DEFICIENCY ALLELE, RS5743854, IS ASSOCIATED WITH LNCRNA TOLLIP-AS1 EXPRESSION, T-CELL MEMORY PHENOTYPE, AND INCREASED TB SUSCEPTIBILITY

February 21, 2018
5th Global Forum on TB Vaccines
MULTIPLE FACTORS INFLUENCE VACCINE IMMUNITY AND TB SUSCEPTIBILITY

- Profile Genes Associated with Mycobacterial Susceptibility
- Determine Pathways Critical to Mycobacterial Control
- Improve Therapies and Vaccines

Case courtesy of Dr. Dalia Ibrahim, Radiopaedia.org, rID: 30824
BCG Activity

TNF; IL-6

TLR 1, 2, 4, 6 IL-1R

TB control/progression

IL-12
IL-23
IL-10

T cell

BCG

TOLLIP
TOLLIP – CRITICAL REGULATOR OF IMMUNE ACTIVATION

Chr 11 (-) 1290000

rs5744015
rs5744016
rs5743890
rs5743899
rs5743942
rs3793964
rs3795223
rs3168046
rs5743890
rs5743942
rs3793964
rs3795223
rs3168046

TBD
C2
CUE

ESCRT
Autophagy
Endosomal Localization
Ub binding
TLR2, TLR4, IL-1R binding

IL-1R
TLR2, TLR

MVDS88
IRAK
IKKs
I-κB
TF activation
Cytokines

TOLLIP
A. CRISPR/Cas9 knockout of TOLLIP in THP1s
Empty vector control (EV) vs TOLLIP-KO

\[ \alpha \text{-TOLLIP} \quad \beta \text{-Actin} \]

Seattle Healthy Control Cohort

\[ \text{TOLLIP mRNA / GAPDH} \]

***

B. TOLLIP mRNA expression in THP1s

\[ \text{TNF (pg/ml)} \]

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\[ \text{IL-6 (pg/ml)} \]

**

\[ \text{PAM3 250ng/ml} \]

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Shah et al. *J. Immunol* 2012
RS5743854 IS A **FUNCTIONAL** TOLLIP PROMOTER VARIANT

**TOLLIP** -362 [G/C] [G/G]

HEK293T cells
TOLLIP promoter transfected with Luciferase protein
Luminescence measured after 24hr

South African cohort
Infant Monocytes
Community Control
N = 155

* p = 0.008, Kruskal-Wallis test

RS5743854 influences TOLLIP-AS1 expression and TOLLIP mRNA transcription.
HYPOTHESIS:
ONE OR MORE FUNCTIONAL TOLLIP VARIANTS REGULATE IN VIVO IMMUNE RESPONSES TO BCG & SUSCEPTIBILITY TO TB

Collaborators:
University of Cape Town

Willem Hanekom
Mark Hatherill
Tom Scriba
Munya Musvosvi
Muki Shey
Evaluating BCG-specific T cell Immunity In TOLLIP-deficient infants

N = 174
Infant Whole Blood
BCG-specific T Cell Cytokines

IL-2
TNF
IFN-γ
IL-17
IL-22
IL-4

TOLLIP DEFICIENCY REDUCES BCG-SPECIFIC IL-2 AND PROLIFERATION IN CD4 T CELLS

* P = 0.02

* P = 0.04

MULTIPLE FACTORS INFLUENCE MEMORY T CELL PHENOTYPES

Inflammation
Ag Exposure
Proliferation/
Duration
IL-2
TNF
IFNγ
Apoptosis

Naïve Tcm Tem Teff Texh Dead
IL-2+  
High  
Low TOLLIP

TNF+  

IL-2+  

TNF+  

Naïve  
TCM  
TEM  
TEMRA

*p = 0.02, partial permutation test
TOLLIP-DEFICIENT INFANTS DEMONSTRATE FEWER TCM OVERALL AT 10 WEEKS OF AGE
TOLLIP DEFICIENCY IS ASSOCIATED WITH ADULT TB SUSCEPTIBILITY

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Cord Blood Controls</td>
<td>762</td>
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<tr>
<td>Adults with Pulmonary TB</td>
<td>385</td>
</tr>
<tr>
<td>Adults with Meningeal TB</td>
<td>307</td>
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<tr>
<td>HIV-infected (%)</td>
<td>0</td>
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<tr>
<td>Age (Mean)</td>
<td>38</td>
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<tr>
<td>Sex (% female)</td>
<td>37</td>
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**Analysis**

<table>
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<tr>
<th>SNP</th>
<th>Group</th>
<th>P</th>
<th>OR</th>
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<tbody>
<tr>
<td>rs5743854</td>
<td>control</td>
<td>6.64E-07</td>
<td>1.7</td>
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<tr>
<td>TB all</td>
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Shah et al. *J. Immunol* 2012
TOLLIP Deficiency is Associated with Latent Tuberculosis Susceptibility

<table>
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<tr>
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<td>No LTBI (N)</td>
<td>116</td>
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<tr>
<td>Adults with LTBI (N)</td>
<td>150</td>
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<tr>
<td>HIV-infected (%)</td>
<td>0</td>
</tr>
<tr>
<td>Age (Mean)</td>
<td>42</td>
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<tr>
<td>Sex (% female)</td>
<td>46</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Group</th>
<th>P (geno)</th>
<th>OR (recessive)</th>
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</thead>
<tbody>
<tr>
<td>rs5743854</td>
<td>No LTBI</td>
<td>7.0E-4</td>
<td>2.6 (1.1 – 7.1)</td>
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<tr>
<td></td>
<td>LTBI</td>
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David Horne; Masa Narita
King County Public Health Department
Harborview Medical Center
Seattle, WA

TOLLIP deficiency

BCG

TNF; IL-6

TLR 1, 2, 4, 6

T cell differentiation

Altered memory response

CD4+ T cell

Reduced vaccine duration?
Increased TB pathology?
SUMMARY

• A functional single nucleotide polymorphism alters TOLLIP promoter activity in monocytes
• Antisense RNA TOLLIP-AS1 influences TOLLIP expression (and perhaps innate immune responses)
• TOLLIP deficiency is associated with altered T cell phenotypes from a very early age
• TOLLIP variation is associated with TB in multiple genetic case control cohorts
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