Cellular immune responses in Asian elephants infected with *Mycobacterium* species

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Current elephant TB challenges

1. Development of accurate diagnostics
2. Establishment of efficacious and tolerable treatment regimens
3. Elucidation of mechanisms underlying disease susceptibility
Tuberculosis in elephants

- *Mycobacterium tuberculosis*
- Documented U.S. zoonosis
- Worldwide prevalence unknown
- Southeast Asia
  - High human TB prevalence
  - Interaction with captive working elephants
Mycobacterium tuberculosis

- One third of world human population infected
- <10% of infected individuals ever develop clinical disease
Tuberculosis immunity

- Disease is secondary to abnormal or inadequate host immune responses that fail to control infection
- Do immune function alterations explain Asian elephant susceptibility to *Mycobacterium* spp.?
Immunity Defined

• The body’s reaction to foreign substances
  – Infectious microbes
  – Noninfectious macromolecules

• Occurs regardless of potentially detrimental physiologic or pathologic consequences
## Innate vs. Adaptive

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
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</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
<td>Structures shared by related groups of microbes</td>
<td>Various microbial and nonmicrobial antigens</td>
</tr>
<tr>
<td><strong>Diversity</strong></td>
<td>Limited</td>
<td>Vast</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Cellular components</strong></td>
<td>Phagocytes, NK cells</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td><strong>Biochemical components</strong></td>
<td>Complement, cytokines, chemokines</td>
<td>Antibodies, cytokines</td>
</tr>
</tbody>
</table>
Cytokines

- Secreted proteins that modulate and coordinate immune responses
- Link between innate and adaptive immunity
- Measurable in blood and tissue samples
Cytokines in adaptive immunity

- Determine whether response cell-mediated ($T_H^1$) or humoral ($T_H^2$)

- IL-12 $\rightarrow T_H^1 \rightarrow$ IL-2, IFN-$\gamma$ $\rightarrow$ macrophage activation

- IL-4 $\rightarrow T_H^2 \rightarrow$ IL-4, IL-10 $\rightarrow$ B cell proliferation & antibody secretion
Immune function and disease

- Tuberculosis
  - Effective immunity = Th1-dominant response
  - Disease = Th1-Th2 imbalance
Resistant

Acute
$T_H1$ response: local and systemic

Latent disease
Sustained local and systemic $T_H1$ response

Susceptible

Acute
$T_H1$ response: local and systemic

Progressive disease
Diminished systemic ($1^{st}$) and local ($2^{nd}$) $T_H1$ response
Human progressive disease

• Decreased levels of $T_H 1$ cytokines systemically: peripheral anergy

• Relative increase in levels of $T_H 2$ cytokines systemically: $T_H 2$ dominated immune response

• Decreased levels of $T_H 1$ cytokines locally: disseminated disease
Bovine TB

- Disease associated with mixed $T_H1/T_H2$ response
- No evidence of divergent local and systemic responses at any stage of disease
- Disease severity correlated with ↓ IFN-$\gamma$:IL-4 and ↑ IL-10
- Studies experimental and involved few animals
Study Goal

• Characterize elephant cellular immune responses by measuring and comparing cytokine levels in TB positive and negative samples
  – Understand susceptibility
  – Improve diagnostics
  – Enhance treatment monitoring
Objective 1

• Develop molecular assays for detection and quantification of elephant cytokine levels
  • Real time, reverse transcriptase (RT)-PCR
Real time RT-PCR

• Utilizes sequence-specific primers and probes to identify and amplify mRNA of interest in sample

• Sensitive technique allowing for detection and quantification of even very low levels of mRNA
mRNA detection

- Analogous to protein detection, but with greater sensitivity
- Also eliminates need for specific antibodies and reagents
Development and validation of RT-PCR assays

• Asian elephant-specific assays for measurement of cytokine levels within samples
  1. Sequencing of cytokine and housekeeping genes (Genbank FJ423082-FJ423112)
  2. Design of real time primers and probes for amplification at gene intron/exon junction sites
  3. Optimization of assay efficiency
Objective 2

• Evaluation of baseline cytokine levels
• RNA-preserved peripheral whole blood samples from 106 captive working Asian elephants in Nepal
  – TB positive: 16 (15%)
  – TB negative: 90 (85%)
• Cytokine quantification using elephant specific real time RT-PCR
Figure 1: Cytokine fold difference means and standard errors

Conclusion

- Elephant systemic immune response to TB is mixed $T_H1/T_H2$

- Important caveats:
  - Elephant TB status based on serology not culture
  - Elephant disease stage unknown
  - Elephant cytokines measured in unstimulated RNA-preserved whole blood
Objective 3

- Evaluation of cytokine levels in mycobacterial antigen-stimulated samples
- Asian elephant peripheral blood mononuclear cell (PBMC) cultures
  - ConA (mitogen positive control)
  - \textit{M. bovis} PPD
  - \textit{M. tuberculosis} CFP-10
Study utility

• Emulates design of majority of human and bovine TB pathogenesis studies
• Results could serve as basis for future development of new diagnostics tests
  • QuantiFERON
  • Bovigam
Progress to date

- Validation and optimization of standard PBMC culture procedures for use with elephant samples
  - Cell concentration
  - Mitogen/antigen concentration
  - Incubation time
Incubation time PPD bovis

Days

Stimulation index

Elephant 1
Samples needed!

- Peripheral whole blood from any and all TB positive Asian elephants
Elephant TB immunity

- Findings thus far suggest elephant immune response to TB is mixed $T_H^1/T_H^2$
- $T_H^2$ component may contribute to disease susceptibility
- Analysis of cytokine expression following mycobacterial antigen stimulation could provide more definitive information
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