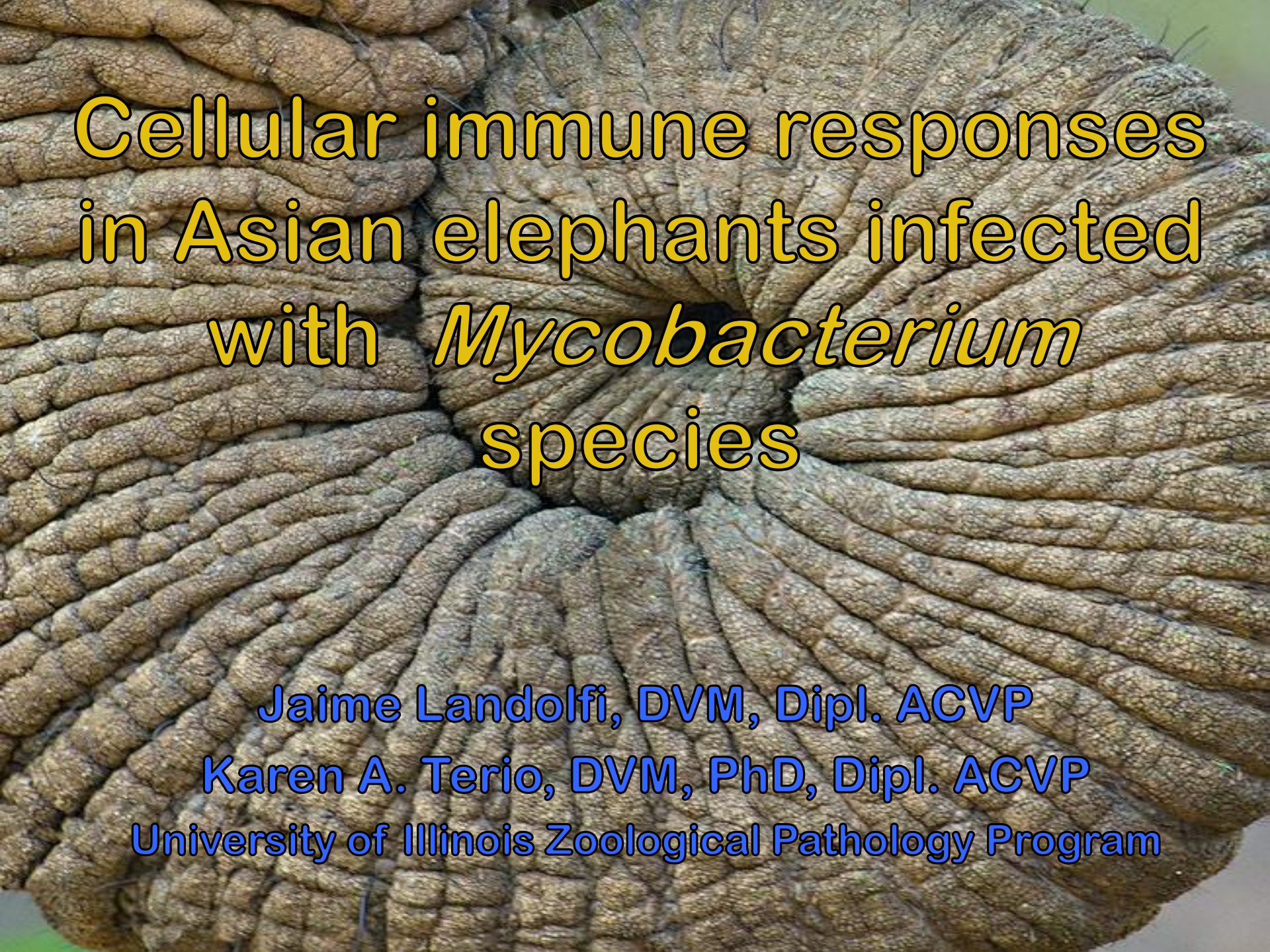

Cellular immune responses in Asian elephants infected with *Mycobacterium* species

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Current elephant TB challenges

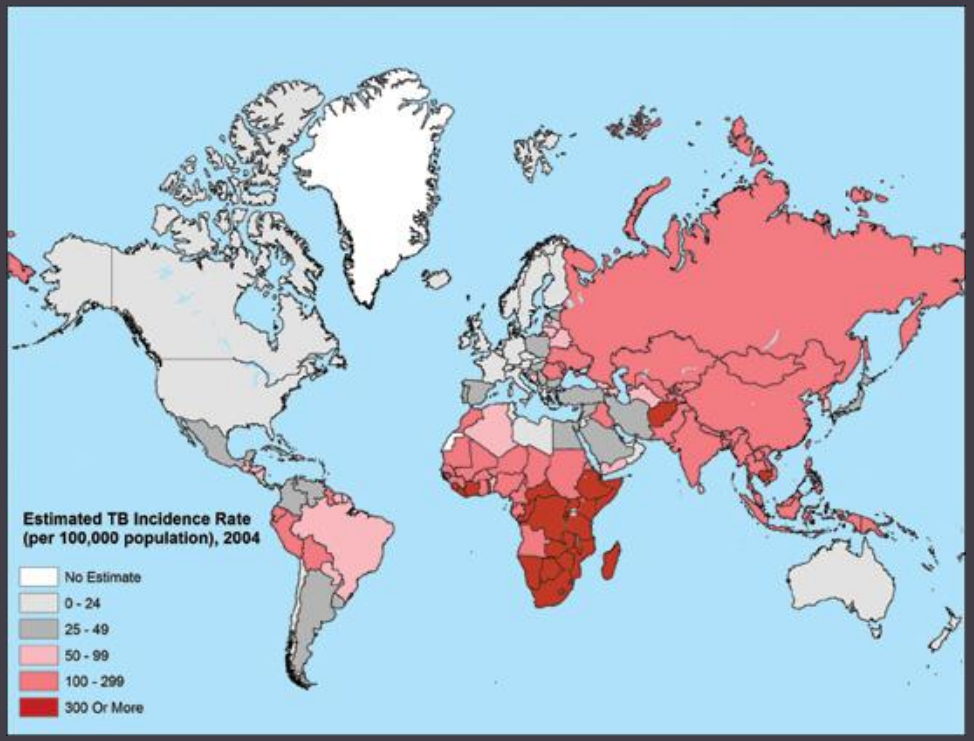
1. Development of accurate diagnostics
2. Establishment of efficacious and tolerable treatment regimens
3. Elucidation of mechanisms underlying disease susceptibility

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

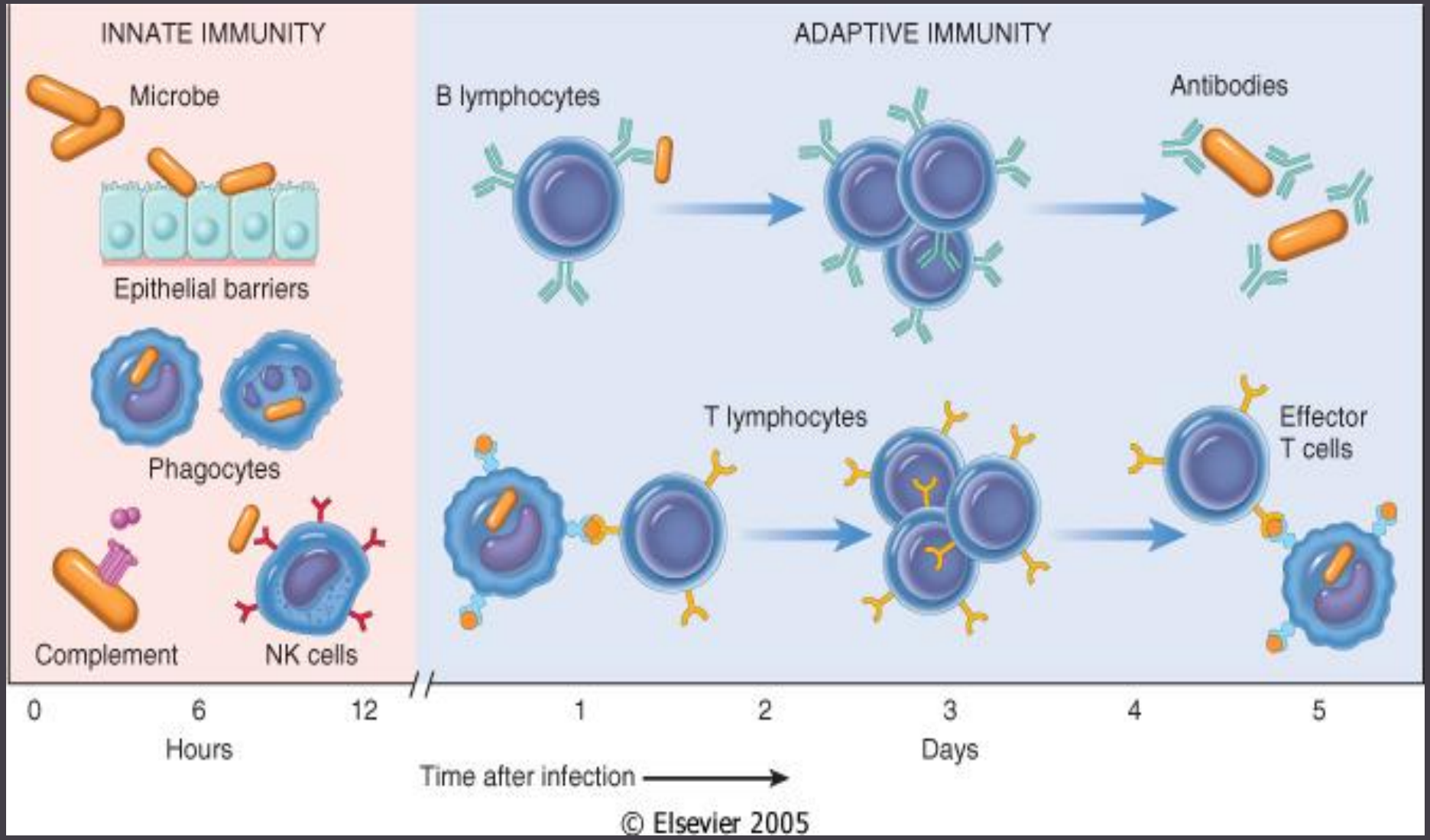


Tuberculosis immunity

- 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 IMMUNITY
ADAPTIVE IMMUNITY
Microbe
Epithelial barriers
Phagocytes
Complement
NK cells
B lymphocytes
Antibodies
T lymphocytes
Effector
T cells
0
6
12
1
2
3
4
5
Hours
Days
Time after infection
© Elsevier 2005

Innate vs. Adaptive

	Innate	Adaptive
Specificity	Structures shared by related groups of microbes	Various microbial and nonmicrobial antigens
Diversity	Limited	Vast
Memory	No	Yes
Cellular components	Phagocytes, NK cells	Lymphocytes
Biochemical components	Complement, cytokines, chemokines	Antibodies, cytokines

Cytokines

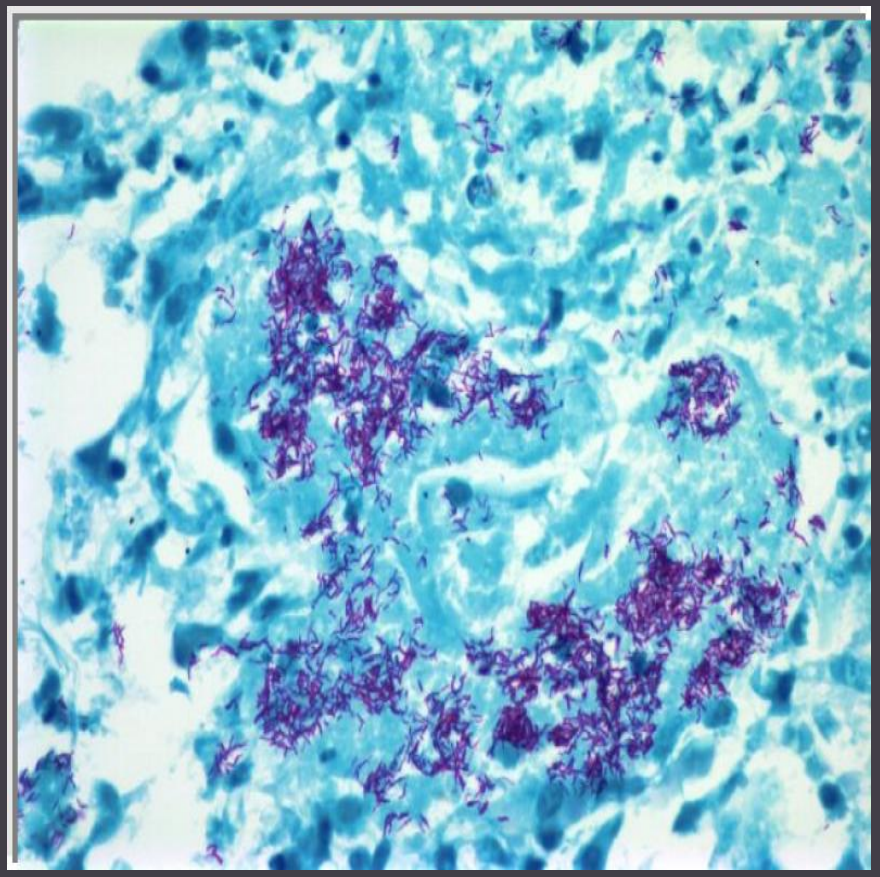
- Secreted proteins that modulate and coordinate immune responses
- Link between innate and adaptive immunity
- Measurable in blood and tissue samples

Cytokines in adaptive immunity

- Determine whether response cell-mediated (T_H1) or humoral (T_H2)
  - IL-12 → T_H1 → IL-2, IFN-γ → macrophage activation
  - IL-4 → T_H2 → IL-4, IL-10 → 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_H1 cytokines systemically: peripheral anergy
- Relative increase in levels of T_H2 cytokines systemically: T_H2 dominated immune response
- Decreased levels of T_H1 cytokines locally: disseminated disease

Bovine TB

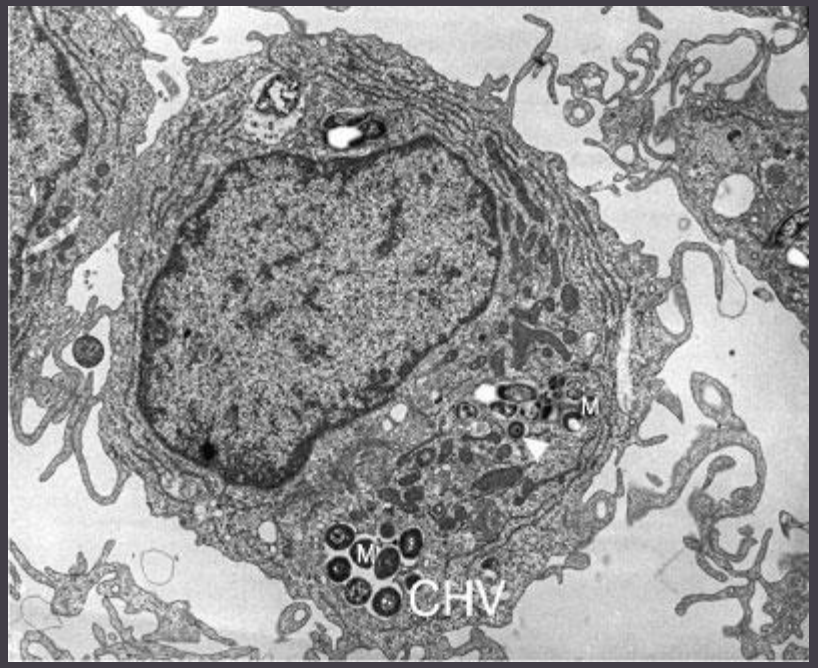
- Disease associated with mixed T_H1/T_H2 response
- No evidence of divergent local and systemic responses at any stage of disease
- Disease severity correlated with ↓ IFN-γ:IL-4 and ↑ IL-10
- Studies experimental and involved few animals

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

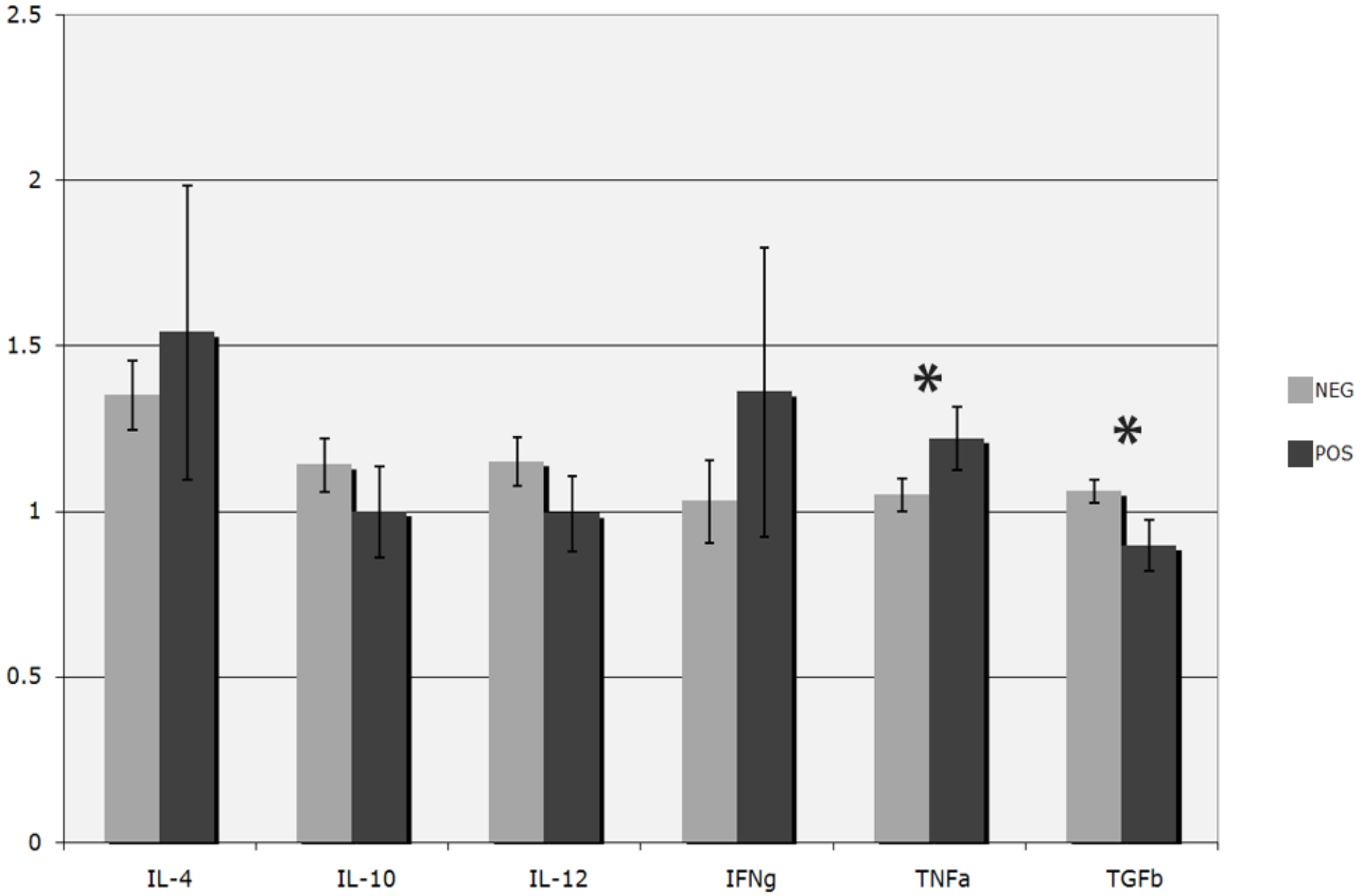
- Asian elephant-specific assays for measurement of cytokine levels within samples
  1. Sequencing of cytokine and housekeeping genes (Genbank FJ423082-FJ423112)
  2. Design of real time primers and probes for amplification at gene intron/exon junction sites
  3. Optimization of assay efficiency

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

Landolfi et al, JZWM 41(3): 445-55, 2010



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

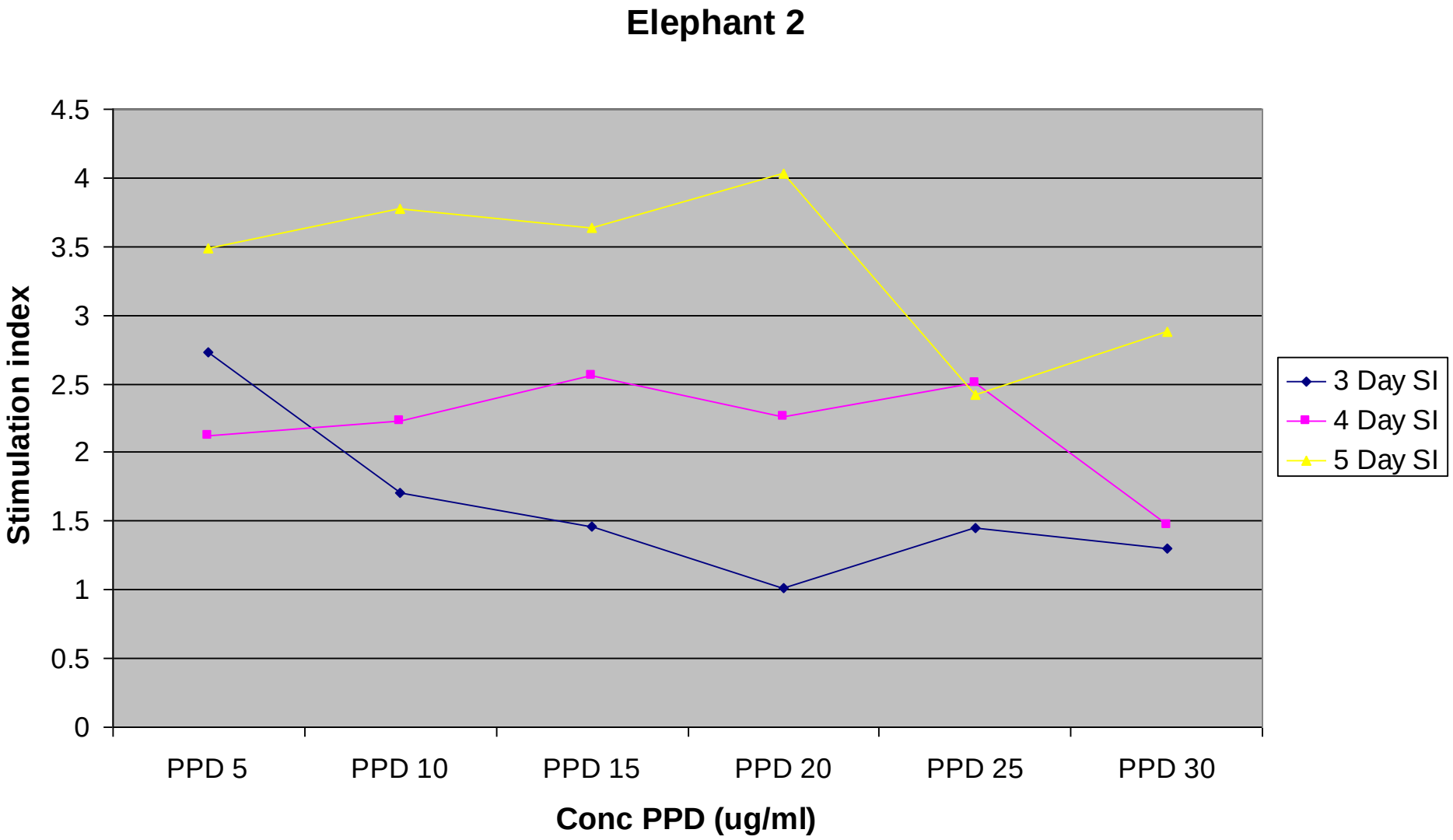
- Evaluation of cytokine levels in mycobacterial antigen-stimulated samples
- Asian elephant peripheral blood mononuclear cell (PBMC) cultures
  - ConA (mitogen positive control)
  - *M. bovis* PPD
  - *M. tuberculosis* CFP-10

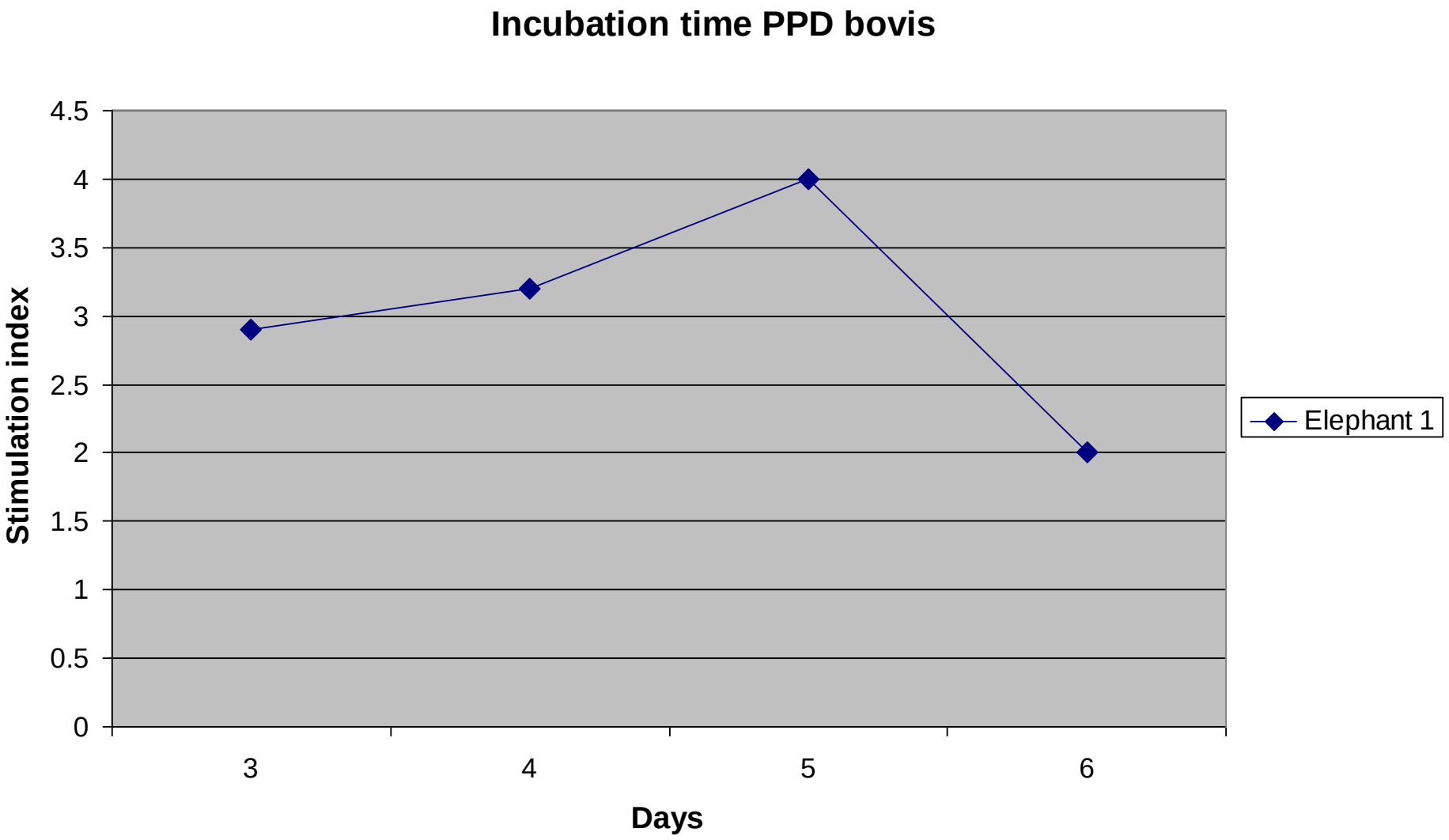
Study utility

- 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


Elephant 2
Stimulation index
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
PPD 5
PPD 10
PPD 15
PPD 20
PPD 25
PPD 30
Conc PPD (ug/ml)
3 Day SI
4 Day SI
5 Day SI


Incubation time PPD bovis
Stimulation index
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3
4
5
6
Days
Elephant 1

Samples needed!

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

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Elephant TB immunity

- Findings thus far suggest elephant immune response to TB is mixed T_H1/T_H2
- T_H2 component may contribute to disease susceptibility
- Analysis of cytokine expression following mycobacterial antigen stimulation could provide more definitive information

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Questions?