



# Immune Responses to *Mycobacterium avium* infection and the role of MAPK pathway in regulation of signal transduction during the infection

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# Diseases caused by Mycobacteria

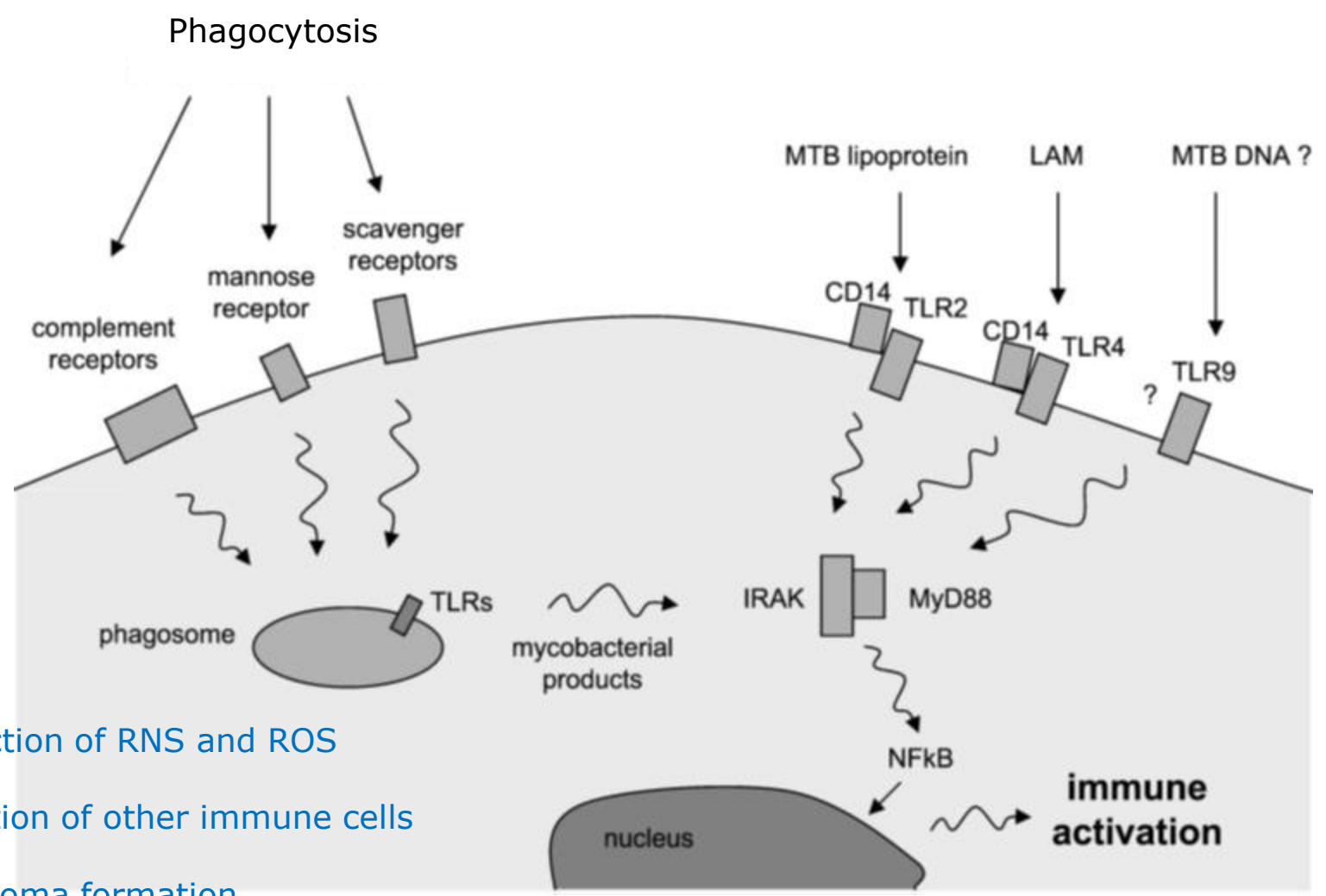
<b><i>Mycobacterium sp.</i></b>	<b>Host</b>	<b>Disease</b>
<b><i>M. tuberculosis</i></b>	Human	Tuberculosis
<b><i>M. bovis</i></b>	Human Bovine	Tuberculosis
<b><i>M. avium complex</i></b>	Birds Immune compromised human, Bovine	Avian Tuberculosis Disseminated Tuberculosis in immune compromised

# Why *M. avium*?

- Avian mycobacteriosis
- Similar to mammalian pathology
- Disseminated disease in HIV patients
- Limited knowledge on the immune processes in poultry
- There are differences between disease process between mammalian and avian infections
  - Progressive disease
- Chickens are easier model for study mycobacterial infection



# Early interactions with Macrophages

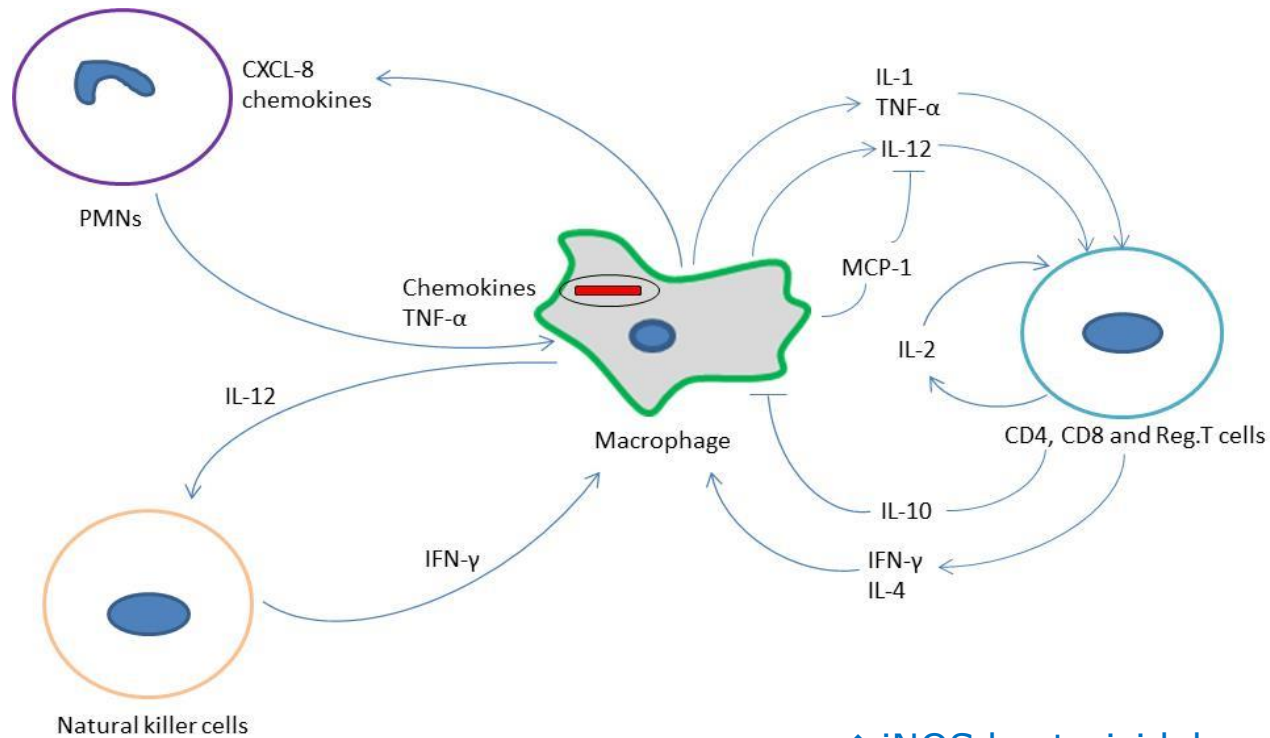


- Production of RNS and ROS
- Attraction of other immune cells
- Granuloma formation

**Macrophage**

VAN Crevel et al., 2002

# Role of cytokines in mycobacterial infection



## ❖ TNF- $\alpha$

- phagocyte activation
- induction of apoptosis
- formation and maintenance of granuloma

## ❖ IFN- $\gamma$

- activation of macrophage
- acidification of phagosome
- promotes antigen presentation process
- stimulation of granuloma formation

## ❖ iNOS bactericidal mechanism

## ❖ IL-1 $\beta$

- regulation of iNOS production,
- maturation and acidification of phagosome
- expression of adhesion molecules

## ❖ CXCL-8

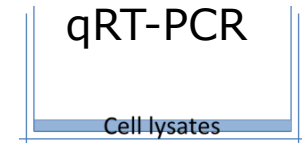
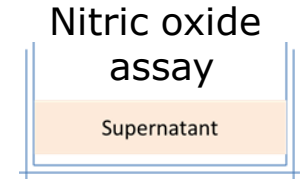
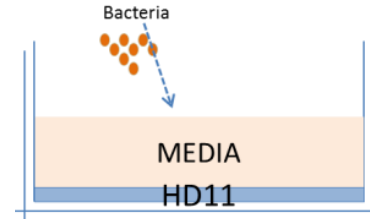
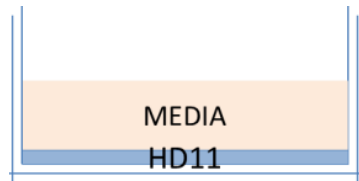
- attraction and activation of many other immune cells

Mammalian cellular immune response to Mycobacteria

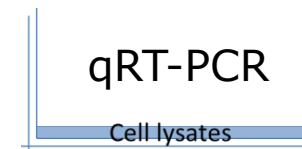
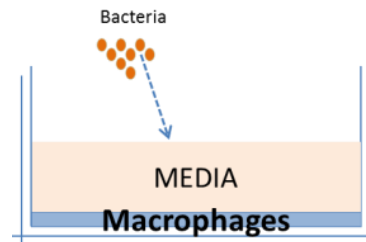
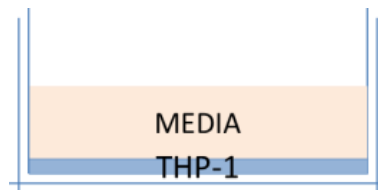
# Aims of the project

- To compare the immunological interactions of *M. avium* subspecies *avium* from different human and animal sources with human and avian infection model.
- To study the role of various MAP kinases in cellular regulation by *M. avium* stimulation.
- To determine the cytokine response to *M. avium* strains during infection of chickens.

## HD11 (Avian Macrophage-like cells)



## THP-1 (Human monocytic cells)



## *M. avium* subspecies *avium*

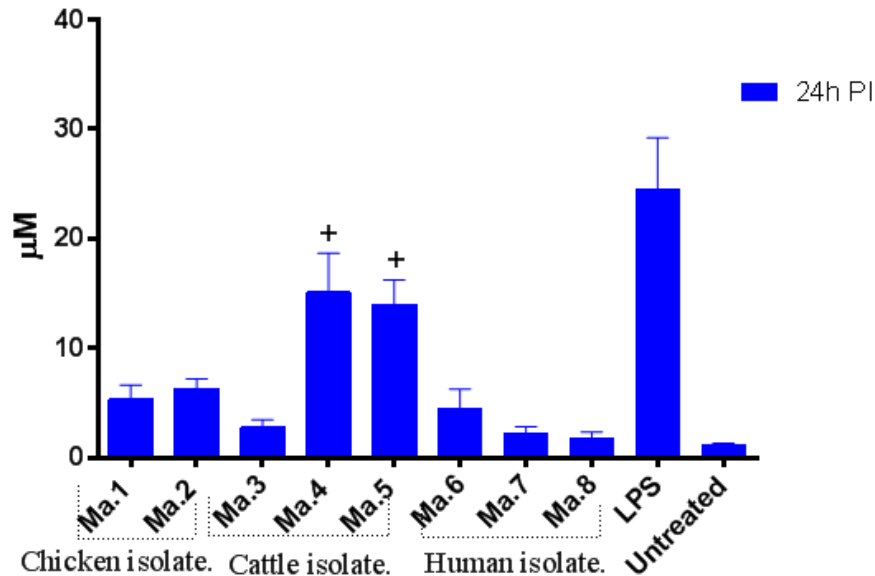
Abb.	Strain	Source
Ma.1	NC.08562-02	Chicken
Ma.2	NC.0855-07	Chicken
Ma.3	61.3623.05	Calf
Ma.4	61.4627.08	Calf
Ma.5	61.3728.08	Calf
Ma.6	430/330	Human
Ma.7	446/301	Human
Ma.8	460/132	Human



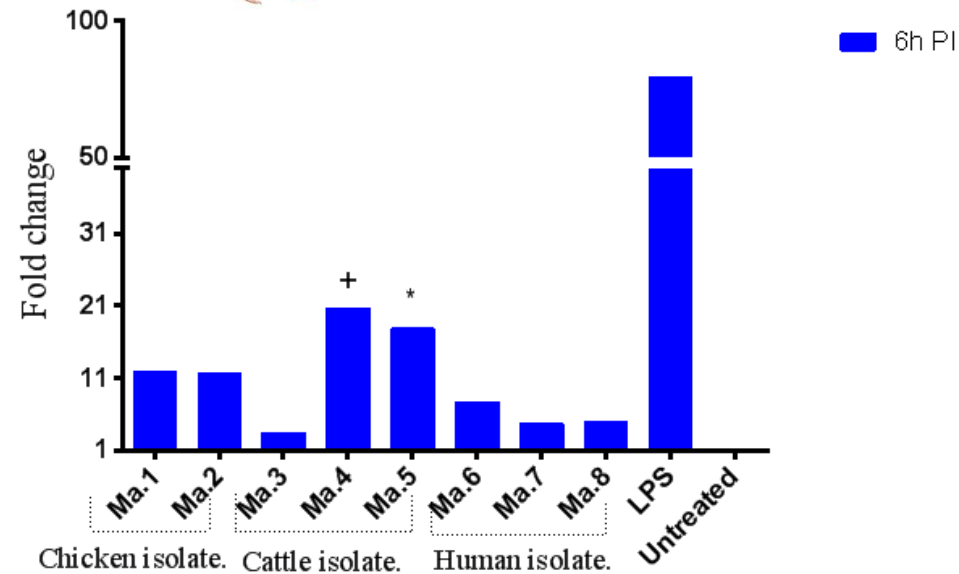
# Examples of differential induction of immune response.



Nitric oxide

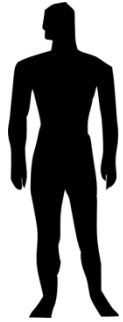


iNOS

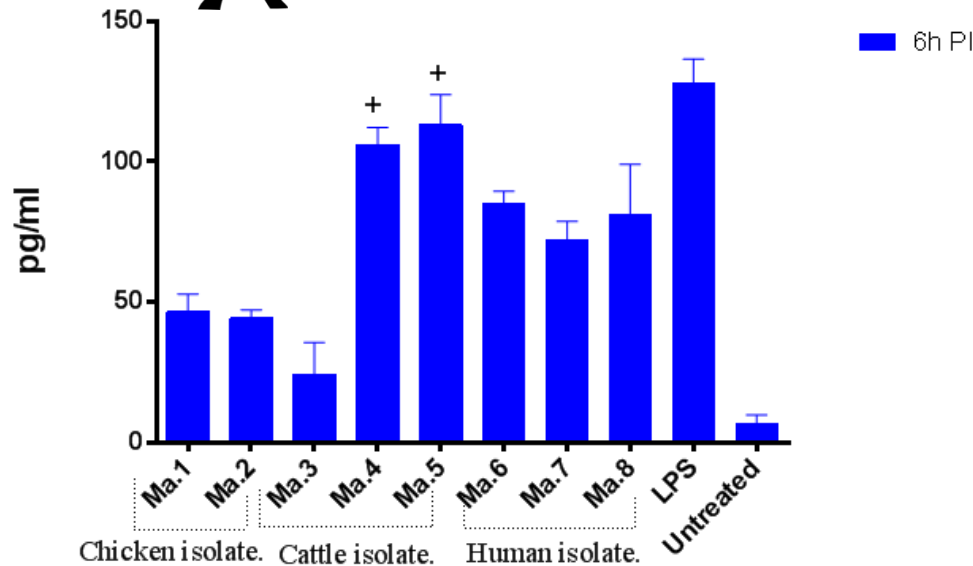


- Similar pattern was observed in other cytokines
- Level does not correlate with source of strains

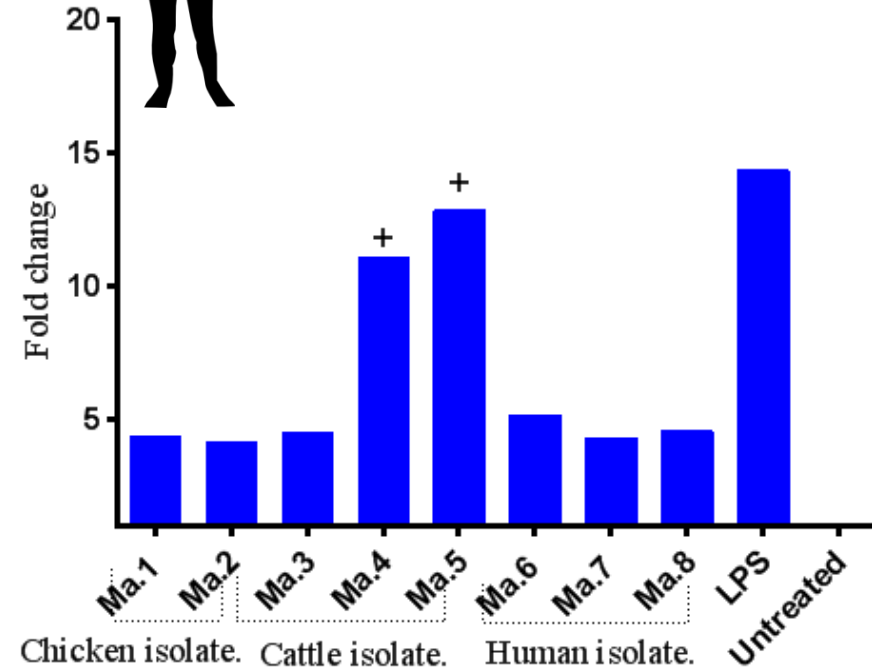
# Examples of differential induction of immune response.



IL-1 $\beta$

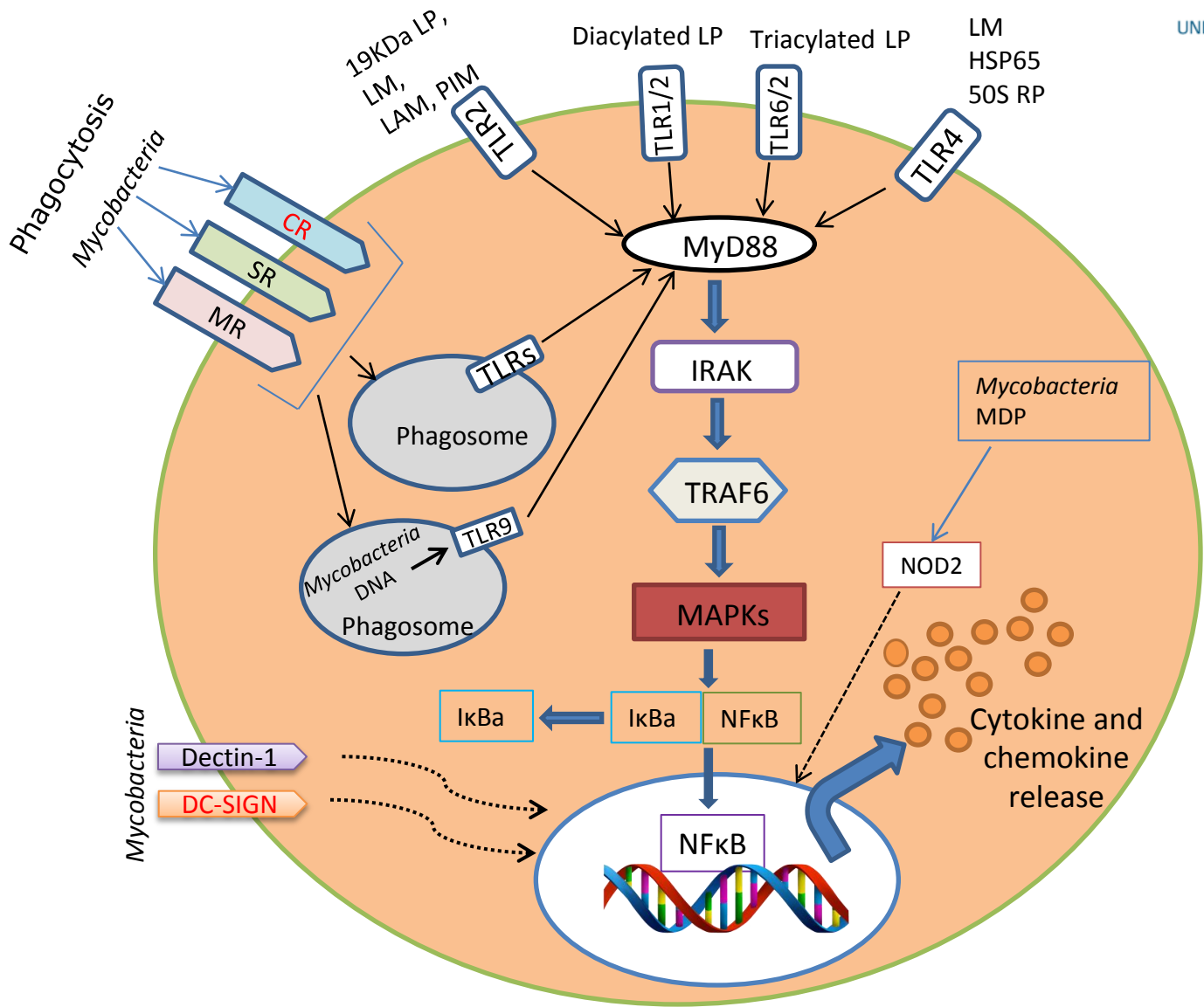


IL-1 $\beta$



- Level does not correlate with source of isolates

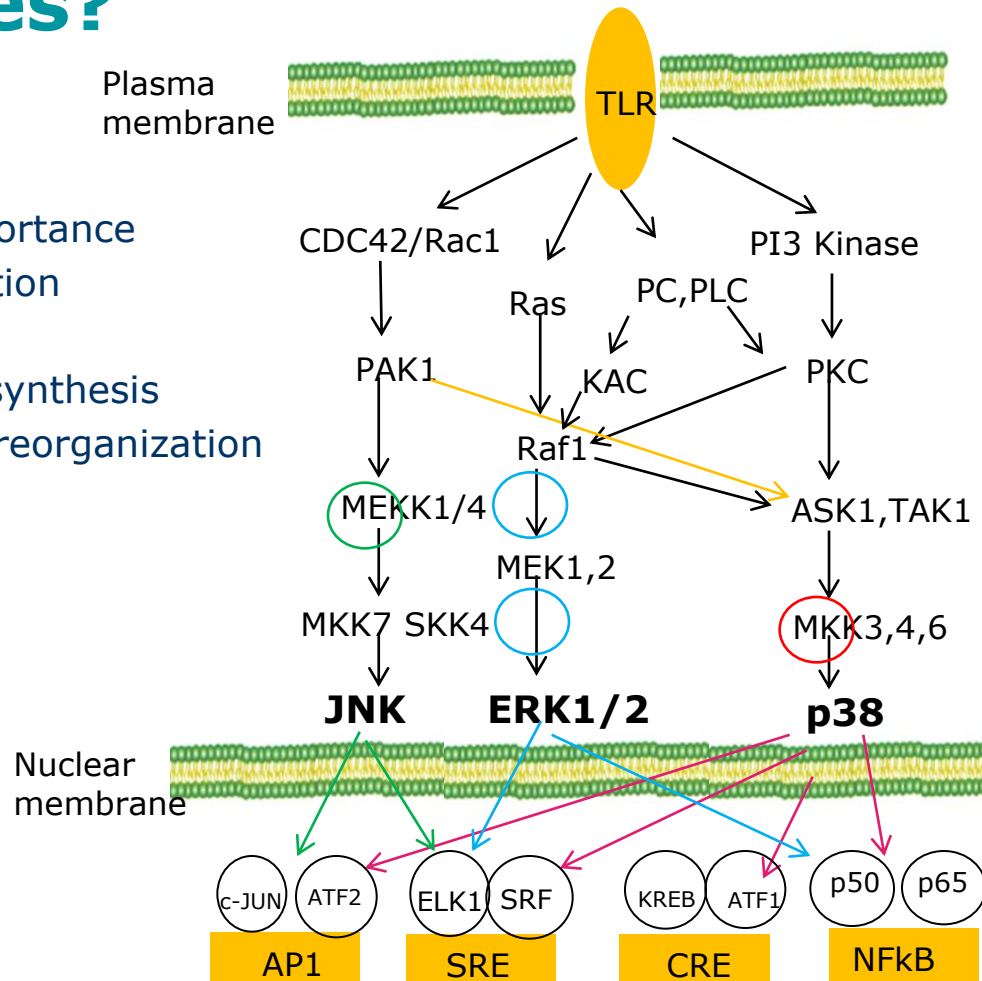
# Regulation of signaling transduction



# Is there a difference between strains and how they signal responses?

## MAP kinases importance

- Cell Proliferation
- Apoptosis
- Cytokine biosynthesis
- Cytoskeletal reorganization



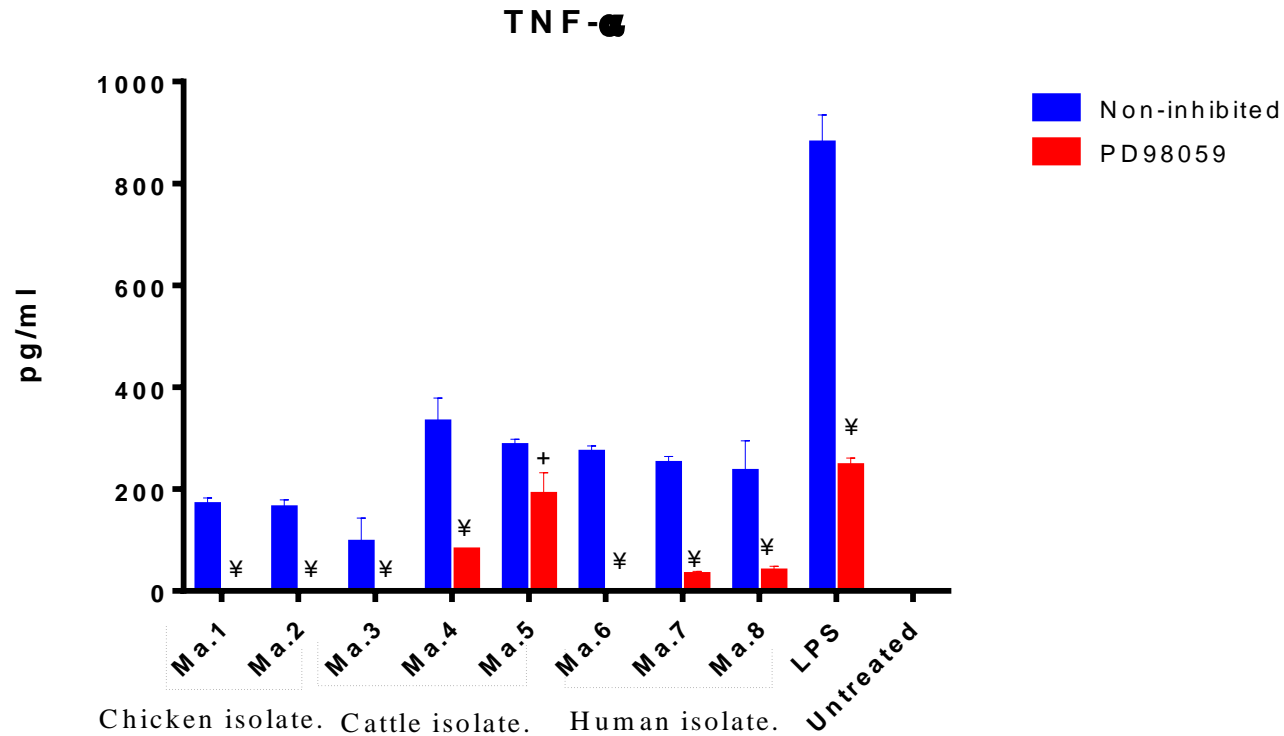
PD98059  
ERK 1/2 pathway inhibitor

U0126  
a significantly  
higher affinity for MEK1

SB203580  
P38 pathway inhibitor

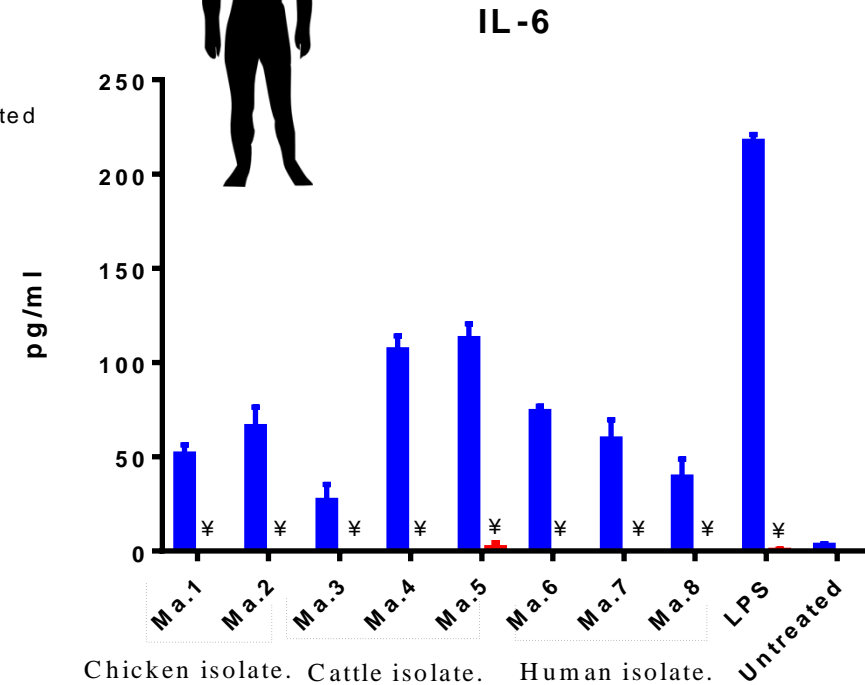
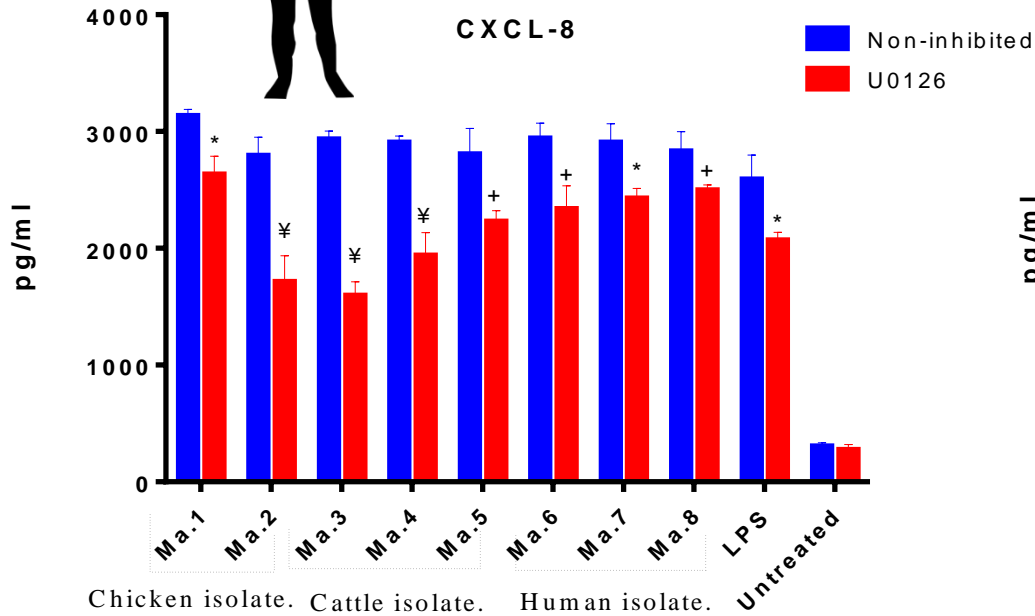
SP600125  
JNK pathway inhibitor

# Inhibition of ERK1/2 by PD98059



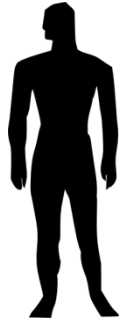
- Signal transduction might be dependent upon mycobacterial species.

# Inhibition of ERK1/2 by U0126

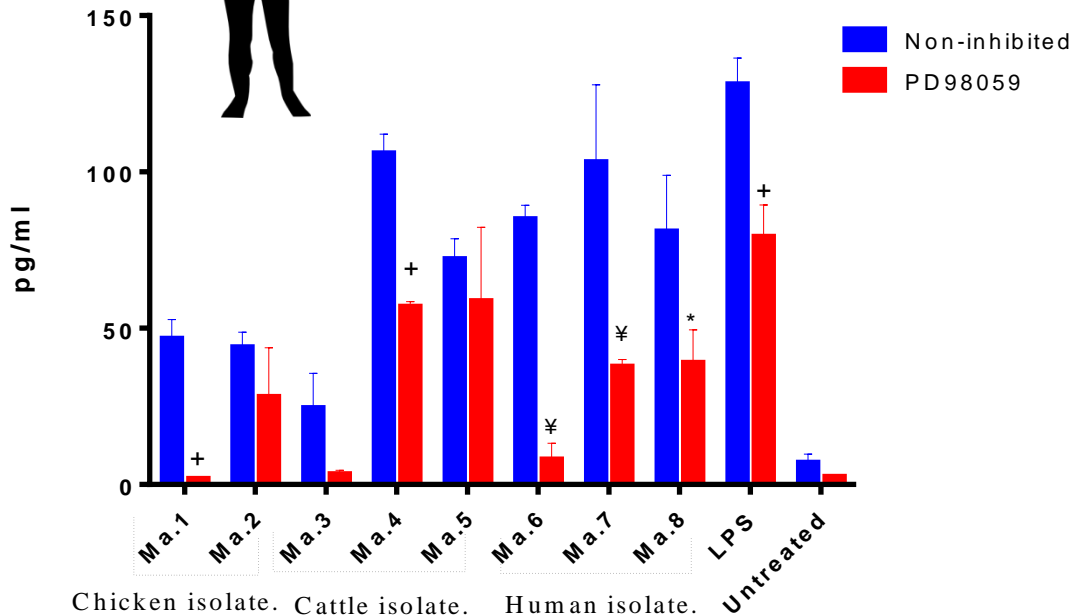


- Signal transduction might be dependent upon cytokine being studied.

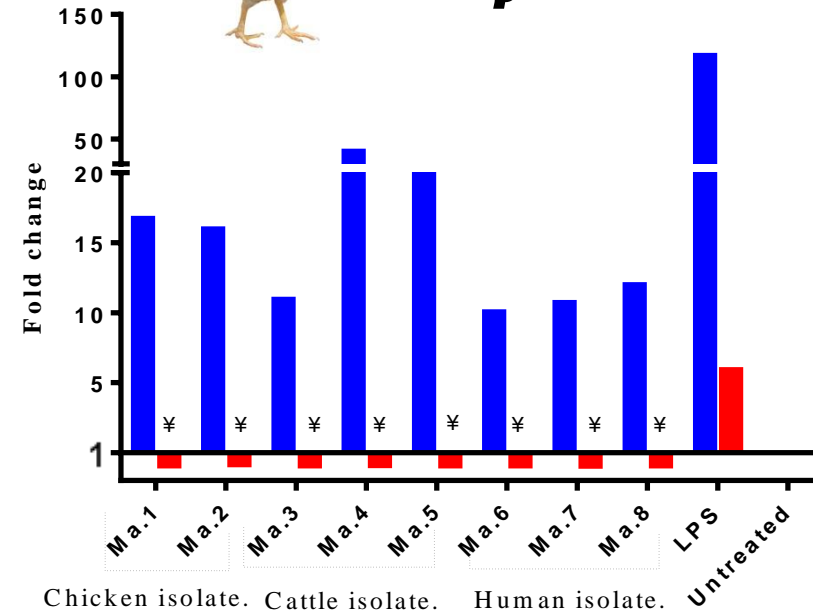
# Inhibition of ERK1/2 by PD98059



IL-1 $\beta$



IL-1 $\beta$



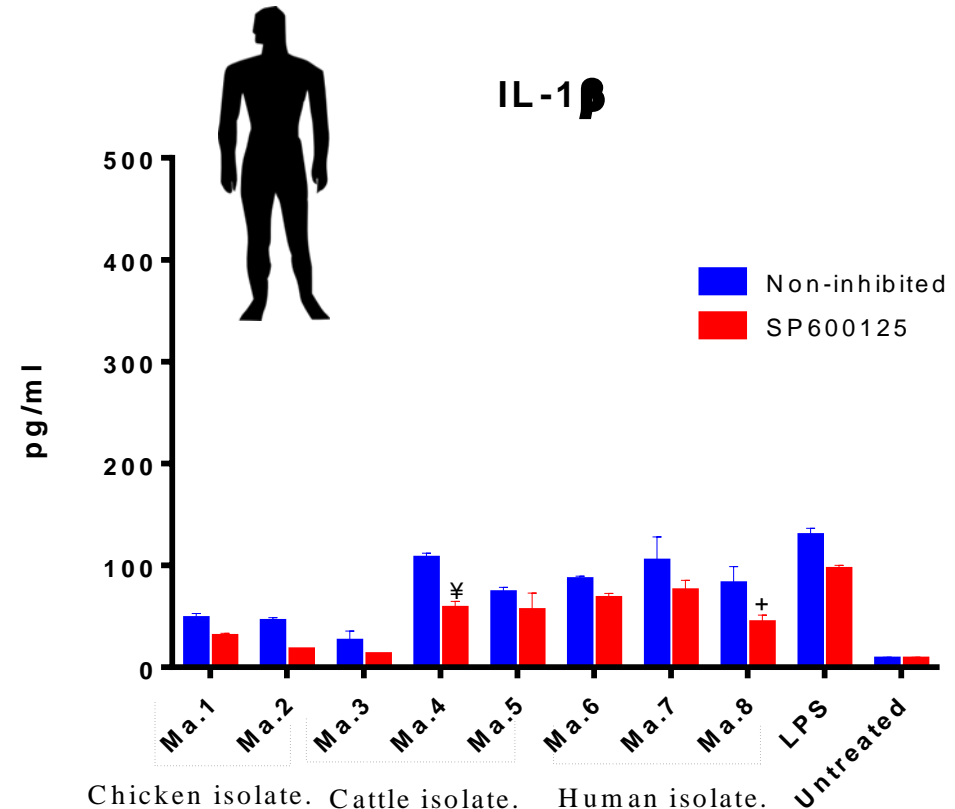
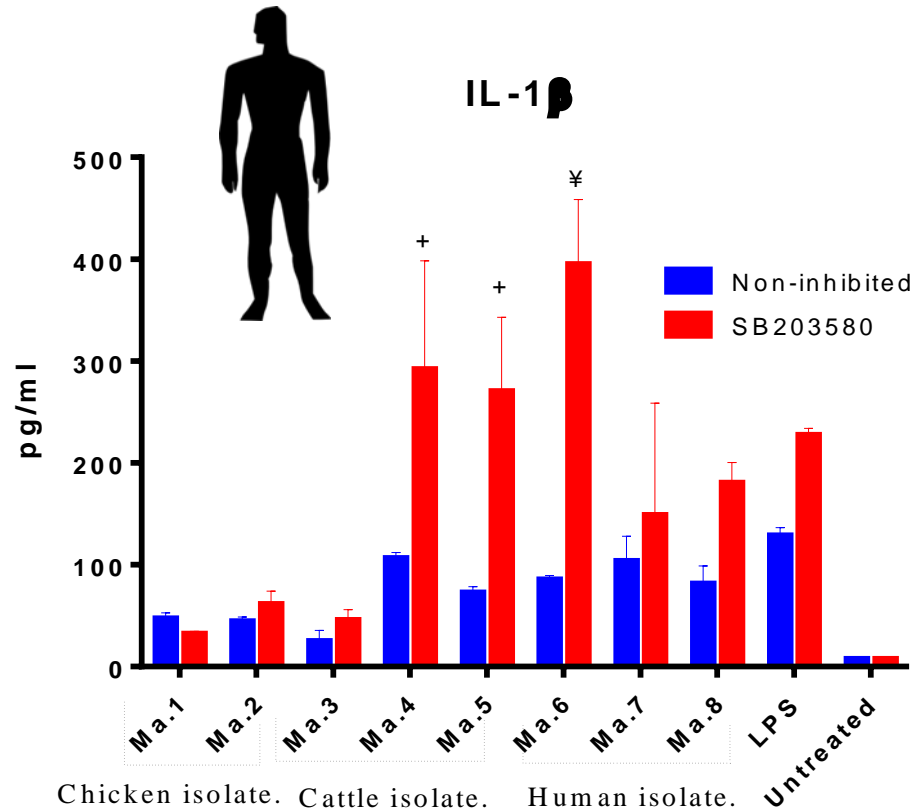
- Signal transduction might be dependent upon cell type being studied.

# Inhibition of p38 by SB203580 and JNK by SP500125



The University of  
Nottingham

UNITED KINGDOM • CHINA • MALAYSIA

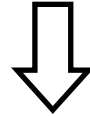


- A cytokine could be differentially regulated by various pathways.



# *In vivo* experiment

Forty eight three week-old commercial chicks

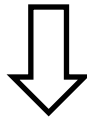


Birds were allocated into 4 groups, twelve birds each in separate rooms

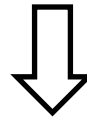
(i.v. inoculation)



Group1  
inoculated with  
Ma.3 (Low)



Group2  
inoculated with  
Ma.4 (High)

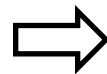





Group3  
inoculated with  
Ma.5 (High)



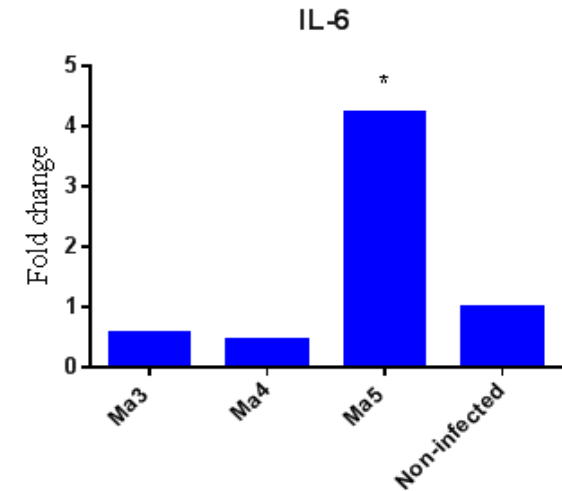
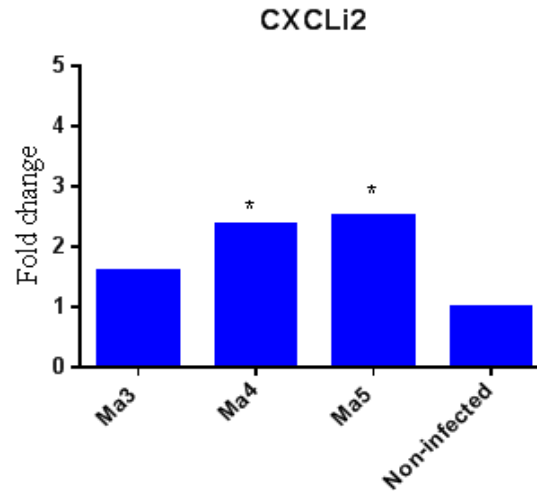
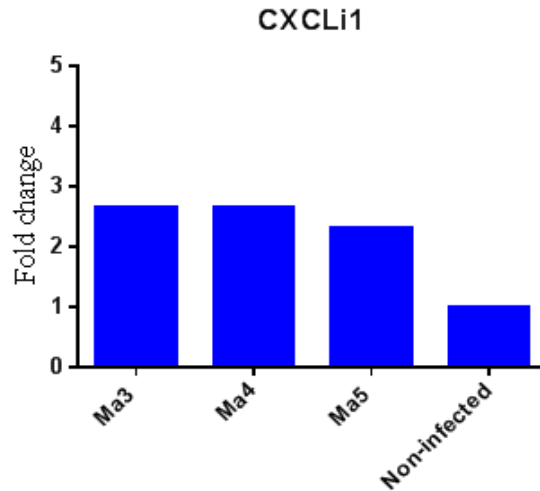
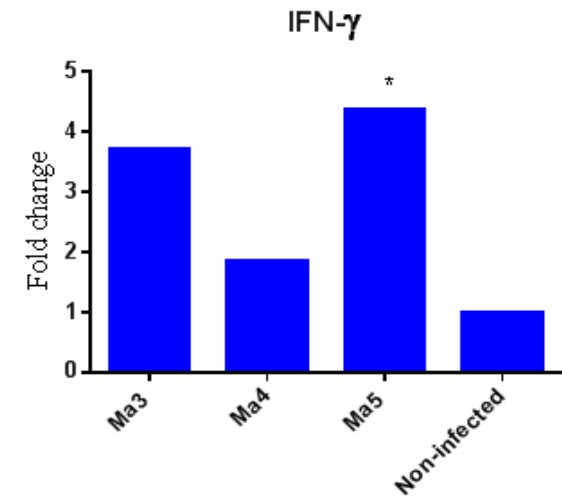
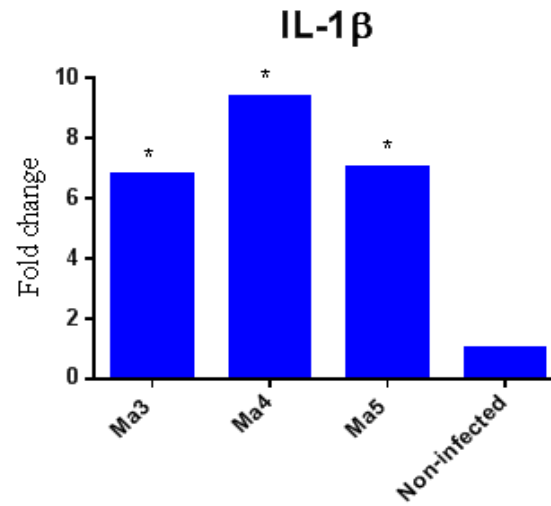
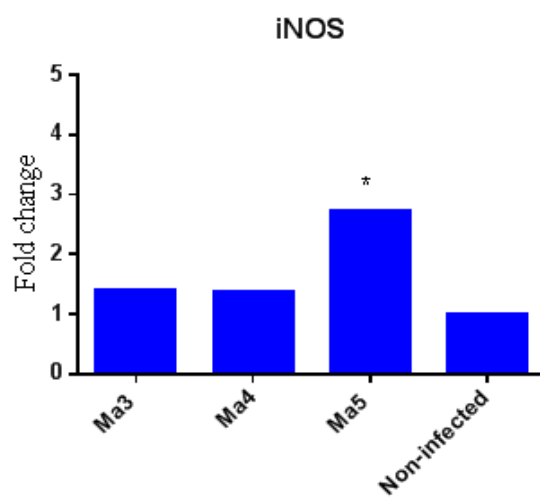
Group4  
Control

Three birds from each group euthanized at the day of infection and at 7, 14 and 21 days post infection.



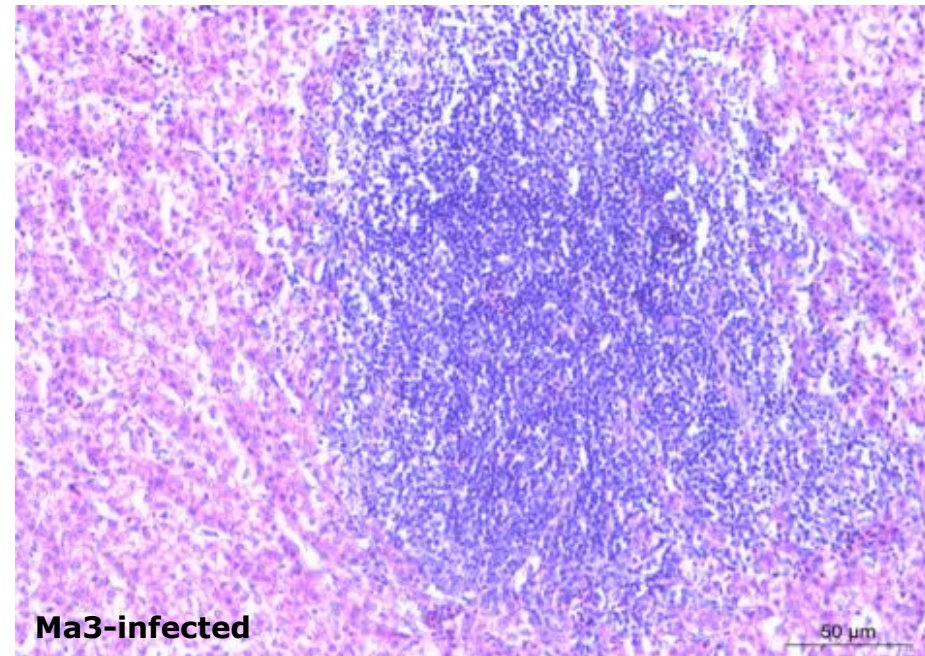
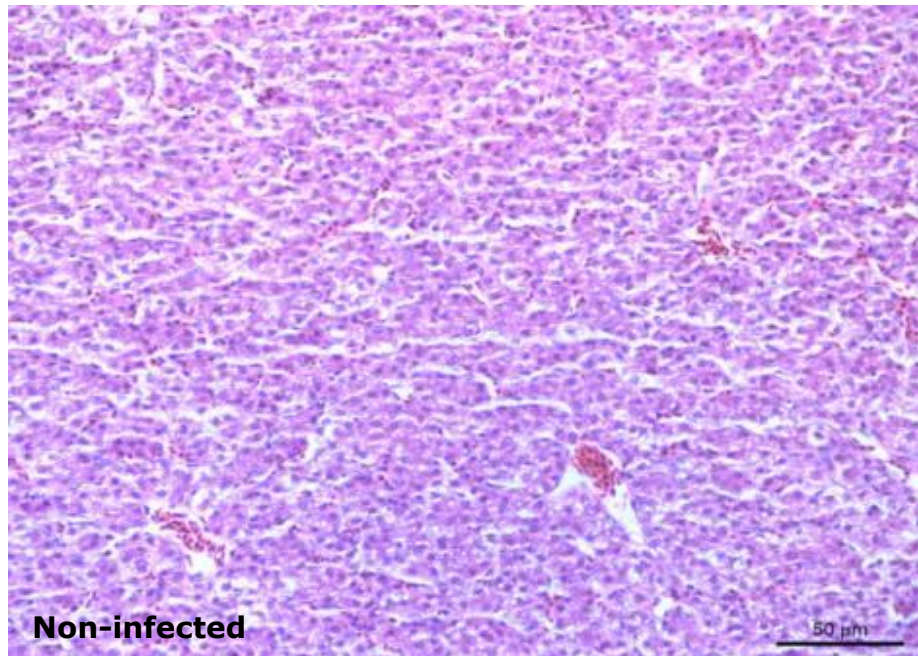
Blood		CFU
Liver		CFU, qPCR, Histpathology
Spleen		CFU, qPCR, Histpathology

# 21 days post infection (Liver)



# Histopathological lesions

Lymphocyte infiltration observed in all infected groups



Representative histopathological micrographs from the liver of chickens 21 days after infection with different *M. avium* isolates. H&E staining, magnification: X20, scale bar: 50μm.

# Summary and Conclusion

- There are *M. avium* strains specific differences in induction of pro-inflammatory cytokine expression.
- These differences do not appear to relate to source of strains
- The differential response does not correlated with granuloma formation.
- Multiple signal pathways participate in regulation of the signal transduction during infection.
- There are several mechanisms of signal transduction depending upon mycobacterial species, type of cytokine and the type of cells being studied.
- Our data support the concept of a general similarity between the immune response to mycobacterial infection in avian species, humans and mice.

# Acknowledgements

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**Thank you!**  
**Any questions?**