# Does pyrazinamide have a host-directed mechanism of action?

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# Rationale

1)

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## The Antituberculosis Drug Pyrazinamide Affects the Course of Cutaneous Leishmaniasis In Vivo and Increases Activation of Macrophages and Dendritic Cells[▽]

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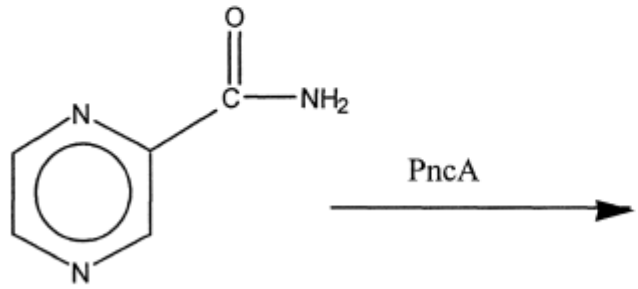
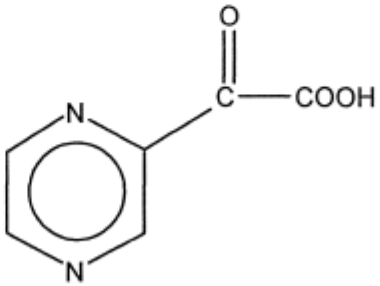
2) Pyrazinamide (PZA) is a nicotinamide analog

**Does PZA have immunomodulatory activity?**

# Nicotinamide and nicotinamide analogs



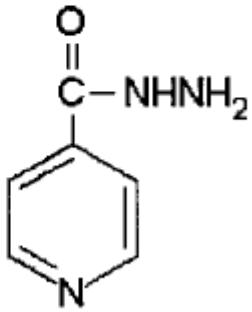

PncA



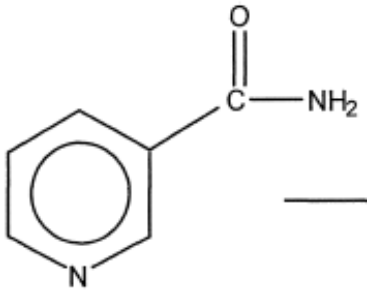

\+ Ammonia

Pyrazinamide

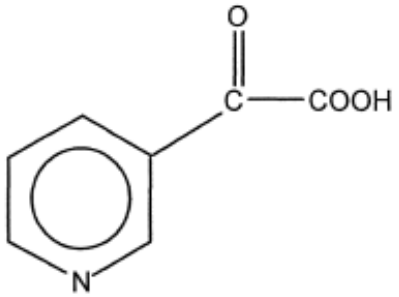
Pyrazinoic acid




**Isoniazid**




PncA




\+ Ammonia

Nicotinamide

Nicotinic acid

# Does PZA stimulate a proinflammatory cytokine response in J774 cells?

**DAY 1:** Count and plate J774 cells

**DAY 2:** Add drugs

- No drug (unstimulated cells, negative control)
- LPS/IFN-γ (positive control)
- Nicotinamide (NAM)
- Nicotinic acid (NIC)
- Pyrazinamide (PZA)
- Pyrazinoic acid (POA)

40 µg/mL

- Isoniazid (INH), 5 µg/mL

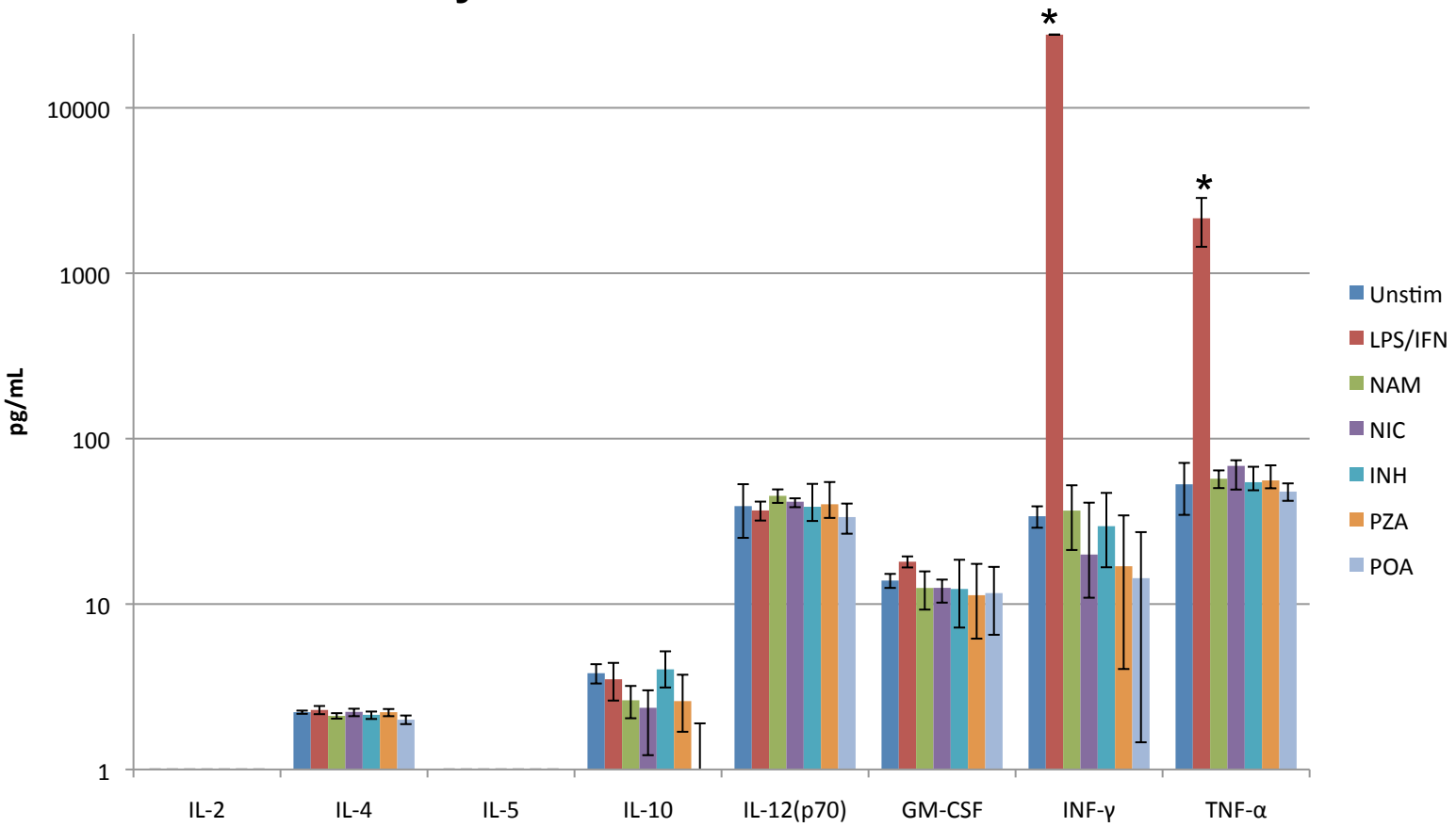
Six hours post-stimulation

- Collect culture supernatants (cytokine analysis)
- Isolate RNA from cells (microarray analysis)

**DAY 3:** Twenty-four hours post-stimulation

- Collect culture supernatants (cytokine analysis)
- Isolate RNA from cells

# Uninfected J774 cells
# Cytokine secretion: 6 hours

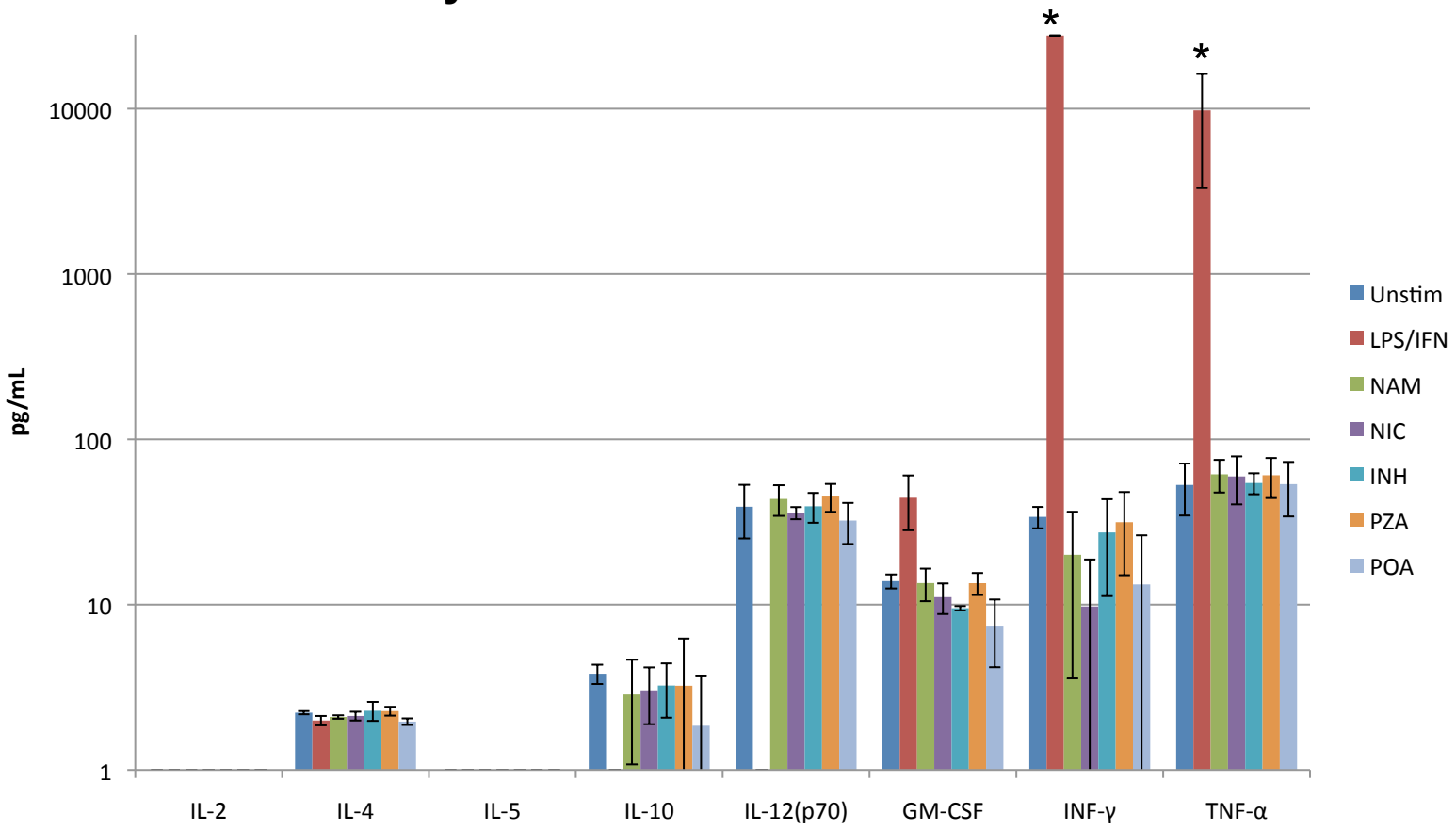



* $p < 0.05$ compared to unstimulated cells, Student's t-test

NAM: nicotinamide NIC: nicotinic acid INH: isoniazid PZA: pyrazinamide POA: pyrazinoic acid

## Uninfected J774 cells

## Cytokine secretion: 24 hours

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NAM: nicotinamide NIC: nicotinic acid INH: isoniazid PZA: pyrazinamide POA: pyrazinoic acid

| | Mendez *et al*. 2009 | This Study |
|---|---|---|
| Cells | J774 | J774 |
| Media | DMEM<br>FBS (10%)<br>L-glutamine (2mM)<br>Penicillin (100U/mL)<br>Streptomycin (100µg/mL) | RPMI<br>FBS (10%)<br>L-glutamine (2mM)<br>HEPES (20mM) |
| Drugs - source | provided by Cynamon and Welch | Sigma |
| Drugs - diluent | DMSO | HBSS |
| [PZA] | 10µM (1.23µg/mL)<br>100µM (12.3µg/mL) | 325µM (40µg/mL) |
| Positive control | LPS (100ng/mL)<br>IFN-γ (10U) | LPS (500ng/mL)<br>IFN-γ (50ng/mL) |
| Negative control | Unstimulated cells | Unstimulated cells |
| Sample | Culture supernatant | Culture supernatant |
| Time points | 24 hours | 6 hours<br>24 hours |
| Assay | ELISA | Multi-Plex |

# Does PZA modulate the cytokine response during an *M. tuberculosis* infection?

**DAY 1:** Count and plate J774 cells

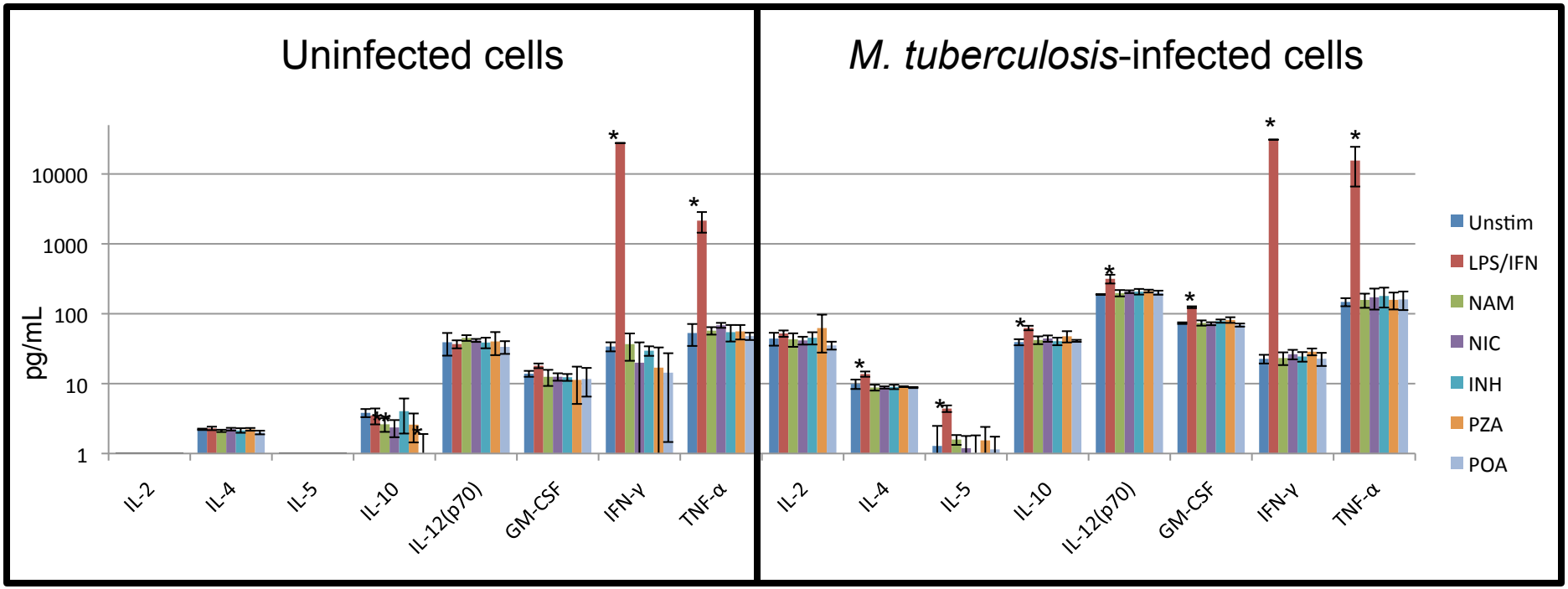
**DAY 2:** Infect cells at MOI = 2

**DAY 3:** Add drugs
- Unstimulated
- LPS/IFN-γ
- PZA, POA, NIC, NAM, INH

Six hours post-stimulation
- Collect culture supernatants (cytokine analysis)
- Isolate RNA from cells (microarray analysis)

**DAY 4:** Twenty-four hours post-stimulation
- Collect culture supernatants (cytokine analysis)
- Isolate RNA from cells

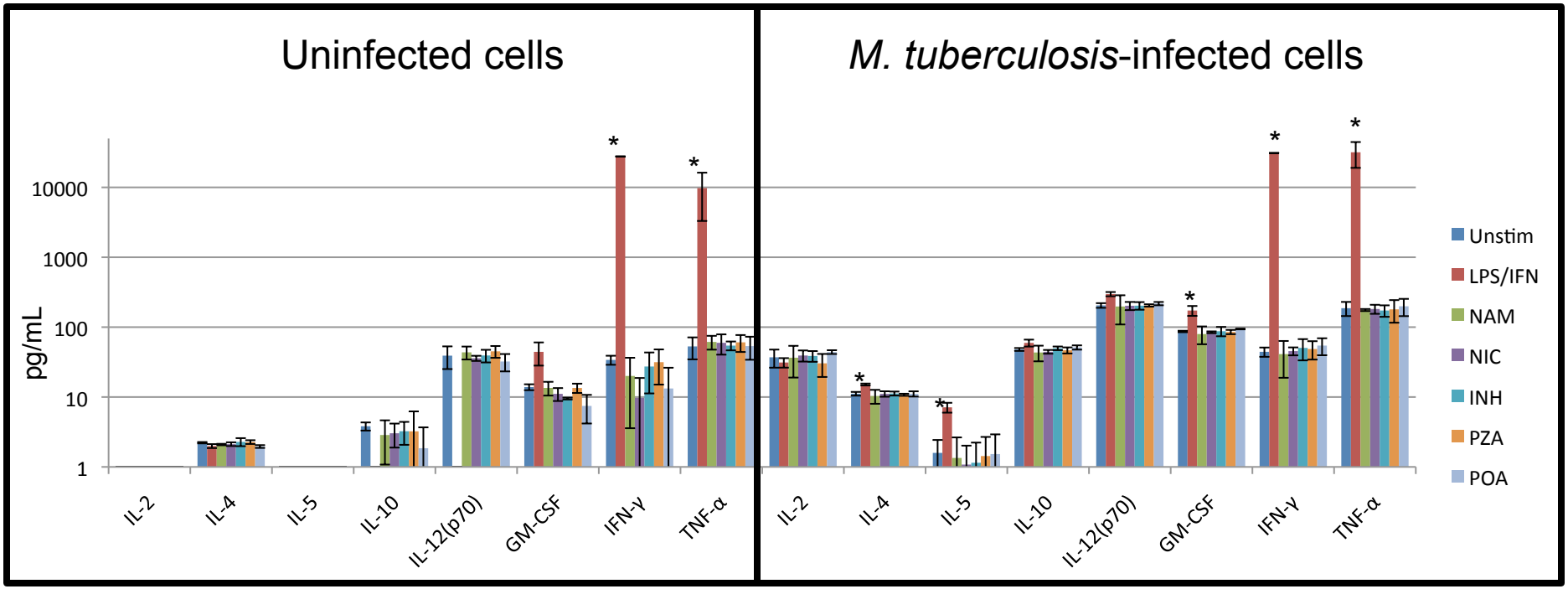
# J774 cells: Cytokine secretion 6-hours post stimulation




* $p < 0.05$ compared to unstimulated cells, Student's t-test

NAM: nicotinamide NIC: nicotinic acid INH: isoniazid PZA: pyrazinamide POA: pyrazinoic acid

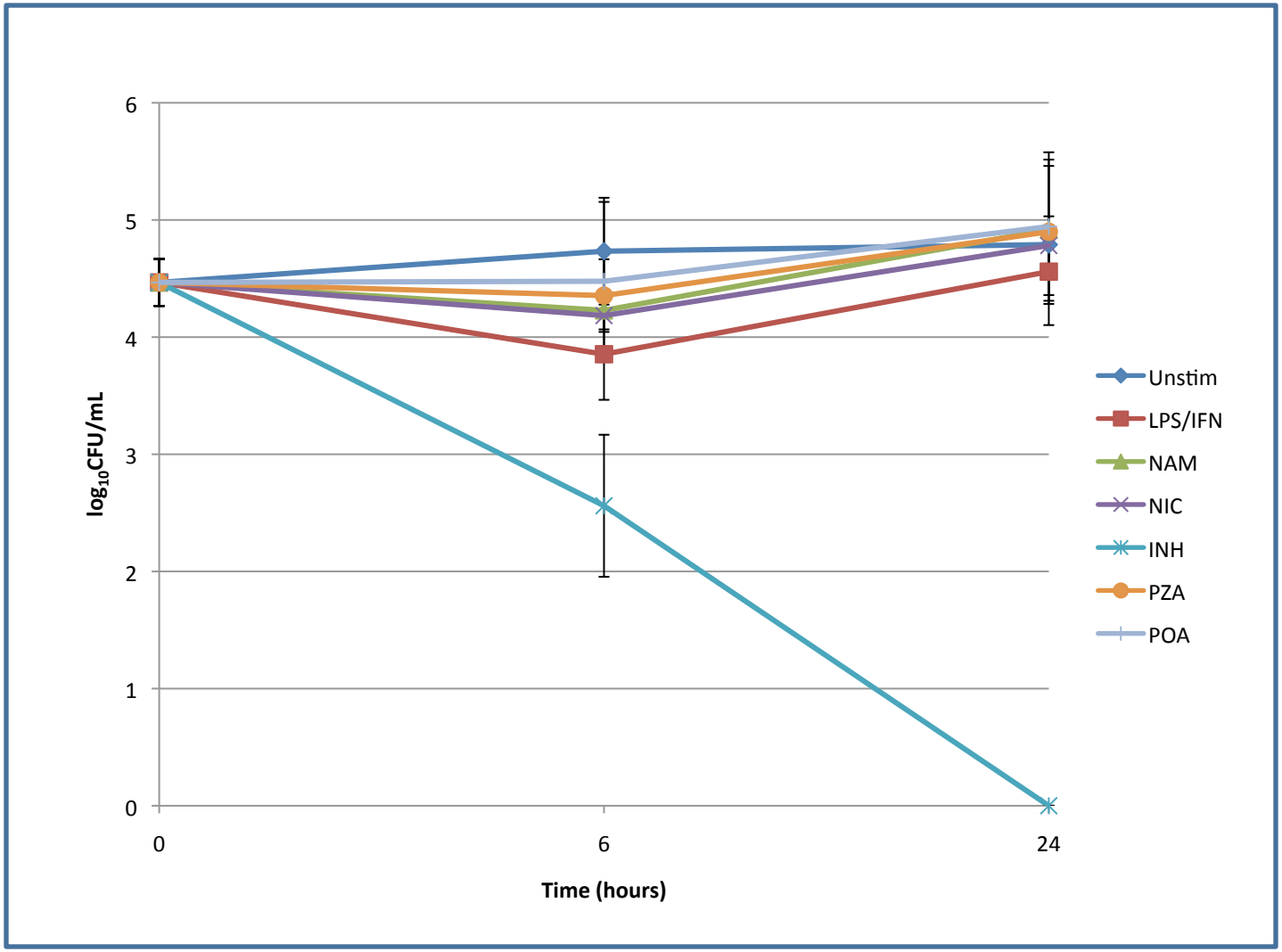
# J774 cells: Cytokine secretion 24-hours post stimulation




* $p < 0.05$ compared to unstimulated cells, Student's t-test

NAM: nicotinamide NIC: nicotinic acid INH: isoniazid PZA: pyrazinamide POA: pyrazinoic acid

# *M. tuberculosis*-infected J774 cells



## Summary I

In this experimental system, we have observed the following:

1) neither PZA, POA, NAM, NIC nor INH stimulated uninfected J774 cells

2) these compounds did not alter cytokine secretion of *M.tb.*-infected J774 cells

3) INH, but none of the other compounds, exhibited *M.tb.*-killing activity.

# Transcriptomic analyses: 24-hour time point

- Total RNA samples were analyzed using Illumina Beadchip Mouse RefSeq-8 microarray platform.
- Drug administration was associated with <20 gene expression changes in uninfected cells (none of which were cytokine-encoding genes).
- Drug administration was not associated with any gene expression changes in *M.tb.*-infected J774 cells.

# Uninfected J774 cells, 24-hours post-exposure
# Genes with >2-fold increase (statistically signficant)

| Fold-change in expression upon exposure to the following drugs: | | | | | | |
|---|---|---|---|---|---|---|
| **INH** | **NAM** | **NIC** | **PZA** | **POA** | **Gene Symbol** | **Gene Product Description** |
| --- | 2.08 | --- | --- | --- | Bhlhb2 | basic helix-loop-helix domain containing class B2 (Bhlhb2) mRNA. |
| --- | 2.99 | --- | --- | --- | Ccl7 | chemokine (C-C motif) ligand 7 (Ccl7) mRNA. |
| --- | 3.67 | --- | --- | --- | Cd40 | CD40 antigen (Cd40) transcript variant 5 mRNA. |
| 4.82 | --- | --- | --- | --- | Cps1 | carbamoyl-phosphate synthetase 1 (Cps1) nuclear gene encoding mitochondrial protein mRNA. XM_993466 |
| --- | 14.38 | --- | --- | --- | Cxcl9 | chemokine (C-X-C motif) ligand 9 (Cxcl9) mRNA. |
| --- | 2.36 | --- | 2.29 | --- | Egr1 | early growth response 1 (Egr1) mRNA. |
| --- | 2.36 | --- | --- | --- | Hdc | histidine decarboxylase (Hdc) mRNA. |
| --- | --- | 2.10 | --- | 2.08 | Id1 | inhibitor of DNA binding 1 (Id1) mRNA. |
| --- | 2.17 | 2.17 | 2.22 | 2.33 | Id3 | inhibitor of DNA binding 3 (Id3) mRNA. |
| --- | 2.03 | --- | --- | --- | Il6 | interleukin 6 (Il6) mRNA. |
| --- | 2.34 | --- | 3.23 | --- | Plau | plasminogen activator urokinase (Plau) mRNA. |
| 2.87 | 2.05 | --- | --- | --- | Serpina3f | serine (or cysteine) peptidase inhibitor clade A member 3F (Serpina3f) mRNA. |
| --- | 6.02 | --- | --- | --- | Serpina3g | serine (or cysteine) peptidase inhibitor clade A member 3G (Serpina3g) mRNA. |
| --- | 4.99 | --- | --- | --- | Serpina3h | serine (or cysteine) peptidase inhibitor clade A member 3H (Serpina3h) mRNA. |
| 2.18 | 2.02 | --- | 2.30 | --- | Sfrs7 | splicing factor arginine/serine-rich 7 (Sfrs7) mRNA. |
| 2.49 | --- | --- | --- | --- | Slc7a2 | solute carrier family 7 (cationic amino acid transporter y+ system) member 2 (Slc7a2) transcript variant 2 mRNA. |
| --- | 3.54 | --- | --- | --- | Ubd | ubiquitin D (Ubd) mRNA. |
| --- | --- | --- | 2.35 | 2.04 | Usp2 | ubiquitin specific peptidase 2 (Usp2) transcript variant 2 mRNA. |

## Summary II

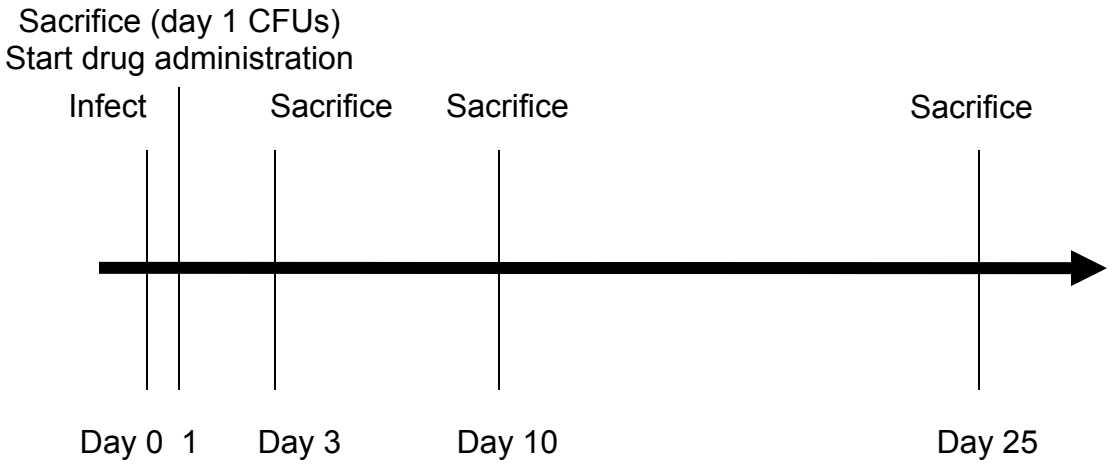
In this experimental system, we have observed the following:

1) PZA, POA, NAM, NIC and INH were associated with few gene expression changes in uninfected J774 cells (none were genes encoding cytokines)

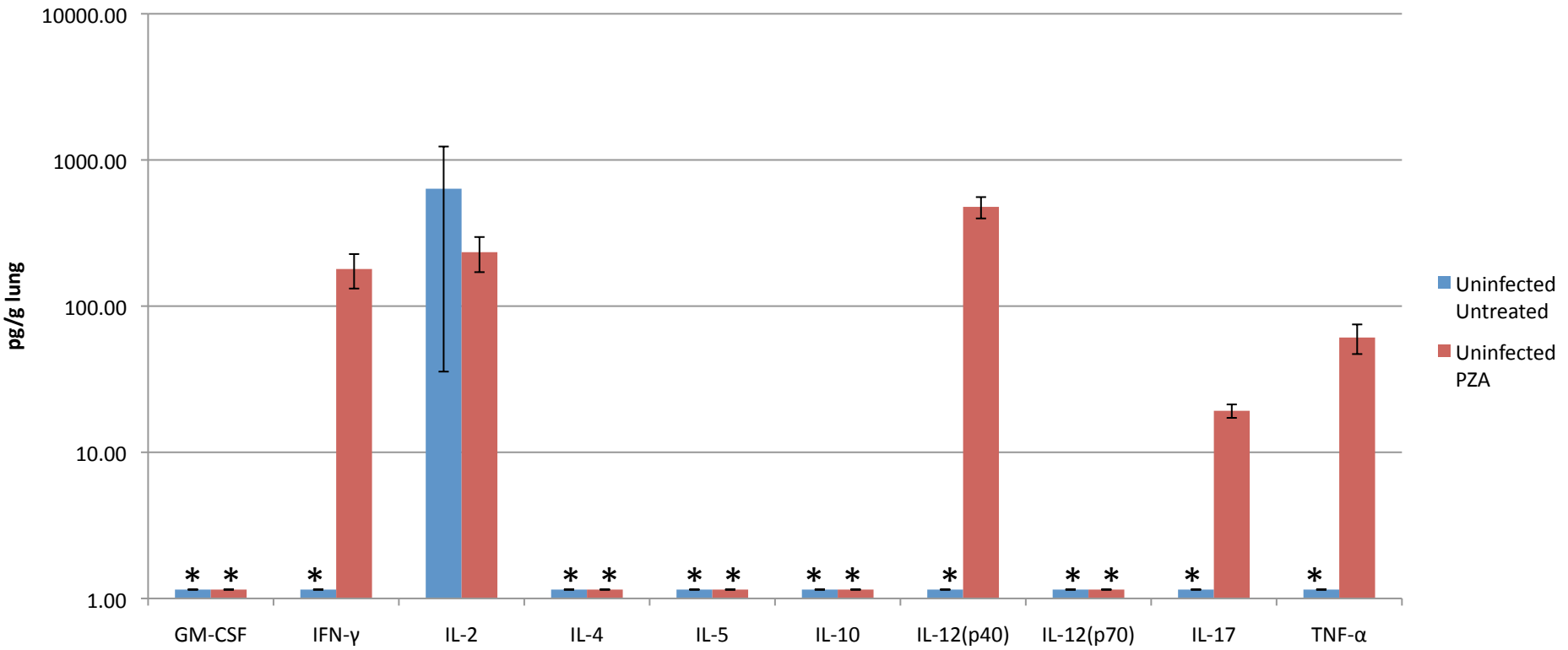
2) these compounds were not associated with any gene expression changes in *M.tb.*-infected J774 cells

# Does PZA stimulate a proinflammatory cytokine response in *in vivo*?

- C3HeB/FeJ (Kramnik) mice
- Uninfected or infected with *M.tb.* CD1551 (aerosol infection with 3.6 $\log_{10}$CFU on day 1)
- PZA (150mg/kg ) administered daily (5/7) by oral gavage
- Sacrifice on days 3, 10, and 25
- Analyze cytokine levels in lung homogenates

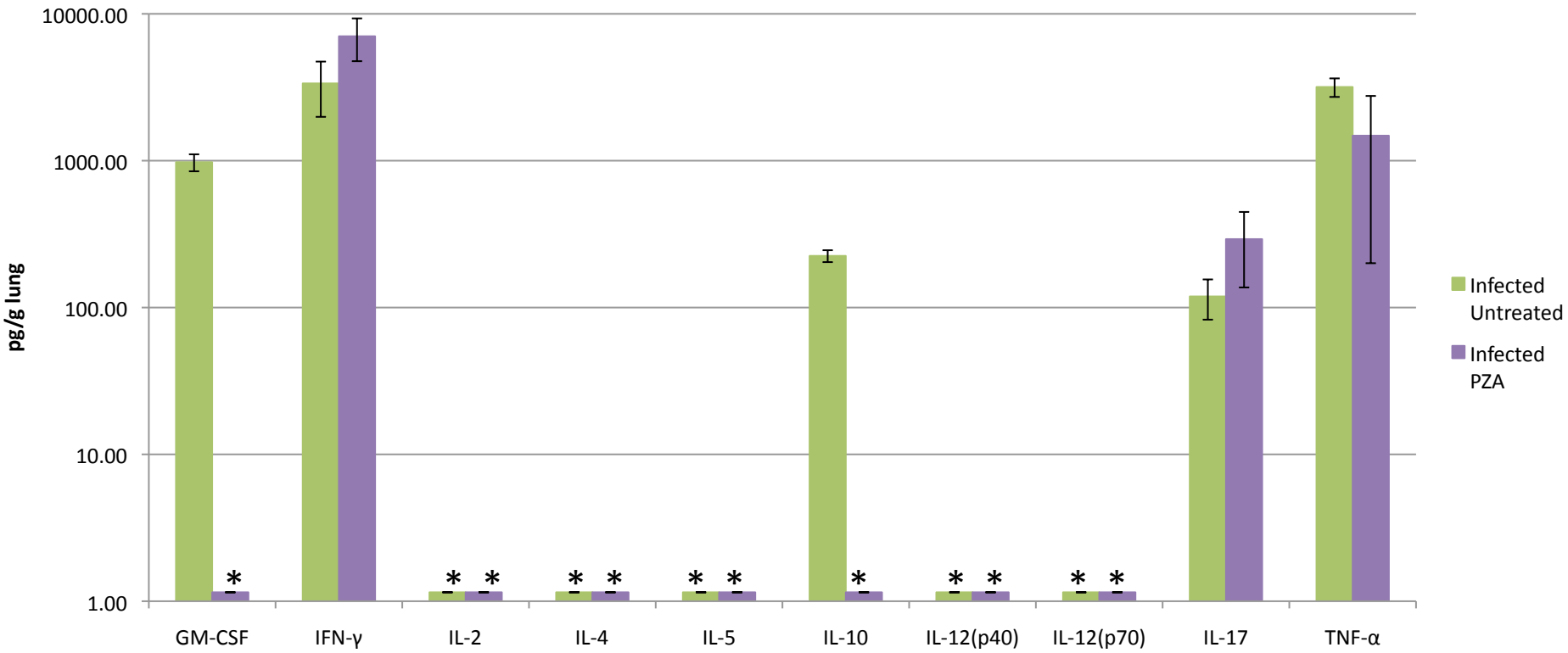


# Cytokine levels in **UNINFECTED** C3HeB/FeJ Mouse Lung Homogenate
## 25 days of daily PZA (150mg/kg via oral gavage)



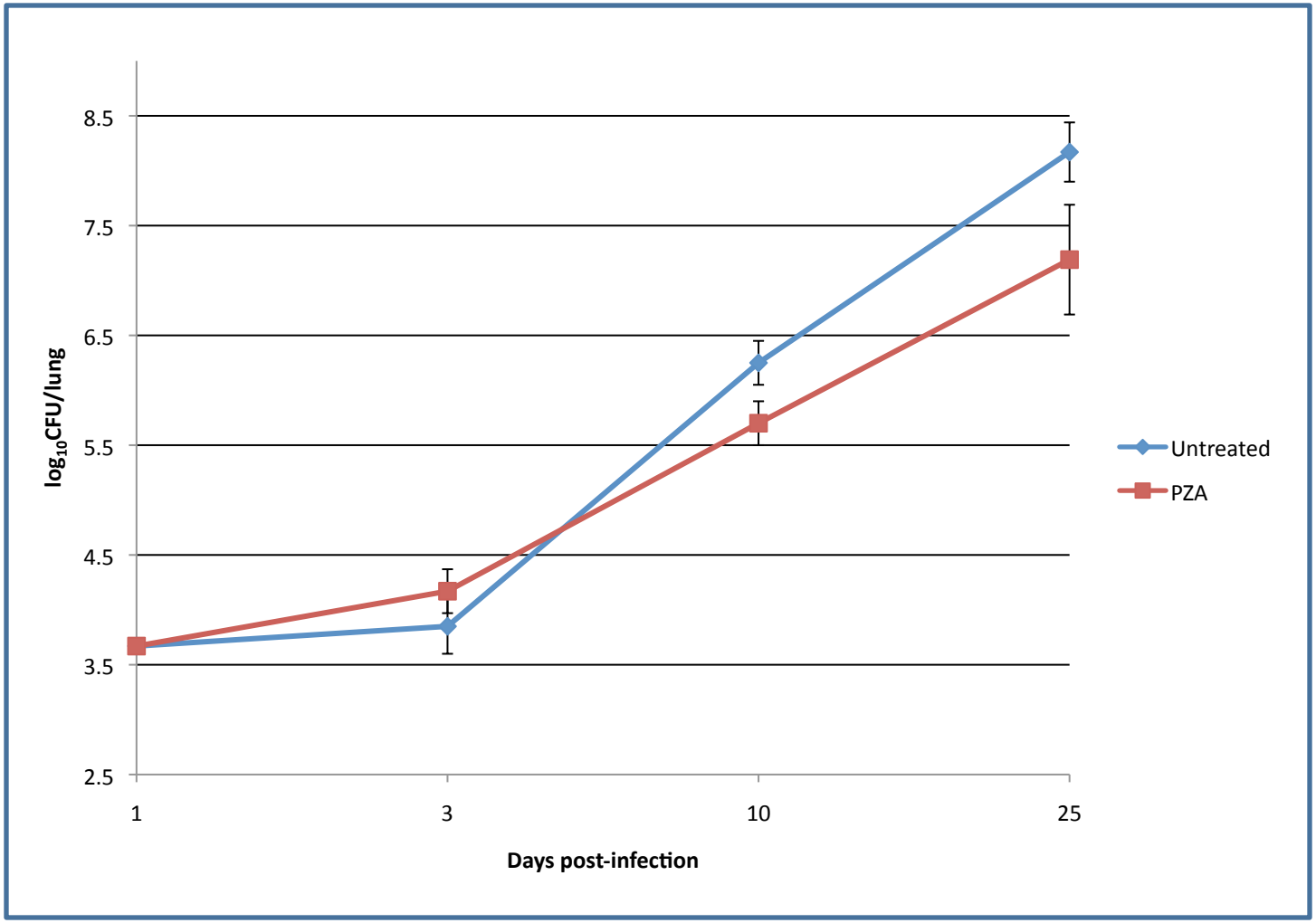

* Below detection limit

# Cytokine levels in ***M.tb.*-INFECTED** C3HeB/FeJ Mouse Lung Homogenate
## 25 days of daily PZA (150mg/kg via oral gavage)




* Below detection limit

# *M. tuberculosis*-infected C3HeB/FeJ mice



## Summary III

In this experimental system, we have observed the following:

1) up to 10 days PZA administration to uninfected or M.tb.-infected C3HeB/FeJ mice did not change the lung cytokine profiles

3) 25 days of PZA administration to uninfected C3HeB/FeJ mice was associated with an increase in INF-γ, IL-12(p40), IL-17 and TNF-α (Th1 cytokines)

3) 25 days of PZA administration to *M.tb.*-infected mice was associated with a decrease in GM-CSF and IL-10 (Th2 cytokines)

4) PZA adminstration was associated with a slightly lower CFU counts in the lungs of *M.tb.*-infected mice.

## Conclusion

Based on these experiments, PZA may have immunomodulatory activity *in vivo* in C3HeB/FeJ mice.

## Next Steps

The mice data need to be confirmed; we may include transcriptional analyses as well.

We will also investigate the effects of NAM, NIC, POA and INH administration on mouse lungs.

We will include different strains of mice.

**THANK YOU!**

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