Lessons for TB Treatment from the Zebrafish

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Global burden of tuberculosis

Multidrug resistance is widespread

Percentage of new TB cases with MDR/RR-TB (WHO - Global TB report 2017)
Genetic susceptibilities influence TB outcomes

The Lubeck Diaster - 1929

251 infants accidentally given three doses of virulent M. tuberculosis as newborns

72 died of TB

173 showed some clinical or radiological features of TB but survived after differing levels of disease
TB is a complex disease

Reviewed in Cambier, Falkow, Ramakrishnan, *Cell* 2015
Rediscovering *Mycobacterium marinum*, a close genetic relative of *Mycobacterium tuberculosis*.

Photo by Paul Edelstein

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**SPONTANEOUS TUBERCULOSIS IN SALT WATER FISH**

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Spontaneous tuberculosis in cold blooded animals has been noted by Sibley in a ringed snake (Tropidonotus matrix), by Bataillon, Dubard and Terre in a carp (Cyprinus carpio), by Friedmann in turtles (Chelone corticata), and by Rupprecht in a frog.

The spontaneous occurrence in salt water fish of lesions associated with acidfast bacilli was first described by Alexander in lupus like ulcers of a cod fish (Gadus callarias) and by Sutherland in the subcutaneous tissue and organs of a halibut (Hippoglossus hippoglossus). In neither case was the organism isolated.

Through the kindness of Dr. Henry Winsor I have had the opportunity of studying a number of salt water fish which have died in the tanks of the Philadelphia Aquarium. At necropsy numerous tubercles were found in the different organs and from these organs I have isolated an acidfast bacillus. The tissue was digested with 2% solution of sodium hydroxide during from 30 minutes to 1 hour at 37 C. and the sediment was neutralized with a 6% solution of hydrochloric acid. After centrifugation, the sediment was inoculated on different culture mediums.

The tubercles were found in the spleen and liver of all of the fish examined and in the eggs, kidneys, testis, pericardium and eye of some of
Mycobacterium marinum infection of the hand
Zebrafish TB Resembles Human TB
Zebrafish larvae form tuberculous granulomas
A high throughput platform for infection studies
Genetic screen reveals host susceptibility loci

Tobin et al., *Cell* 2010
A Genetic Screen Reveals Hypersusceptible Mutants

*LTA4H* Mutants Exhibit Bacterial Cording

WT  

*LTA4H* mutant
The cording revealed that the granulomas had become necrotic.
Leukotriene A4 hydrolase is a rheostat
LTA4H deficiency and excess both increase TB susceptibility in zebrafish and humans.

Tobin et al., *Cell* 2010
Tobin et al., *Cell* 2012
A human *LTA4H* promoter polymorphism regulates gene expression.
TB meningitis is the most severe form of disease
*LTA4H* genotype influences inflammation and survival in TB meningitis

Overall survival

Days after enrollment

(Thwaites et al., *NEJM*, 2004)
**LTA4H** genotype influences inflammation and survival in TB meningitis

**Overall survival**

**LTA4H Genotype-Adjusted Survival**

P=0.02

(Thwaites et al., *NEJM*, 2004)
*LTA4H* genotype influences treatment response

**Overall Response**
(Thwaites et al., *NEJM*, 2004)
*LTA4H* genotype influences treatment response

**Overall Response**
(Thwaites et al., *NEJM*, 2004)

**Genotype-adjusted Response**

![Graph showing survival over days after enrollment for Placebo and Dexamethasone with different genotypes.](image-url)
**LTA4H genotype influences treatment response**

Overall Response
(Thwaites et al., *NEJM*, 2004)

Genotype-adjusted Response

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**Days after enrollment**

**Survival**

**DEXAMETHASONE**

**PLACEBO**

**P=0.005**

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Mapping Tuberculosis Susceptibility
Review
Dead Cell Clearance
What makes LTA4H extremes susceptible?
Low and high LTA4H activity *both* cause infected macrophage necrosis by dysregulating TNF.
TNF excess triggers microbicidal reactive oxygen species in the mitochondrion which are bactericidal.
Mitochondrial ROS promote first increased bactericidal activity, then necrosis.

Roca and Ramakrishnan, *Cell* 2013
Mitochondrial ROS promote necrosis through the mitochondrial matrix protein Cyclophilin D

Roca and Ramakrishnan, Cell 2013
Surprise! Lysosomal components are also required for this necrosis pathway.
TNF-induced mitROS induce lysosomal ceramide production
Lysosomal cathepsin D activates BAX
BAX mediates apoptosis by oligomerizing at and piercing pores in the MOM, which releases pro-apoptogenic factors.

BAX also mediates mitochondrial necrosis independent of oligomerization and in collaboration with cyclophilin D.
Lysosomal cathepsin D activates BAX

- BAX mediates apoptosis by oligomerizing at and piercing pores in the MOM, which releases pro-apoptogenic factors.
- BAX also mediates mitochondrial necrosis independent of oligomerization and in collaboration with cyclophilin D.

However mitochondrially-tagged BAX fails to cause TNF-mediated necrosis.
BAX targets the ER to cause Ca2+ translocation into the mitochondrion
BAX causes mitochondrial Ca\(^{2+}\) overload in infected macrophages
Mitochondrial calcium overload requires upstream components also.
BAX activates Ryanodine receptors in the ER to cause mitochondrial calcium overload
Glucocorticoids worsen outcome of LTA4H low TB meningitis

Elucidation of the impact of glucocorticoids on the outcome of LTA4H low TB meningitis. The graph illustrates survival rates over time for different treatment groups: placebo, dexamethasone, and various dose levels (HET, LOW, HIGH). The data suggests that glucocorticoids may have a detrimental effect on the survival rates in this condition.

Vietnam
Thwaites et al, NEJM 2004
Glucocorticoids worsen outcome of LTA4H low TB meningitis

**Diagram Description:**
- The diagram shows survival rates over different days after enrollment for various conditions.
- The x-axis represents days after enrollment, ranging from 0 to 400.
- The y-axis represents survival probability, ranging from 0.4 to 1.0.
- There are three conditions: HET/LOW, HET, and HIGH.
- The survival rates are compared across different conditions and are presented with line graphs.

**References:**
- Vietnam Thwaites et al, NEJM 2004
- Vietnam Thuong et al, JID 2017
- Indonesia van Laarhoven et al, JID 2017
LTA4H/TNF-high pathway suggests downstream drugs
Reduction of cellular calcium with $\text{Ca}^{2+}$ channel blocking drugs inhibits necrosis.
Conclusions

- Studying TB can uncover fundamental new biology

- This pathway may be a common mechanism of necrosis in TB regardless of LTA4H genotype:
  - Evidence of high content of ceramide in necrotic TB granulomas (Kim et al., EMBO Mol Med 2010)
  - Key components of pathway (RIPK3, ROS and cyclophilin D) cause necrosis of human macrophages (Zhao et al., Mucosal Immunol 2017)

- Safe and inexpensive drugs can be repurposed as host-targeting drugs for both drug sensitive and resistant TB
Verapamil targets *M. tuberculosis* by inhibiting macrophage-induced antibiotic tolerance

Adams et al., *Cell* 2011
Adams, Szumowski, Ramakrishnan, *JID* 2014
Verapamil targets *M. tuberculosis* by inhibiting macrophage-induced antibiotic tolerance

Verapamil also targets the host by inhibiting a pathogenic macrophage necrosis pathway
Boiling River, Yellowstone National Park
Photo: Neeta Satam
Tricyclic antidepressants
Dantrolene
Nifedipine
Diltiazem
Flunarizine
Verapamil

Boiling River, Yellowstone National Park
Photo: Neeta Satam