Using zebrafish to understand inflammation in TB meningitis

Cressida Madigan (UCLA)
Lalita Ramakrishnan (Cambridge)
David Tobin (Duke)

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What are the initial events in TBM pathogenesis?

- Host factors: TNF, laminin A, TIRAP, TLR2, LTA4H
- Bacterial factors: pknD
- Route of CNS entry? Cells involved?
- What initially triggers the neuroinflammation?
- *In vivo* models: rabbit, mouse, guinea pig
1. Why zebrafish?

2. LTA4H modulates inflammation in TBM

1. Modeling CNS invasion
1. Why zebrafish?

2. LTA4H modulates inflammation in TBM

1. Modeling CNS invasion
Advantages of the zebrafish model to study mycobacterial pathogenesis

1. Orthologs for 70% of human genes
2. Genetics: CRISPR, morpholinos, transgenics
3. Established TB model (*M. marinum*)
4. Optically transparent → live imaging

Live imaging neuroinflammation in an intact animal

brain - action potentials

intracellular calcium

spinal cord - infection

M. marinum
myelin
macrophages

from Ahrens 2013

Madigan, submitted
1. Why zebrafish?

2. LTA4H modulates inflammation in TBM

1. Modeling CNS invasion
Humans and fish: low or high *lta4h* = increased mycobacterial disease severity

Zebrafish + *M. marinum*

- *lta4h* low
- *lta4h* high

Humans + TB meningitis

- *lta4h* intermediate
- *lta4h* low
- *lta4h* high

**Survival vs. Days after enrollment**

- low LTA4H → TNF
- high LTA4H → dexamethasone

*LTA4H* genotype of TB meningitis patients predicts treatment response to dexamethasone

**without dexamethasone**

- *Ita4h* low
- *Ita4h* intermediate
- *Ita4h* high

**with dexamethasone**

- *Ita4h* low
- *Ita4h* intermediate
- *Ita4h* high

*Ita4h* low $\rightarrow$ low TNF $\rightarrow$ low inflammation $\rightarrow$ dexamethasone hurts

*Ita4h* high $\rightarrow$ high TNF $\rightarrow$ high inflammation $\rightarrow$ dexamethasone helps

1. Why zebrafish?

2. LTA4H modulates inflammation in TBM

1. Modeling CNS invasion
1. Why zebrafish?

2. LTA4H modulates inflammation in TBM

1. Modeling CNS invasion
   – entry?
   – first mediators of inflammation?
How do mycobacteria enter the CNS?

**Blood**
- *E. coli* K1 strain
- *N. meningitidis*
- *S. agalactiae* (group B)
- *S. pneumoniae*
- *L. monocytogenes*
- *Trypanosoma spp.*
- *Borrelia sp.*
- *C. albicans*
- *C. neoformans*
- *H. influenzae* type b

**Endothelial cells**

**CNS**

**Transcellular**
- *Borrelia sp.*
- *Treponema pallidum*
- *Trypanosoma spp.*
- *S. pneumoniae* (?)

**Paracellular**
- *C. neoformans*
- *L. monocytogenes*
- *S. pneumoniae* (?)

**“Trojan horse”**

Adapted from Barichello 2013
Zebrafish larvae have a functional blood-brain barrier

Hallmarks of mammalian blood-brain barrier:
1. Endothelial tight-junction proteins
2. Size-selective

Madigan, unpublished
M. marinum attach to CNS blood vessels and replicate

Madigan, unpublished
Live imaging infection in an intact animal

(1) tail vein *M. marinum*

(2) tail vein dextran

(3) brain

Madigan, unpublished
Mycobacterial entry into CNS: working model

1. vessel attachment
2. vessel exit
3. vessel leakage

0-2 days
3-4 days
5 days

M. marinum
dextran

Madigan, unpublished
M. marinum attach to vessels without macrophages...

M. marinum
macrophages (mpeg1-dsRed)
endothelial cells (kdrl-GFP)

Madigan, unpublished
...but after vessel exit, macrophages/microglia arrive

*M. marinum* macrophages/microglia (*mpeg1-dsRed*) dextran

Madigan, unpublished
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Summary

1. LTA4H modulates inflammation in TBM
   • high \( lta4h \) = high TNF = give dexamethasone
   \( \rightarrow \) New therapies?

2. Modeling CNS invasion in zebrafish
   – Mycobacterial entry does not require macrophages
   – Blood-brain barrier breakdown
   \( \rightarrow \) Mechanism of entry: endothelial cell tight junctions?
   \( \rightarrow \) How does inflammation contribute to vessel leakage?
   \( \rightarrow \) Host/pathogen factors?
Summary

1. LTA4H modulates inflammation in TBM
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     $\rightarrow$ New therapies?

2. Modeling CNS invasion in zebrafish
   – Mycobacterial entry does not require macrophages
   – Blood-brain barrier breakdown
     $\rightarrow$ Mechanism of entry & vessel leakage?
Future Directions:
whole animal screening for new TBM therapies

- 96-well plate + compounds
- vessel attachment
- bacteria in CNS

image wells

total bacterial burden
Screen identifies zebrafish mutants with increased *M. marinum* susceptibility

Mutagen + *M. marinum*

| 25% +/+ | 50% mutant/+ | 25% mutant/mutant |

*maternal genes only*

caudal vein *M. marinum*

Tobin 2010, Tobin 2012
Other genotype-specific therapies?

inadequate inflammation: 15-LOX inhibitor, LXA₄R antagonist

excess inflammation: aspirin, LTB4DH inducer, LTB₄R antagonist
Vessels near *M. marinum* become permeable

Intact

Leaking

| dextran | M. marinum dextran macrophages/microglia |

% animals with dextran leakage

days post infection

0 2 3 4 5 6

80 60 40 20 0
Zebrafish- *M. marinum* model shows 
*LTA4H* activity increases TNF and inflammation

Prediction:

*ltA4h* low → low TNF → low inflammation → dexamethasone hurts

*ltA4h* high → high TNF → high inflammation → dexamethasone helps

Tobin 2010, Tobin 2012