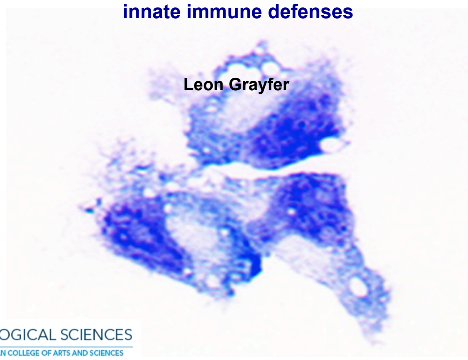
Successes and pitfalls of amphibian anti-ranaviral innate immune defenses



Leon Grayfer

BIOLOGICAL SCIENCES
COLUMBIAN COLLEGE OF ARTS AND SCIENCES

Ranaviruses: emerging cold-blooded killers

Ranavirus (family *Iridoviridae*):

Icosahedral, dsDNA viruses

Wide susceptible host range

Juveniles most susceptible

Frog Virus 3 (FV3) disseminating to new hosts

davidstang.com

Xenopus laevis - FV3: a model of amphibian anti-ranaviral immunity

X. laevis is an ideal platform for FV3 research

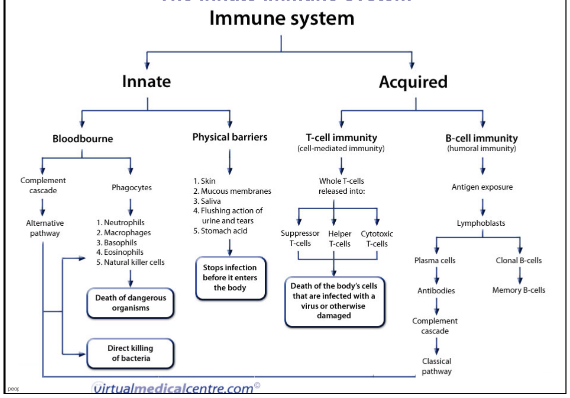
X. laevis adults successfully clear FV3 infections

Tadpoles succumb to FV3 infection within a month

What amphibian innate immune components confer susceptibility and resistance to FV3?

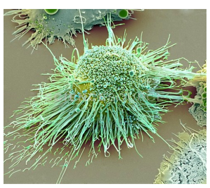
http://en.wikipedia.org/

The innate immune svstem



Cytokine regulation of innate immunity

Proinflammatory cyokines
IL-1β
IL-8
TNFα



Antiviral cyokines
IFNs

Immunosuppressive cyokines
IL-10
TGFβ
IL-4

sciencesource

"Susceptibility of *Xenopus laevis* tadpoles to infection by the ranavirus Frog Virus 3 correlates with a reduced and delayed innate immune response in comparison with adult frogs"

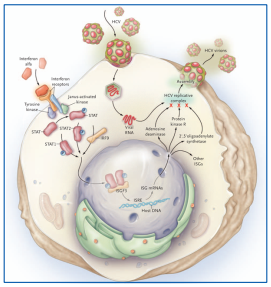
Tadpoles exhibit modest and delayed leukocyte and tissue expression of inflammation-associated (TNF-α, IL-1β and IFN-γ) and antiviral (Mx1) genes

The same tadpole genes are readily unregulated following heat-killed *E. coli* stimulation

"Our study suggests that tadpole susceptibility to FV3 infection is partially due to poor virus-elicited innate immune responses"

- De Jesús Andino *et al.*, 2012

What are the roles of amphibian antiviral interferons (IFNs) during FV3 infections?



Mammals possess IFNα, β, κ, ω, ε (δ only in pigs and τ in ruminants)

Most are multi-gene families

Fish and amphibians do not have IFNα, β …

Instead possess unique type I IFNs

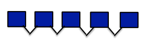
The amphibian IFN system remains largely not described

p://depts.washington.edu/hepstudy/hepC/mgmt/meda/discussion.html

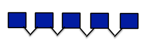
The evolution of vertebrate IFN immunity

Reptiles, birds and mammals:
- possess intron-less type I IFN genes

Bony fish:
- possess 5 exon / 4 intron type I IFN genes

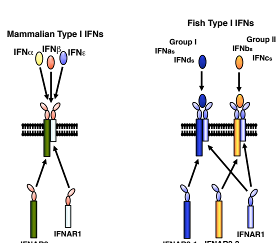


Amphibians:
- possess 5 exon / 4 intron type I IFN genes



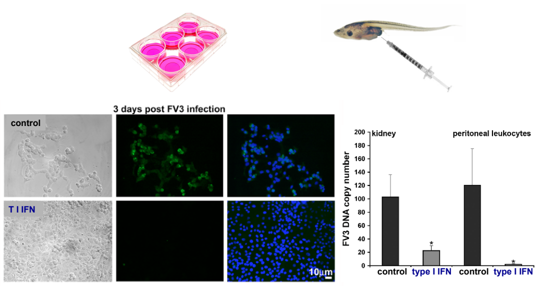
odh.ohio.gov; petfinder; dailymail; sites;

Mammalian and fish type I IFNs



Adopted from: Zou and Secombes (2012) Teleost fish interferons and their role in immunity

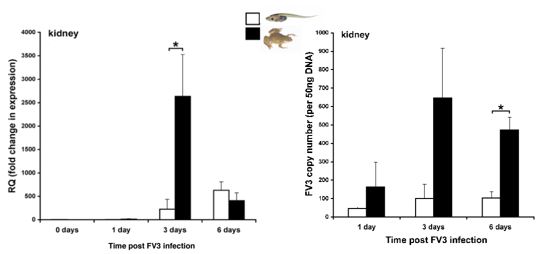
X. laevis type I IFN confers anti-FV3 protection



α-53R Ab courtesy of Dr. V. Greg Chinchar

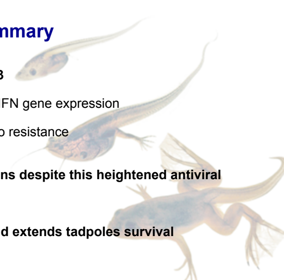
Grayfer *et al.*, 2014

X. laevis type I IFN confers anti-FV3 protection



Grayfer *et al.*, 2014

Summary



Adult *X. laevis* are resistant to FV3

- mount faster and more robust IFN gene expression
- could be a factor contributing to resistance

Adults possess higher viral burdens despite this heightened antiviral response

Type I IFN lowers viral burdens and extends tadpoles survival

- Inefficient antiviral immunity?
- Possible determinant of susceptibility
- Likely other contributing factors

Why do tadpoles possess lower FV3 loads but still die from infection?

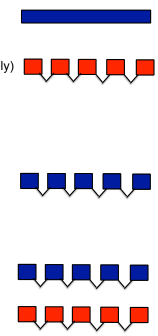
Grayfer *et al.*, 2014

The evolution of vertebrate IFN immunity

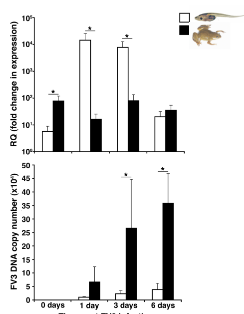
Reptiles, birds and mammals:
- possess intron-less type I IFN genes
- IFN-λ1, IFN-λ2 and IFN-λ3 (IL29, IL28A and IL28B, respectively)
- encoded by 5 exon / 4 intron genes
- similar antiviral effects to those conferred by type I IFNs

Bony fish:
- possess 5 exon / 4 intron type I IFN genes
- currently believed to lack type III IFNs

Amphibians:
- possess 5 exon / 4 intron type I IFN genes
- possess 5 exon / 4 intron type III IFN genes

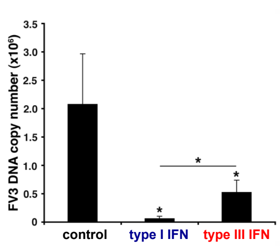


FV3-infected tadpoles rapidly upregulate their kidney Type III IFN gene expression



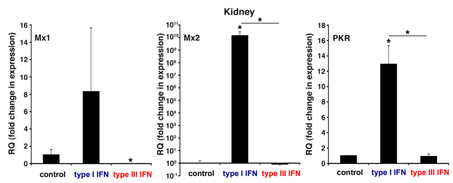
Grayfer *et al.*, 2015

Type I IFN confers greater anti-FV3 protection of A6 cells than type III IFN



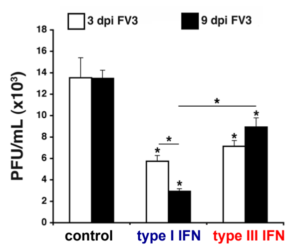
Grayfer *et al.*, 2015

Type I IFN elicits greater expression of tadpole kidney IFN-stimulated genes than the type III IFN



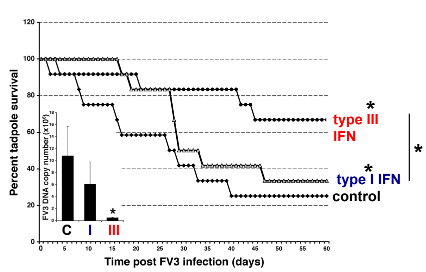
Grayfer *et al.*, 2015

Type I IFN provides greater tadpoles anti-FV3 protection than type III IFN



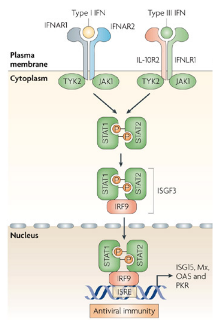
Grayfer *et al.*, 2015

Type I IFN coffers greater anti-FV3 protection than the type III IFN



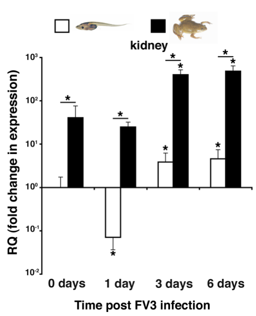
Grayfer *et al.*, 2015

Interferon cytokine signaling



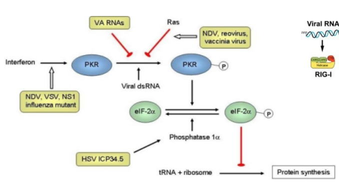
Anthony J. Sadler & Bryan R. G. Williams
Nature Reviews Immunology **8**, 559–568

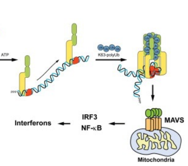
FV3-infected tadpole kidney type III IFN receptor gene expression



Grayfer *et al.*, 2015

The induction of cellular antiviral state





http://openi.nlm.nih.gov/detailedresult.php?img=2883714_viruses-02-00078f1&req=4

http://www.cell.com/immunity/abstract/S1074-7613(12)00230-0

Frog Virus 3:
a formidable foe of amphibian immunity

98 putative open reading frames

Function of ~1/3 of these known or inferred

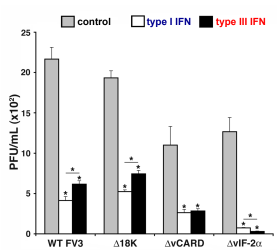
Several of these are putative immune evasion genes

vCARD and vIF-2*α*

Improved knockout methodology reveals that Frog Virus 3 mutants lacking either the 18K immediate-early gene or the truncated vIF-2alpha gene are defective for replication and growth *in vivo*.

- Chen *et al.*, 2011

r*X*/IFNλ confers equal to greater protection than r*X*/IFN against *Δ*vCARD- and *Δ*vIF-2α-FV3



Grayfer *et al.*, 2015

Summary

Tadpoles upregulate type III over type I IFN expression during FV3 infections

FV3 dampens the tadpole type III IFN responses (vIF-2α and vCARD)

Relative anti-FV3 efficacies of type I Vs type III IFNs may reflect this

Insights into amphibian type I and type III IFN responses will help defined immune limitations of these animals and enhance our appreciation for the evolutionary origins of our own antiviral defenses

Why do significantly lowered FV3 burdens still lead to tadpole mortality?

Early evidence for FV3 pathogenesis and cell tropism

Aubertin A.M., Hirth C., Travo C., Nonnenmacher H., Kirn A. **Preparation and properties of an inhibitory extract from frog virus 3 particles.** J. Virol. 1973;11:694–701.

- Solubilization of FV3 prepackaged components
- Soluble components inhibit host nucleic acid synthesis
- Neutralization of the activity by anti-FV3 Ab

Gut J.P., Anton M., Bingen A., Vetter J.M., Kirn A. **Frog virus 3 induces a fatal hepatitis in rats**. Lab. Invest. 1981;45:218–228.

Kirn A., Gut J.P., Elharrar M. **FV3 (Frog Virus 3) toxicity for the mouse.** Nouv. Presse. Med. 1972;1:19–43.

Elharrar M., Hirth C., Blanc J., Kirn A. **Pathogenesis of the toxic hepatitis of mice provoked by FV3 (frog virus 3): Inhibition of the liver macromolecular synthesis.** Biochem. Biophys. Acta. 1973;319:91–102.

The truth is out there!

Kirn A., Steffan A.M., Bingen A. **Inhibition of erythrophagocytosis by cultured rat Kupffer cells infected with frog virus 3**. J. Reticuloendothel. Soc. 1980;28:381–388.

Gendrault J.L., Steffan A.M., Bingen A., Kirn A. **Penetration and uncoating of frog virus 3 (FV3) in cultured rat Kupffer cells.** Virology. 1981;112:375–384.

Kirn A., Bingen A., Steffan A.M., Wild M.T., Keller F., Cinqualbre J. **Endocytic capacities of Kupffer cells isolated from the human adult liver**. Hepatology. 1982;2:216–222.

Hagmann W., Steffan A.M., Kirn A., Keppler D. **Leukotrienes as mediators in frog virus 3-induced hepatitis in rats.** Hepatology. 1987;7:732–736.

Murine Hepatitis Induced by Frog Virus 3 (FV 3)

A. Kirn, J.L. Gendrault, A.M. Steffan, J.P. Gut, and A. Bingen
Laboratoire de Virologie
Unitè INSERM U 74
3 rue Koeberlè
67000 Strasbourg
France

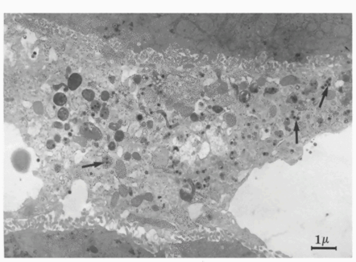
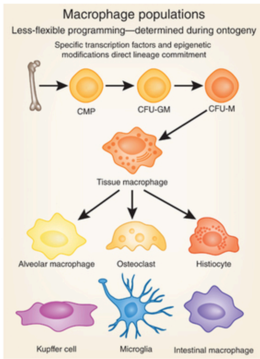


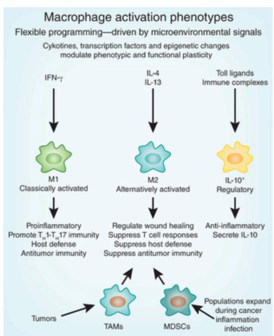
Figure 1 : Mouse Kupffer cell 15 min after the infection with FV 3. Numerous virus particles may be observed within the cellular cytoplasm (⟵).

Macrophage development and differentiation



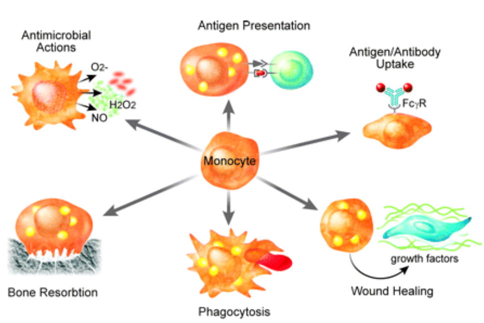
Galli *et al.*,2011

Macrophage development and differentiation

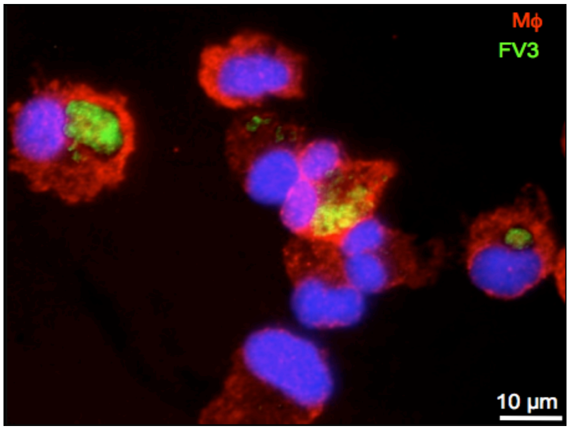


Galli *et al.*,2011

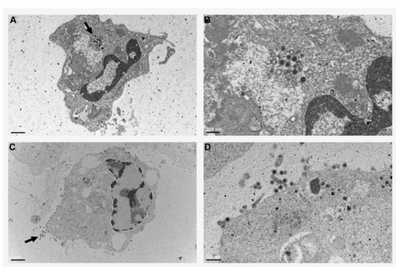
The Mighty Macrophage



Chawla, 2016



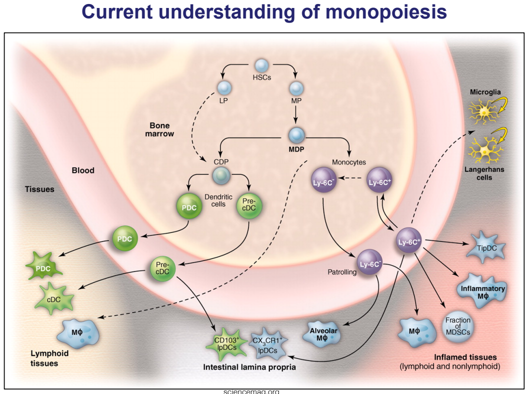
Amphibian macrophage vectors of ranaviral disease



Electron micrographs of peritoneal macrophages from FV3-infected *Xenopus laevis* adults.

Morales HD, Abramowitz L, Gertz J, Sowa J, Vogel A, Robert J. **Innate immune responses and permissiveness to ranavirus infection of peritoneal leukocytes in the frog *Xenopus laevis.*** J Virol. 2010 May; 84(10):4912-22.

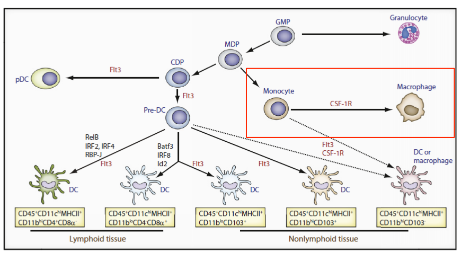
Current understanding of monopoiesis



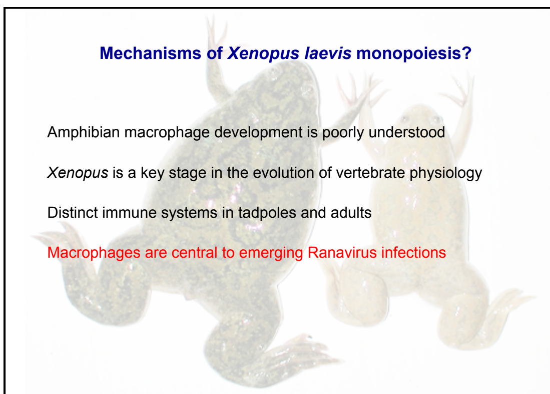
sciencemag.org

Current understanding of amphibian monopoiesis

Colony-Stimulating Factor-1 (CSF-1) is a principal macrophage growth factor



Hashimoto, D. et al., 2011 Dendritic cell and Macrophage Heterogeneiety In Vivo. Immunity 35: 323-35



Mechanisms of *Xenopus laevis* monopoiesis?

Amphibian macrophage development is poorly understood

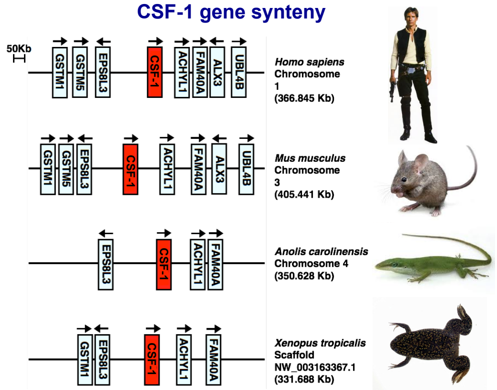
Xenopus is a key stage in the evolution of vertebrate physiology

Distinct immune systems in tadpoles and adults

Macrophages are central to emerging Ranavirus infections

Xenopus.com

CSF-1 gene synteny



Grayfer and Robert, 2013

www.sewinguptheneighbourhood.com; www.warrenphotographic.co.uk; www.petco.com; www.bio.utexas.edu

CSF-1 is a central *X. laevis* macrophage growth and differentiation factor

Grayfer L., Robert, J. **Colony-stimulating factor-1-responsive macrophage precursors reside in the amphibian (*Xenopus laevis*) bone marrow rather than the hematopoietic sub-capsular liver.** J. Innate Immunity. 2013;5:531-542.

Interleukin-34 (IL-34)

CSF-1 is integral to macrophage heterogeneity

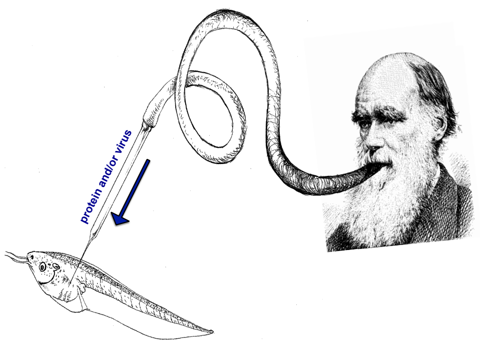
IL-34 has no sequence identity with CSF-1

Binds the CSF-1R and contributes to monopoiesis

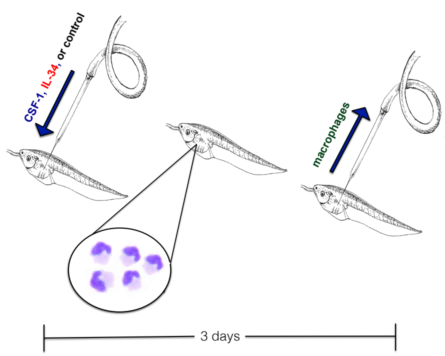
What is the immunological necessity for a second CSF1-R ligand?

What (if any) are the roles of frog IL-34?

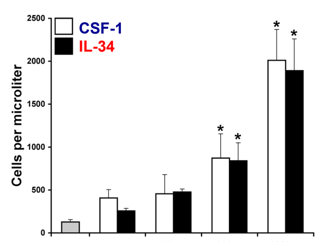
Isolation of peritoneal macrophages



Isolation of peritoneal macrophages

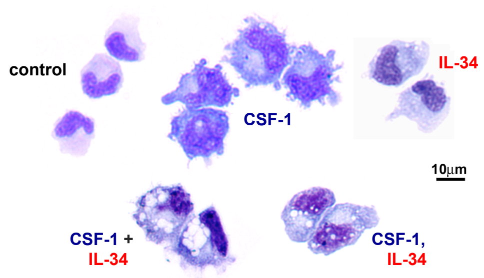


CSF-1 and IL-34 chemo-attract and differentiate *X. laevis* tadpole macrophages



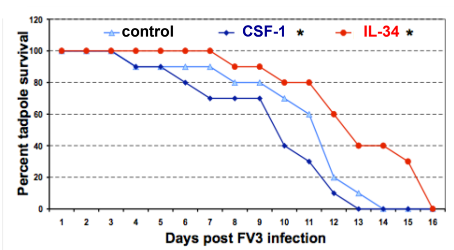
Grayfer and Robert, 2014

X. laevis tadpole CSF-1 and IL-34 derived macrophages are cytologically distinct



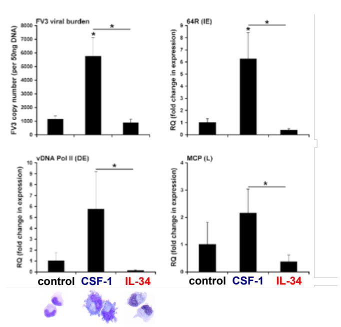
Grayfer and Robert, 2014

CSF-1 compounds tadpole FV3 infections
IL-34 extends FV3-infected tadpole survival



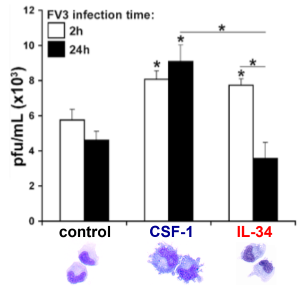
Grayfer and Robert, 2014

CSF-1 macrophages are more susceptible to FV3



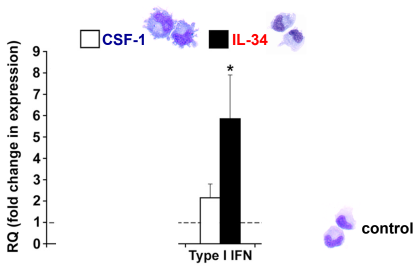
Grayfer and Robert, 2014

IL-34 macrophages exhibit greater resistance to FV3



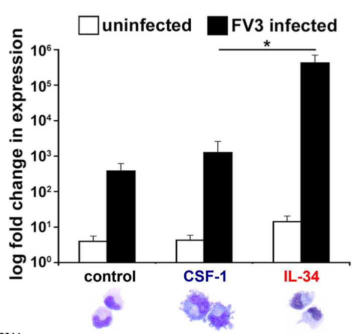
Grayfer and Robert, 2014

IL-34 macrophages express greater type I IFN



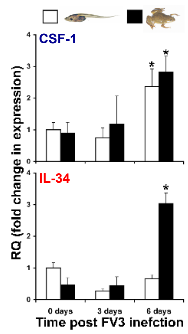
Grayfer and Robert, 2014

IL-34 macrophages express greater type I IFN



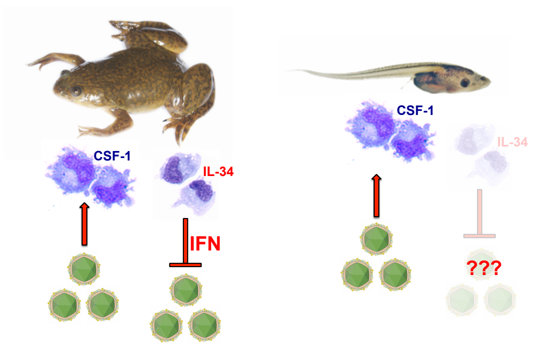
Grayfer and Robert, 2014

Tadpole susceptibility to FV3 coincides with inadequate kidney IL-34 gene expression

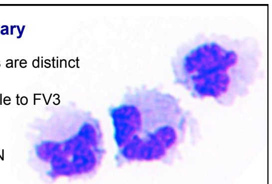


Grayfer and Robert, 2014

Immune efficacies of *X. laevis* tadpoles and adult frogs



Summary



X. laevis CSF-1 and IL-34 macrophages are distinct

CSF-1 renders tadpoles more susceptible to FV3

IL-34 confers anti-FV3 protection
- production of the antiviral type I IFN

During FV3 challenge, tadpoles upregulate their kidney gene expression of CSF-1 but not IL-34
- thus, they increase the numbers of FV3 susceptible, but not antiviral Mϕ
- IL-34 macrophages are prominent type I IFN producers
- lack of tadpole kidney IL-34 Mϕ explains inadequate IFN expression

Tadpole resistance to FV3 may be enhanced by amending their kidney expression of IL-34 and IFN

Extending tadpole survival and lowering FV3 burdens would significantly reduce the ecological devastation caused by ranaviruses

Concluding remarks

Suffice it to say, aquatic and terrestrial vertebrate species evolved from a common ancestor but have been subject to distinct pressures

The immune system as an important component of vertebrate physiology

In turn, physiology (and environment) dictate immunity

The amphibian immune system has both similarities and disparities from those of mammals

Gaining greater understanding into the pressures, efficacies and inefficacies of these animals will lend to understanding the successes and pitfalls of their immune systems

Studies of this nature will grant us greater insight into the evolutionary origins of our own immune systems

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Departments
URMC Dept. of Micro and Immunol
GWU Dept. Biological Sciences

BIOLOGICAL SCIENCES
COLUMBIAN COLLEGE OF ARTS AND SCIENCES

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

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Andino Fde J., Chen G, Li Z, Grayfer L, Robert J.Susceptibility of Xenopus laevis tadpoles to infection by the ranavirus Frog-Virus 3 correlates with a reduced and delayed innate immune response in comparison with adult frogs.Virology. 2012;432:435-43.

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