Les bactériophages thérapeutiques

pourquoi et comment ?

Laurent DEBARBIEUX

POUR LA RECHERCHE, POUR LA SANTÉ,
POUR DEMAIN

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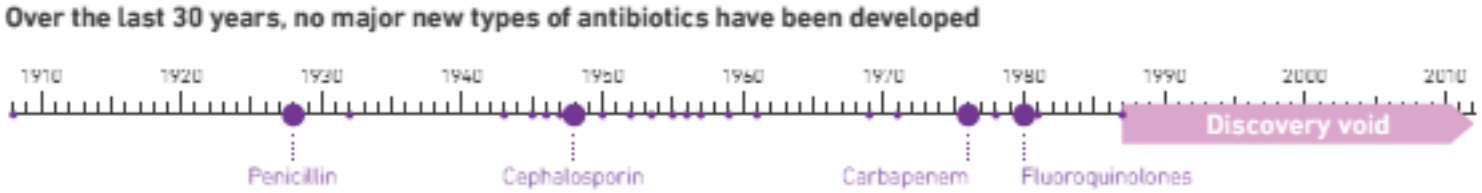
WHO: we've got a problem ! (March 30, 2014)

World Health Organization

WHO's first global report on antimicrobial resistance, with a focus on antibiotic resistance, reveals that it is no longer a prediction for the future. Antibiotic resistance - when bacteria change and antibiotics fail - is happening right now, across the world.

Without urgent action we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill.



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Here is a (100 years old) solution !

In Belgium:

Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury-a case report.

Jennes S, Merabishvili M, Soentjens P, Pang KW, Rose T, Keersebilck E, Soete O, François PM, Teodorescu S, Verween G, Verbeken G, De Vos D, Pirnay JP.
Crit Care. 2017 Jun 4;21(1):129. PMID: 28583189

In USA:

Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection.

Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM, Taplitz R, Smith DM, Kerr K, Kumaraswamy M, Nizet V, Lin L, McCauley MD, Strathdee SA, Benson CA, Pope RK, Leroux BM, Picel AC, Mateczun AJ, Cilwa KE, Regeimbal JM, Estrella LA, Wolfe DM, Henry MS, Quinones J, Salka S, Bishop-Lilly KA, Young R, Hamilton T.
Antimicrob Agents Chemother. 2017 Sep 22;61(10). PMID:28807909

In France, 2 patients treated in 2016/2017

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Phage Therapy, birth, neglecting and come back

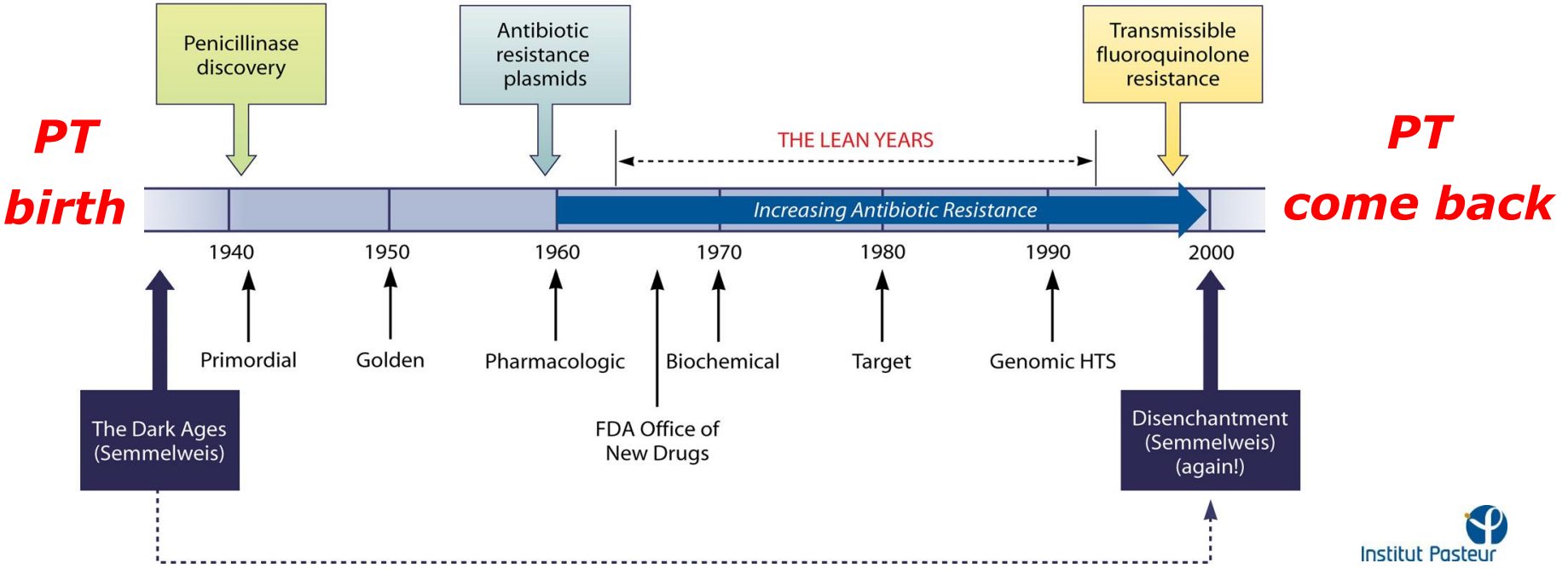
The use of bacteriophages to kill pathogenic bacteria

Date: 1917

Father: Félix d'Herelle (1873-1949)

Location: Institut Pasteur, Paris, France

Particular signs: first specific antibacterial treatment



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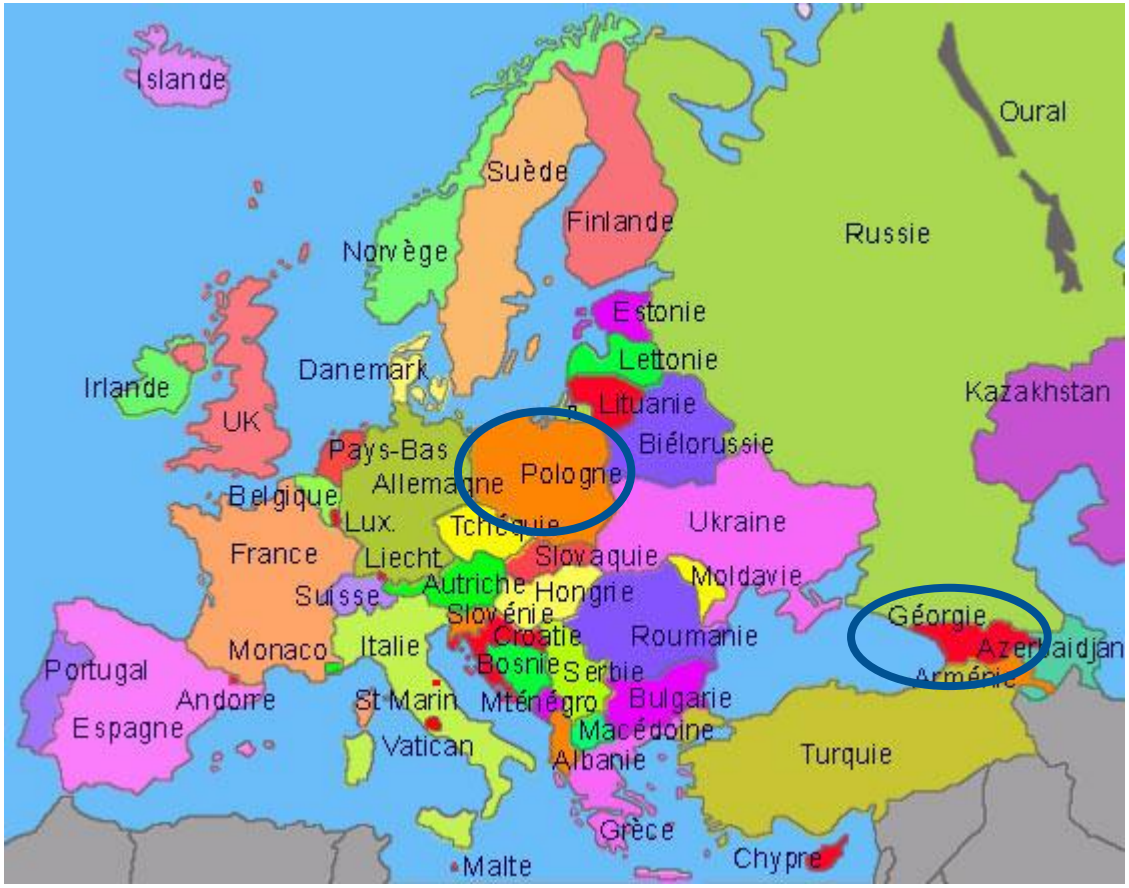
Phage Therapy, the forgotten cure

In Eastern Europe, several countries developed phage therapy



… and today patients are still being treated in Georgia and Poland

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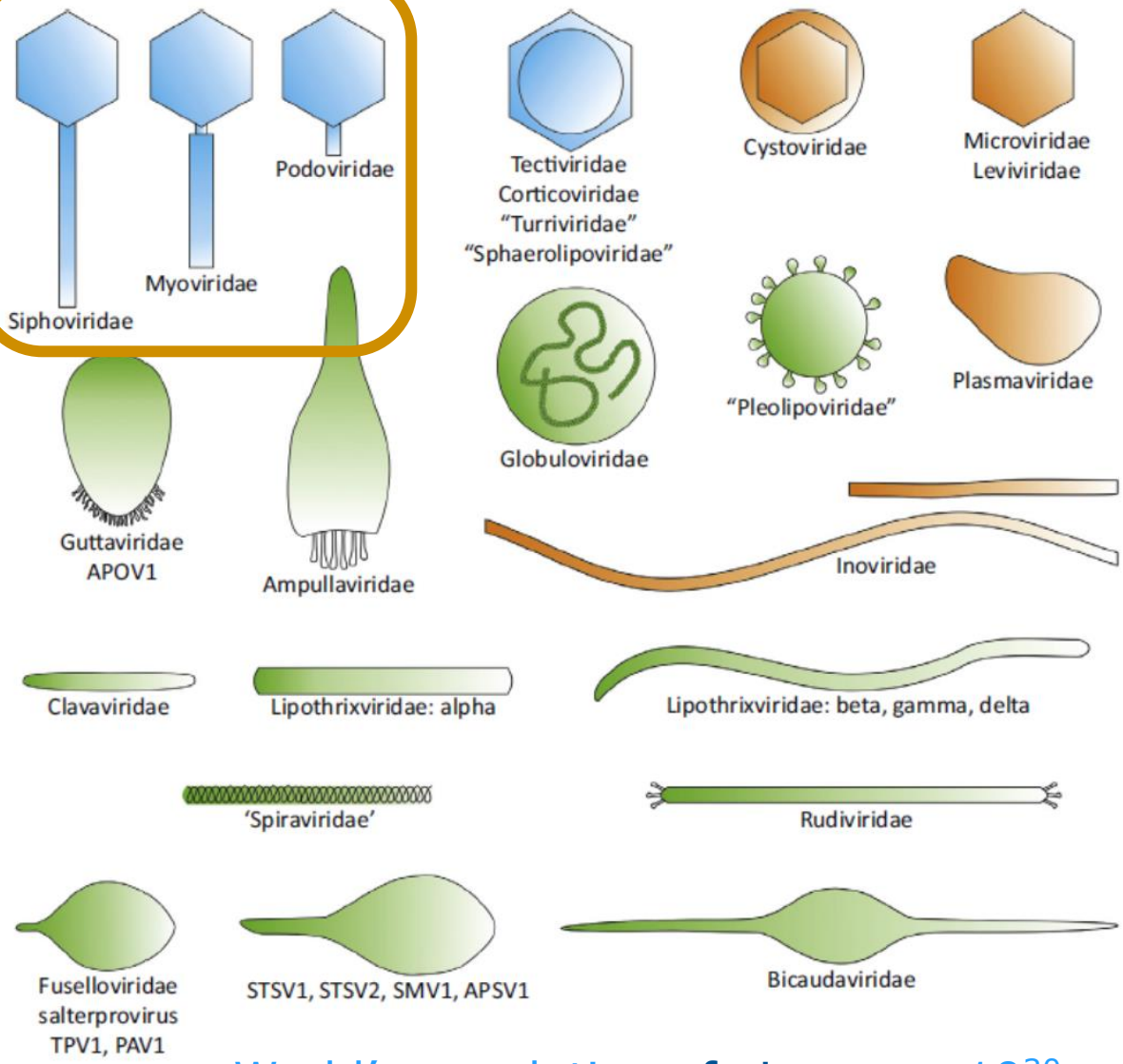
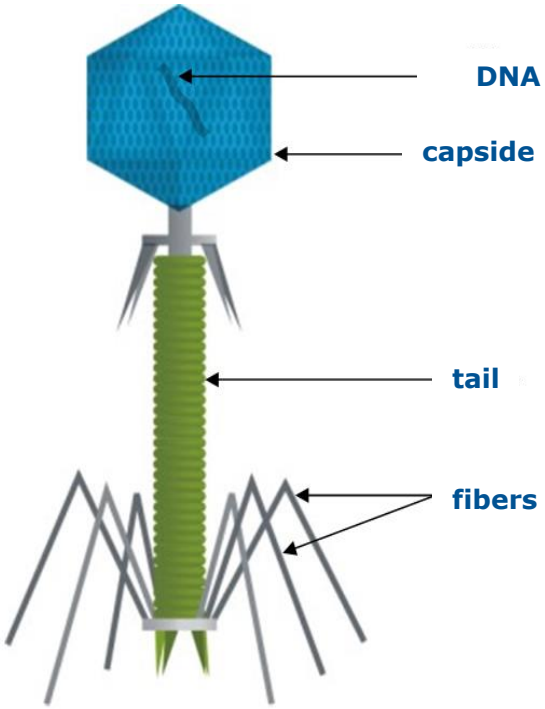
Bacteriophages belong to viruses infecting microbes



96% of bacteriophages belong to *Caudovirales*



The T4 phage model

World's population of viruses ~ 10^{30} particles

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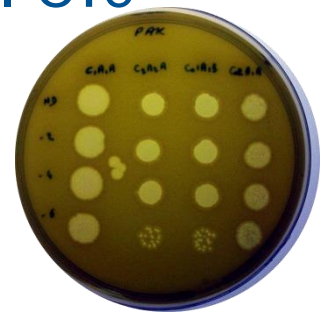
Host range and coevolution

P.ae. O:6

ND
-2
-4
-6

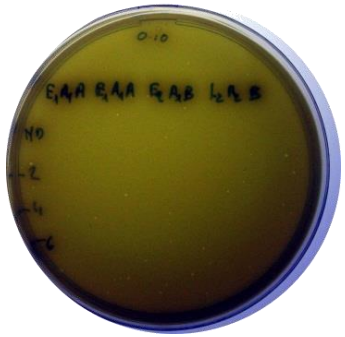


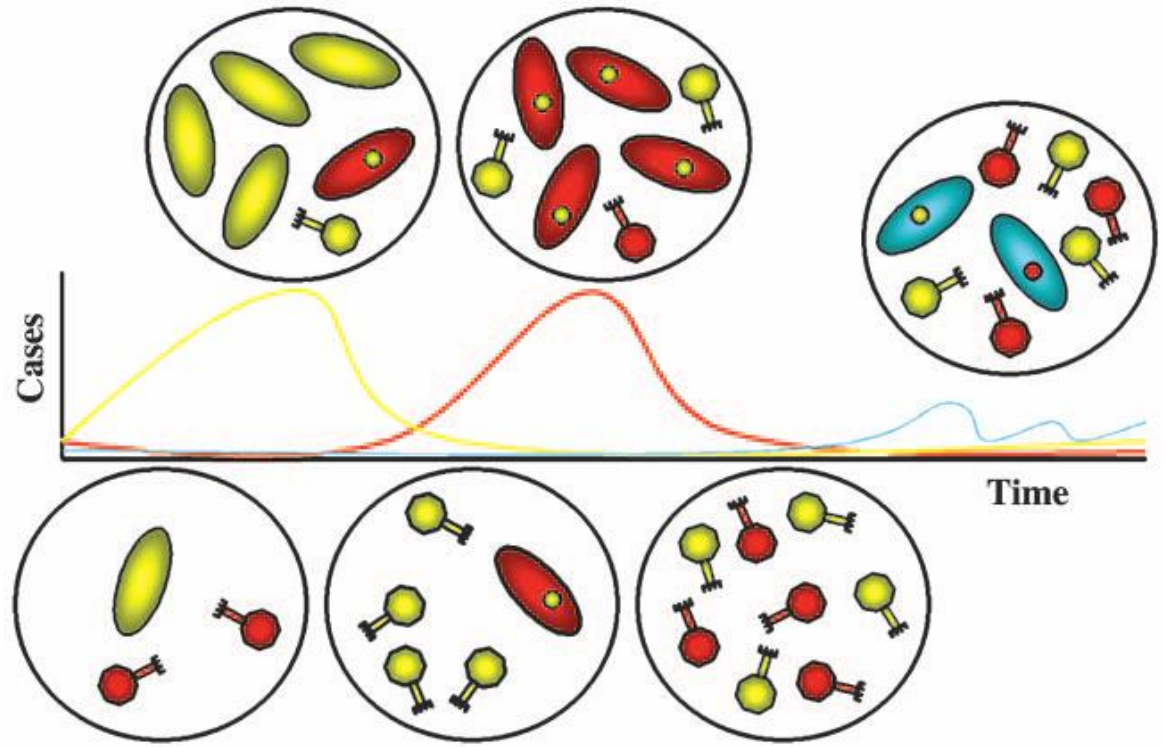
P.ae. O:9

ND
-2
-4
-6

P.ae. O:10

ND
-2
-4
-6





Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages
Faruque et al., 2005, PNAS, vol 102, p1702

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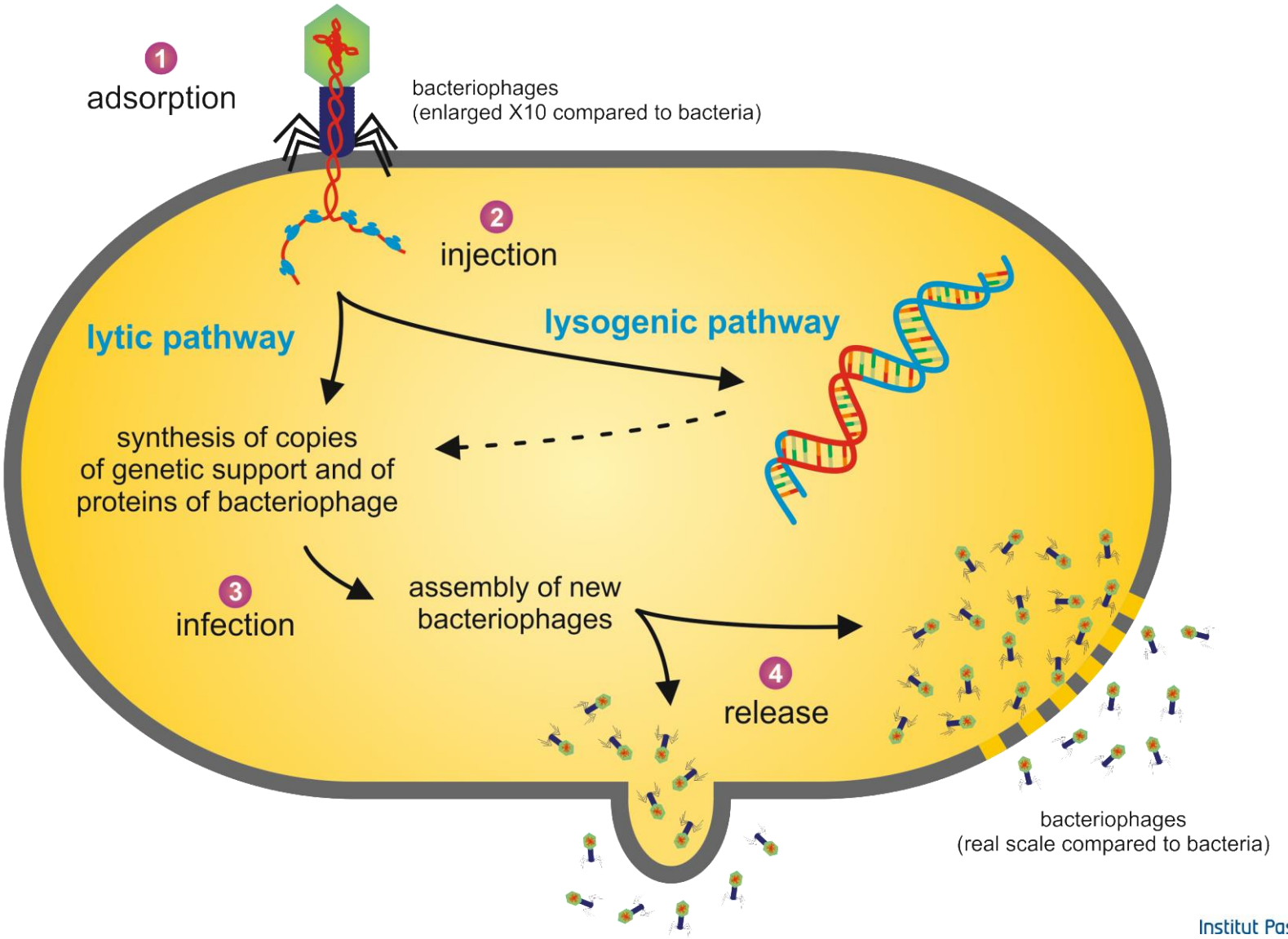
Bacteriophages: virulents vs. temperate



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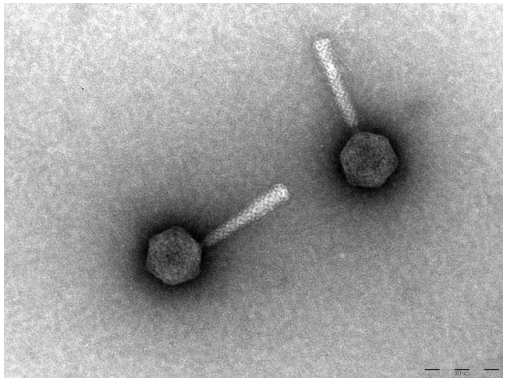
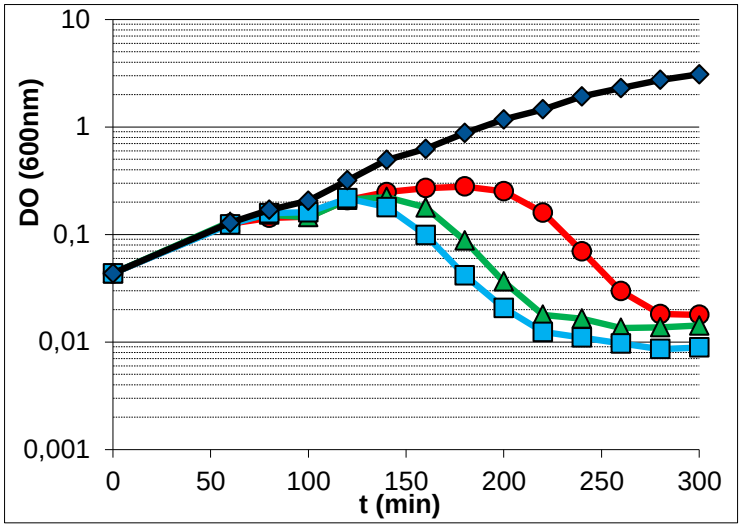
Bacteriophages isolation and characterization

Plaques

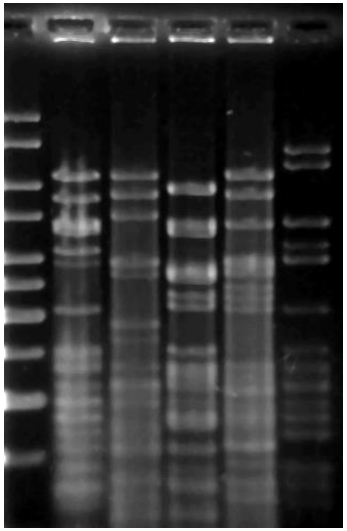
EM



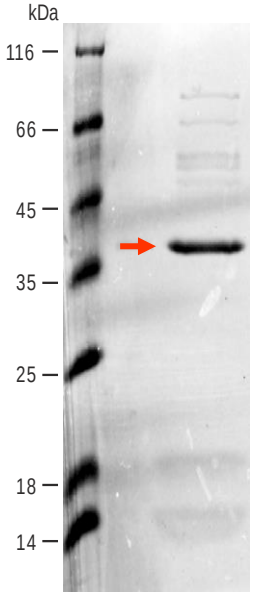
Lysis kinetics



RFLP



Mass Spec



Genome sequencing

↓

Virus classification

↓

Molecular studies

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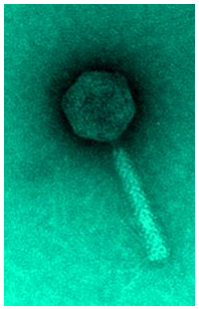
Phage Therapy, solving the therapeutic equation

1) Animal models

2) Clinical strains

3) Bacteriophages



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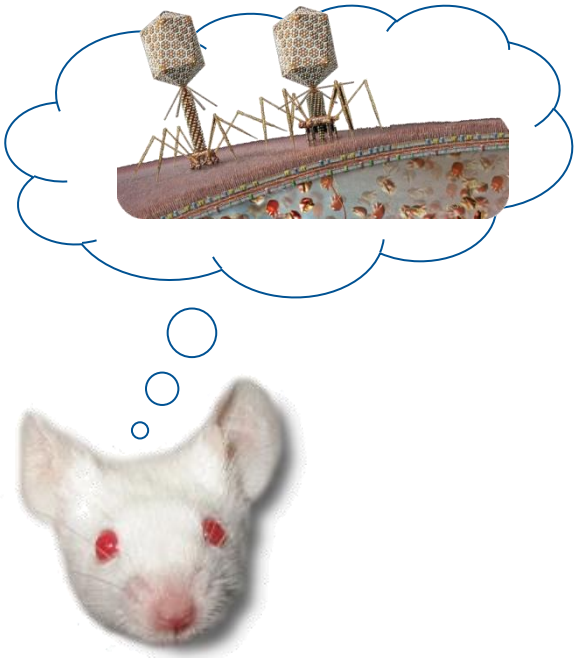

Lung infections / *Pseudomonas aeruginosa* and *Escherichia coli*

Digestive tract environment / *Escherichia coli*

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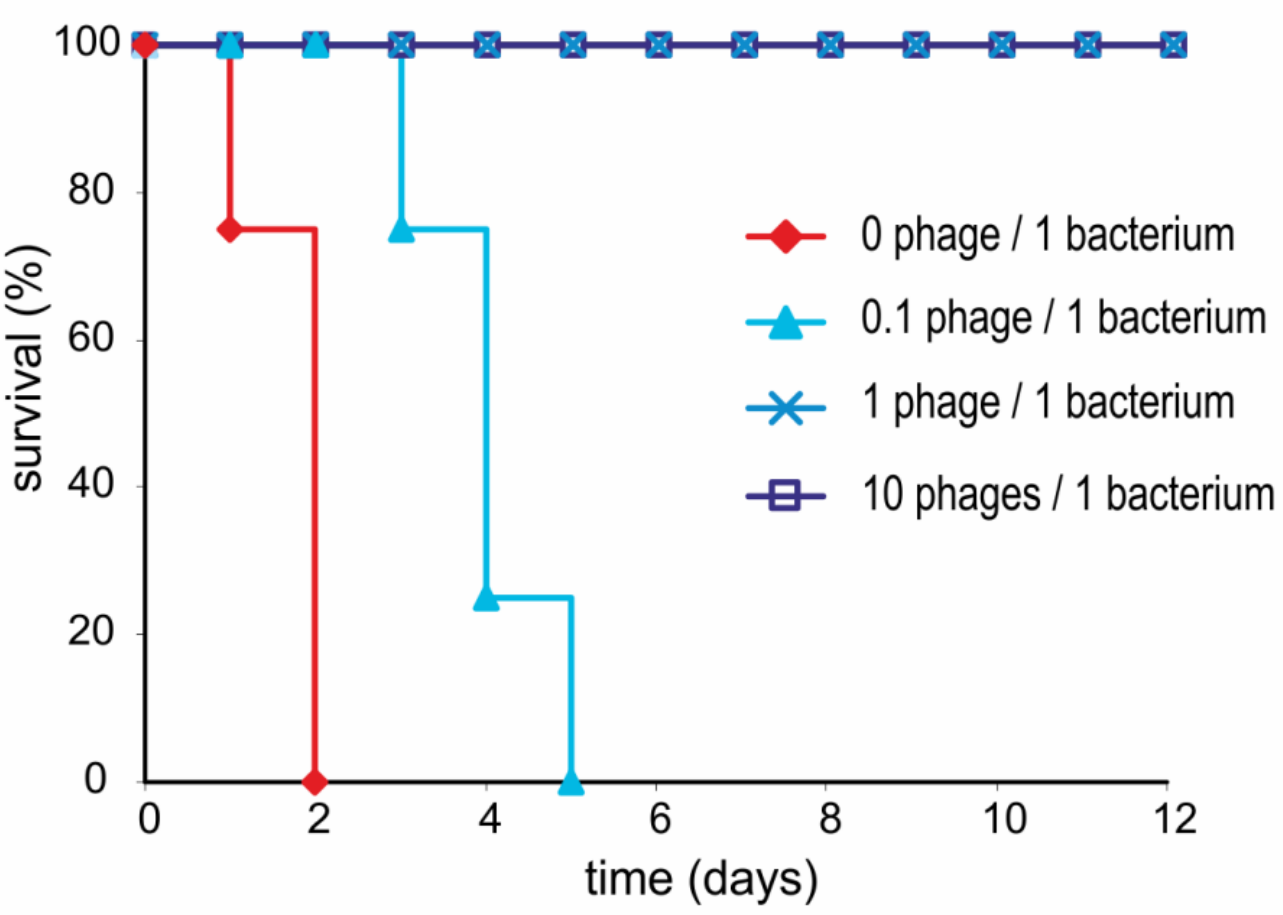
Bacteriophages treatment of lung infection in mice

Infection by $1.0x10^7$ bacteria and 2H later different doses of phages



PAK_P1

Debarbieux et al., JID, 2010

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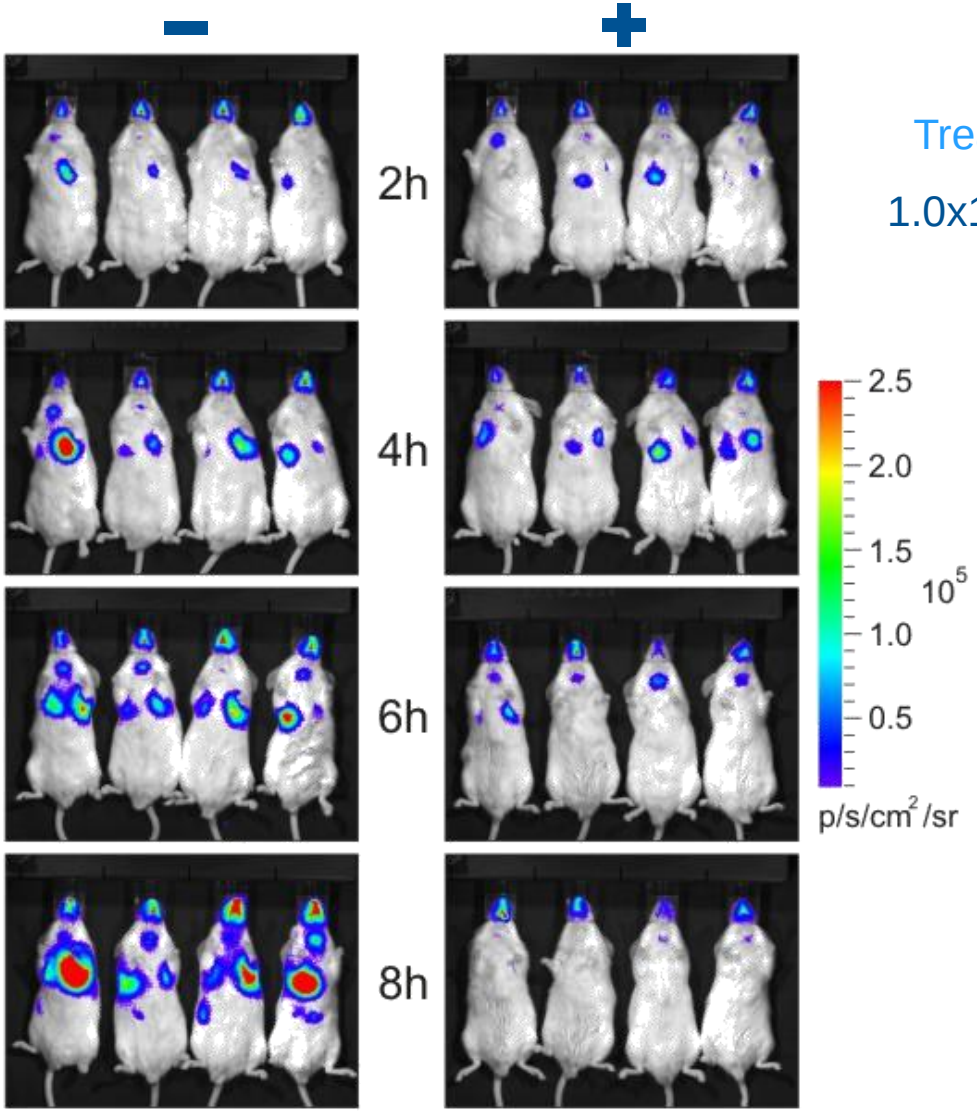
In vivo kinetics of pulmonary phage therapy

Infection at time 0 by $1.0x10^7$ bacteria



Treatment at 2H with

$1.0x10^8$ bacteriophages

Debarbieux et al., JID, 2010

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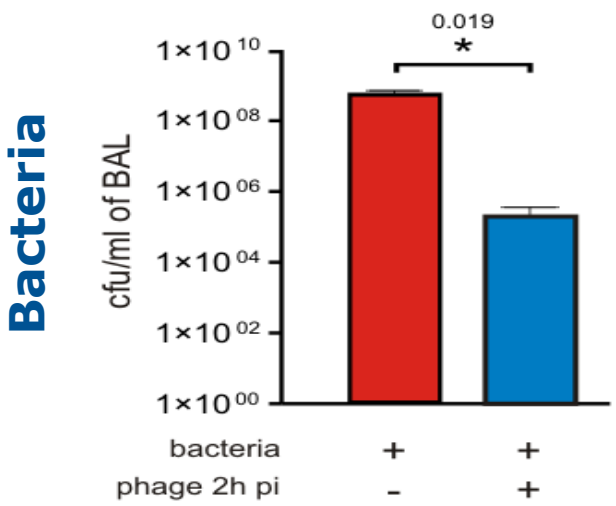
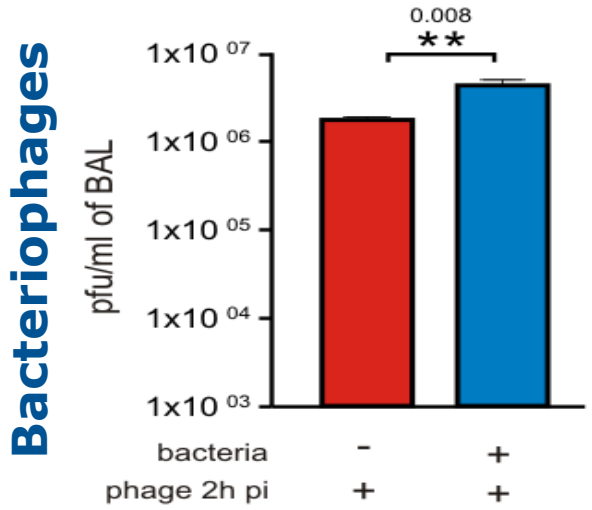
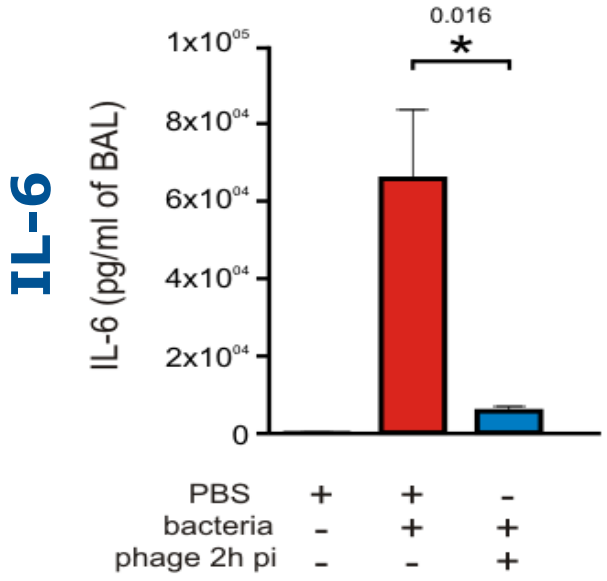
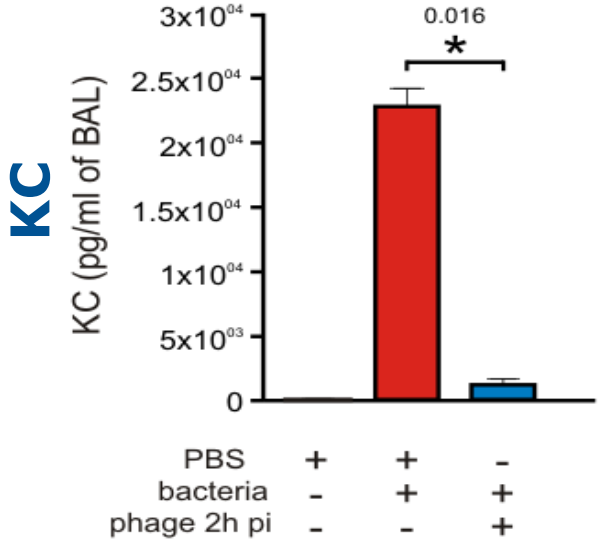
How works pulmonary phage therapy ?









Bacteria ↘

Bacteriophages ↗

Inflammation ↘

Survival ↗

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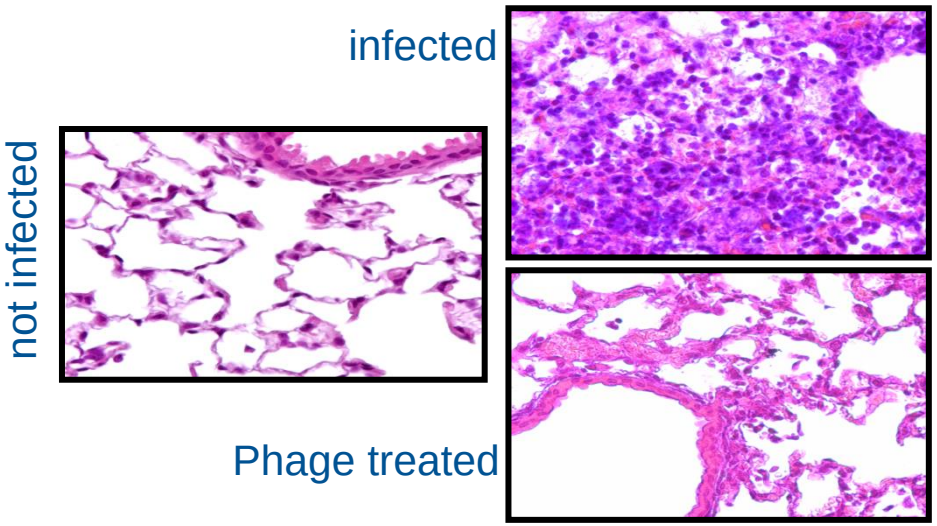
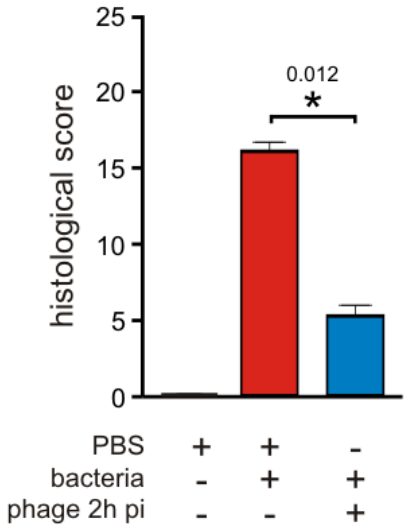
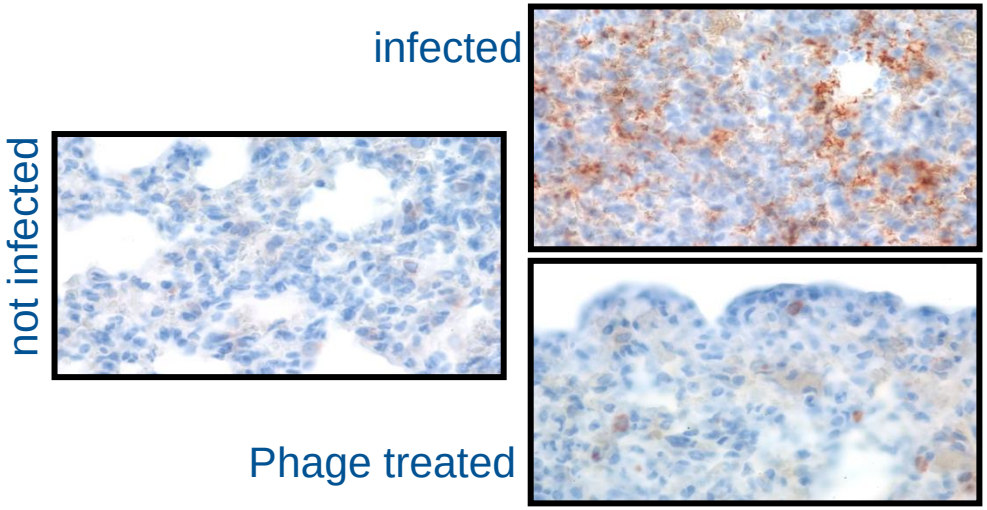
Histology and Immuno-histochemistry analyses





PMNs,
Lymphocytes,
Infiltration,
Alveolitis,
Brochitis,
Necrosis



P. aeruginosa antibodies

(Ina Attree-Delic, Grenoble)

Bacteria clearance in phage-treated samples

Morello et al., PLoS One, 2011

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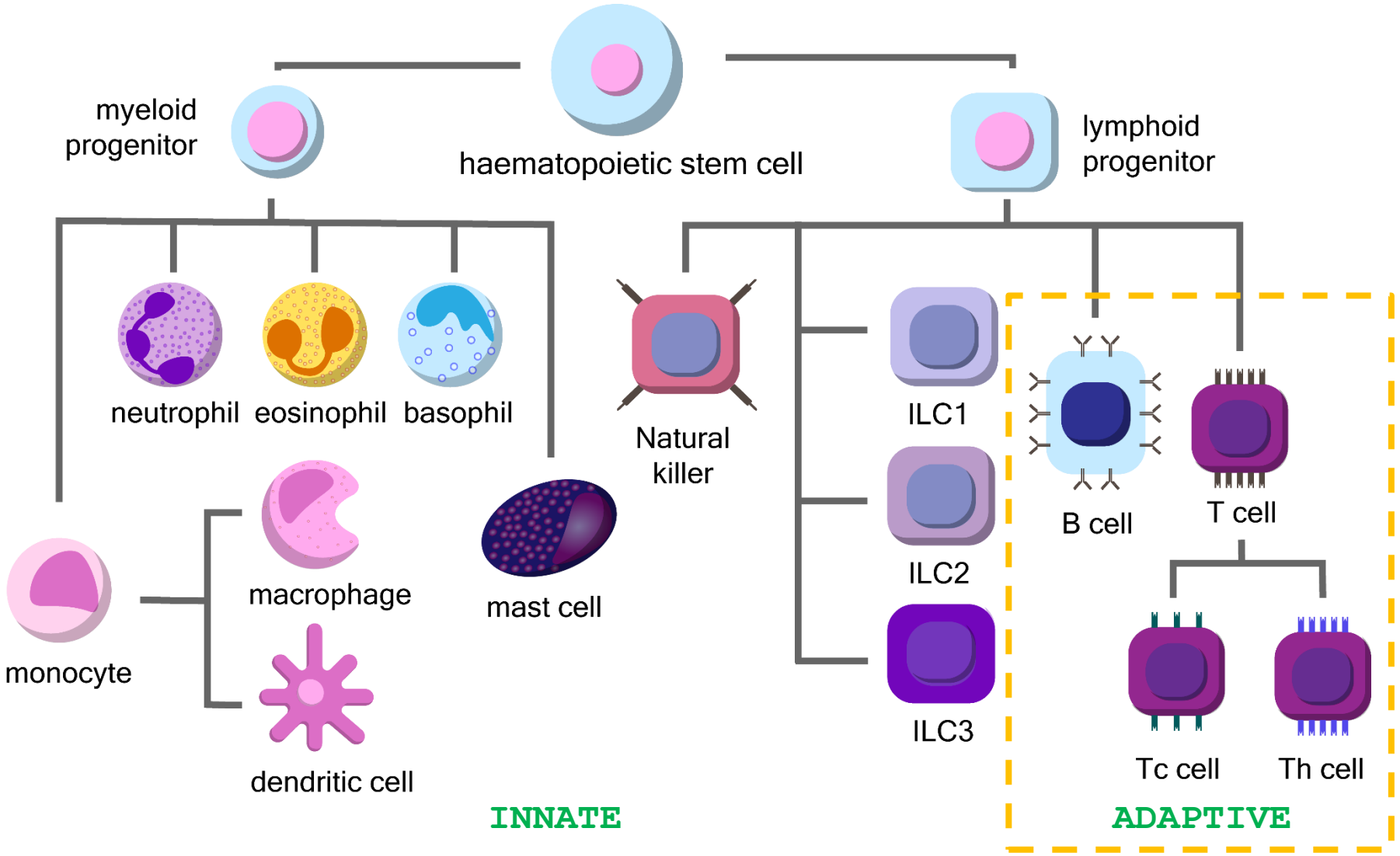
Is the immune system needed for PT?



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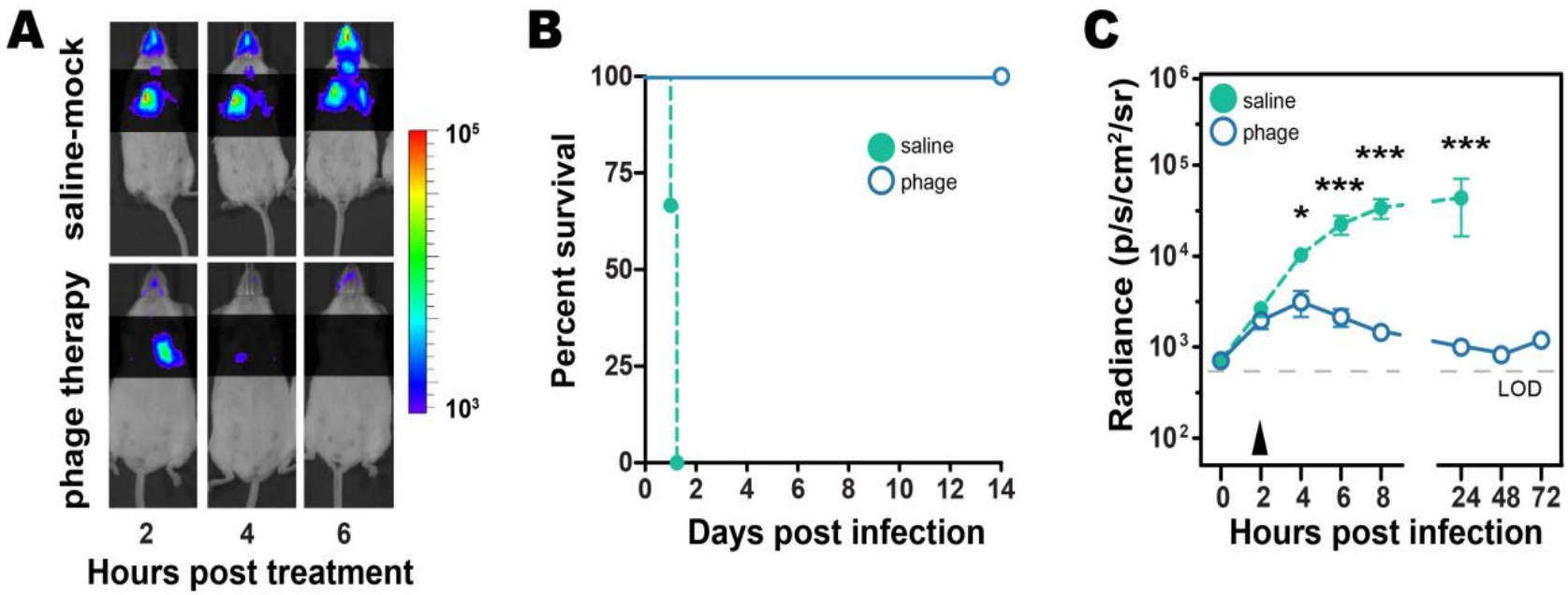
Settings: strain PAK, phage PAK_P1, *in vivo* imaging

Survival and kinetics of treatment in WT mice



infectious dose 10^7 cfu, treatment MOI 10 (10^8 pfu)

Roach *et al.*, Cell Host Microbe 2017

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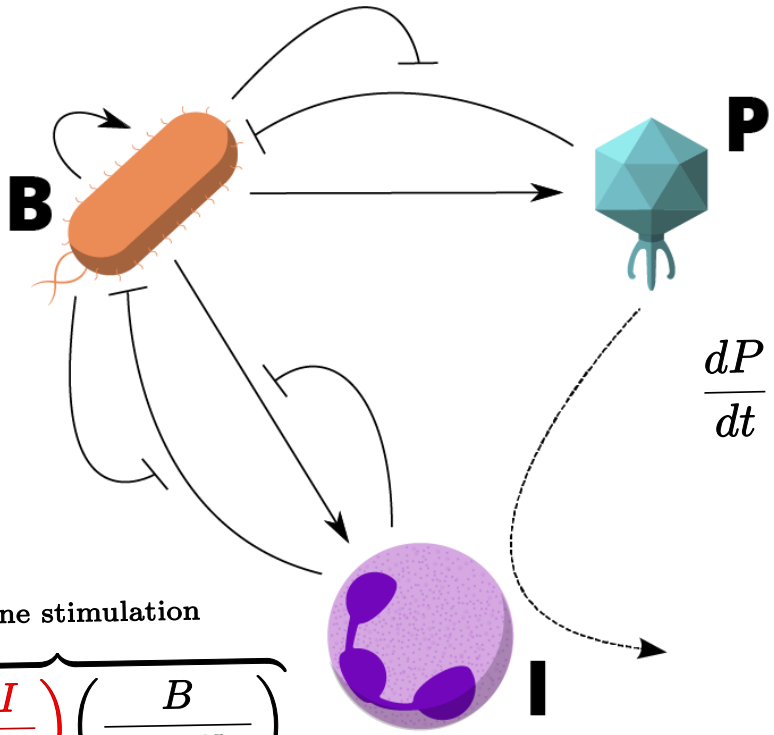
Mathematical modelling of tripartite interaction process

$$\frac{dB}{dt} = \overbrace{rB\left(1-\frac{B}{K_C}\right)}^{\text{Growth}} - \overbrace{\frac{\epsilon IB}{1+B/K_D}}^{\text{Immune killing}} - \overbrace{SF(P)}^{\text{Lysis}} + \overbrace{\mu rS\left(1-\frac{S+R}{K_C}\right)}^{\text{Mutation}}$$

bacteria can develop resistance to phage

bacteria can evade the immune response



$$\frac{dP}{dt} = \overbrace{\beta\phi BP}^{\text{Viral release}} - \overbrace{\omega P}^{\text{Decay}}$$

immune cell response to phages ??

$$\frac{dI}{dt} = \overbrace{\alpha I\left(1-\frac{I}{K_I}\right)\left(\frac{B}{B+K_N}\right)}^{\text{Immune stimulation}}$$

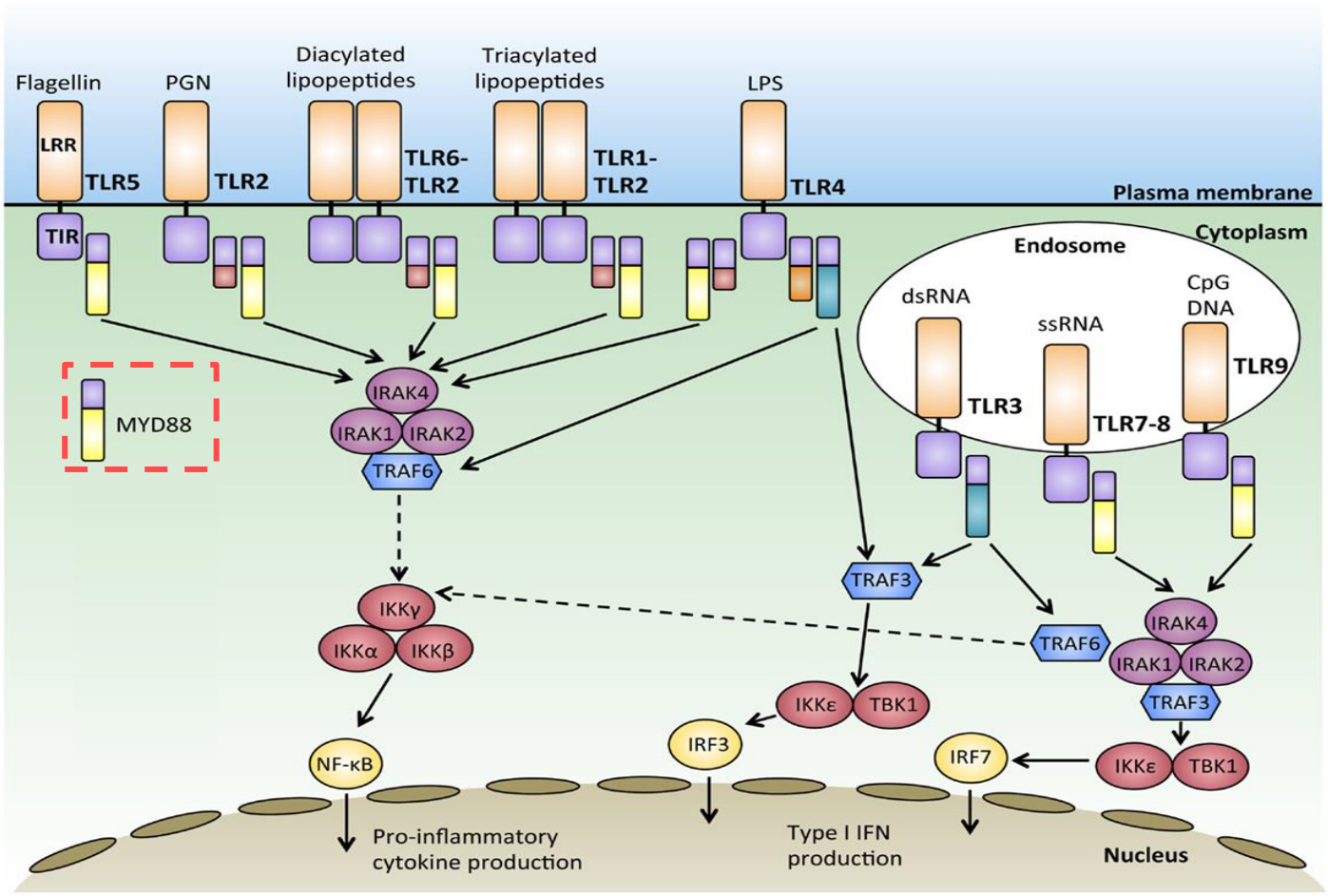
Immune stimulation has a maximum "carrying capacity"

Georgia Tech School of Biological Sciences

Joey Leung &
Joshua Weitz

MyD88 is an essential protein in immune signaling

MyD88 is a Toll-like receptor signaling molecule for innate immune cell activation and recruitment



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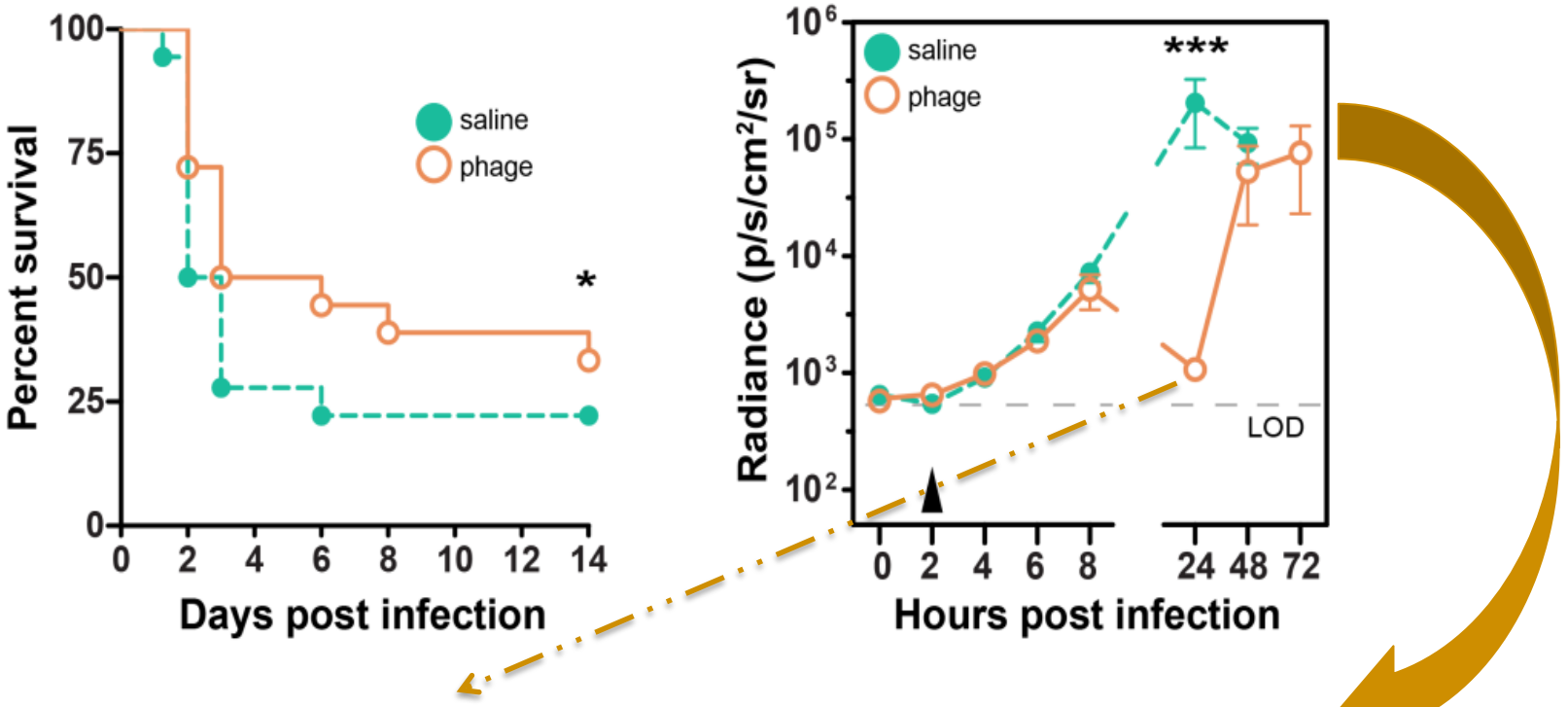
Phage therapy failed in Myd88$^{-/-}$



Initial reduction but resurgence of infection because of the growth of phage-resistant bacteria.

NB: mice hypersensitive to infection, lower dose but same MOI

Roach *et al.*, Cell Host Microbe 2017

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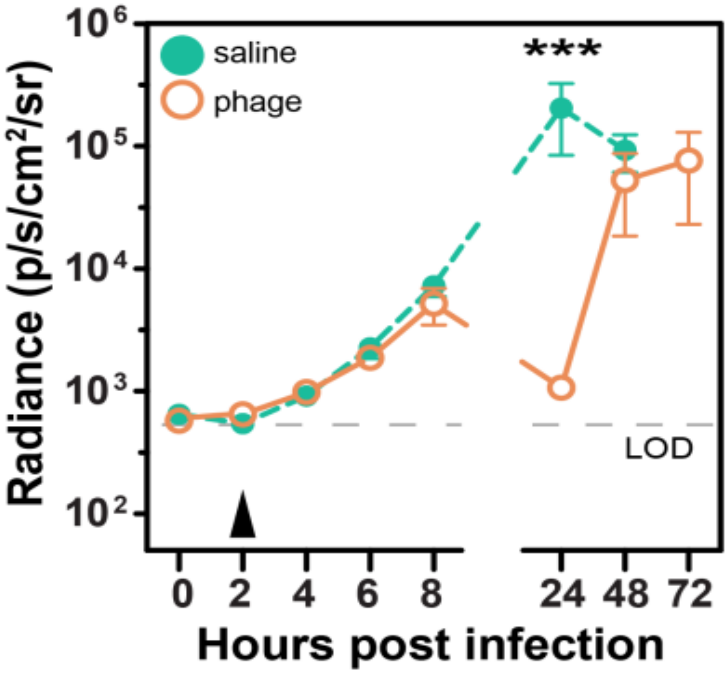
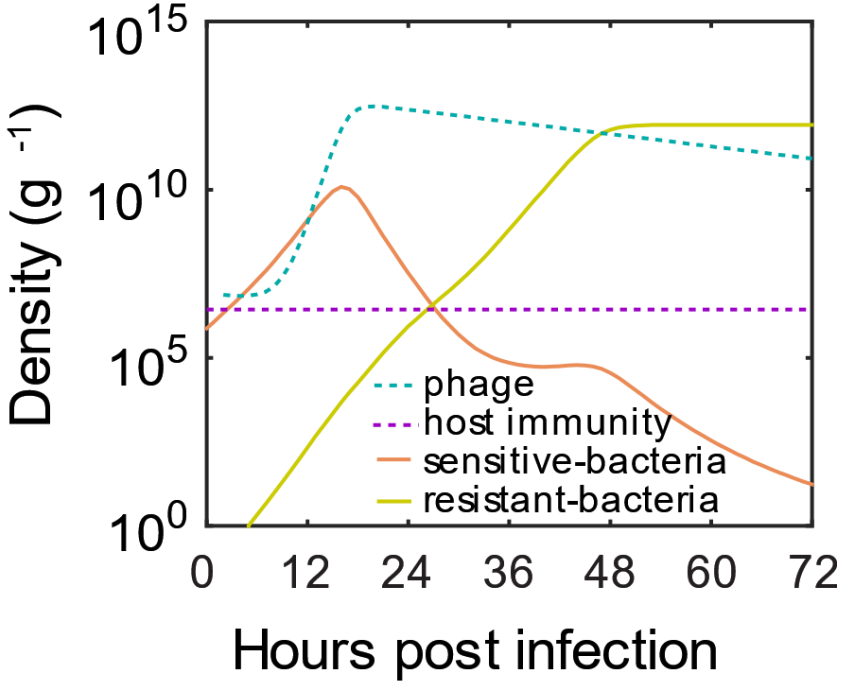
Failure in MyD88$^{-/-}$ mice is predicted by simulation



simulation



In vivo, these are phage-resistant

PT efficacy relies on immunophage synergy

Roach *et al. Cell Host Microbe, 2017*

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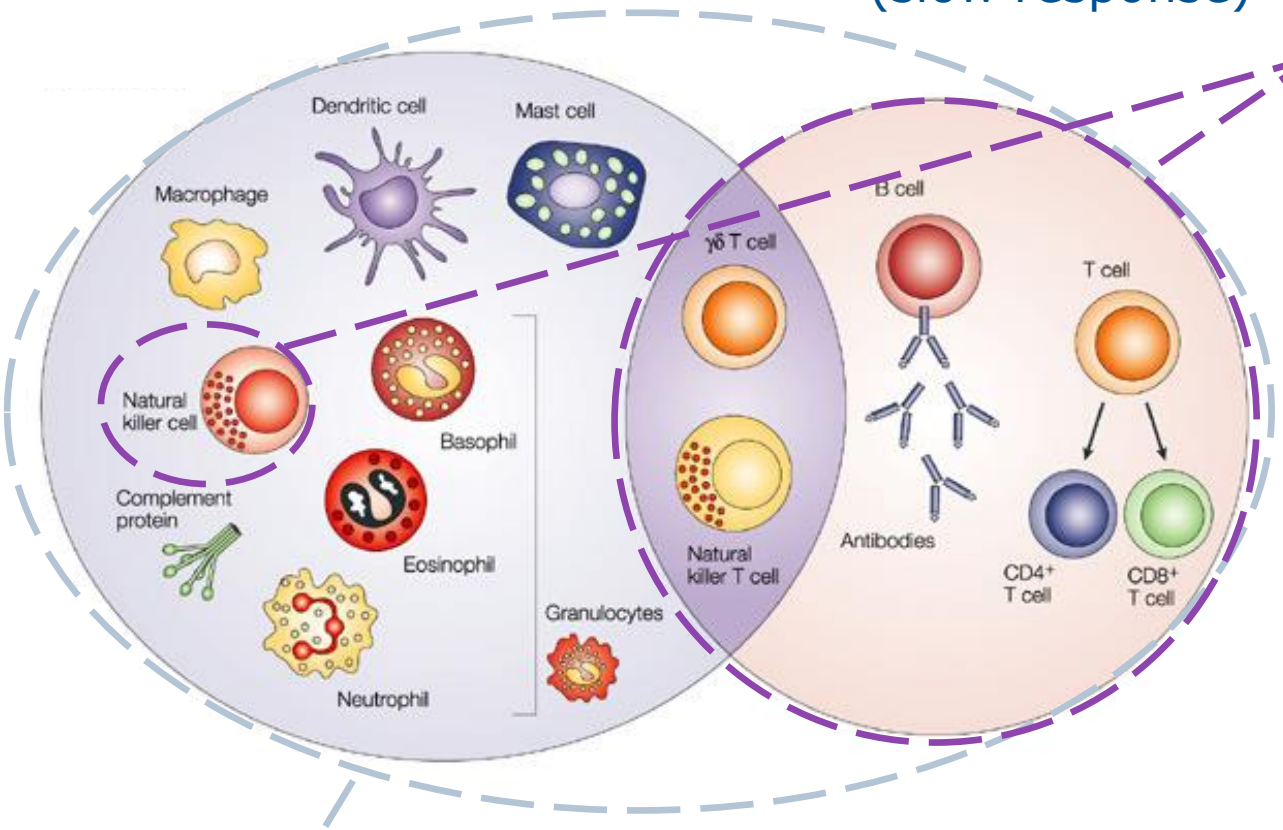
Which immune cells are involved ?

INNATE IMMUNITY
(rapid response)

ADAPTIVE IMMUNITY
(slow response)



2) lymphocyte deficiency (Rag2/Il2rg$^{-/-}$)

1) pathogen receptor signaling deficiency (MyD88$^{-/-}$)

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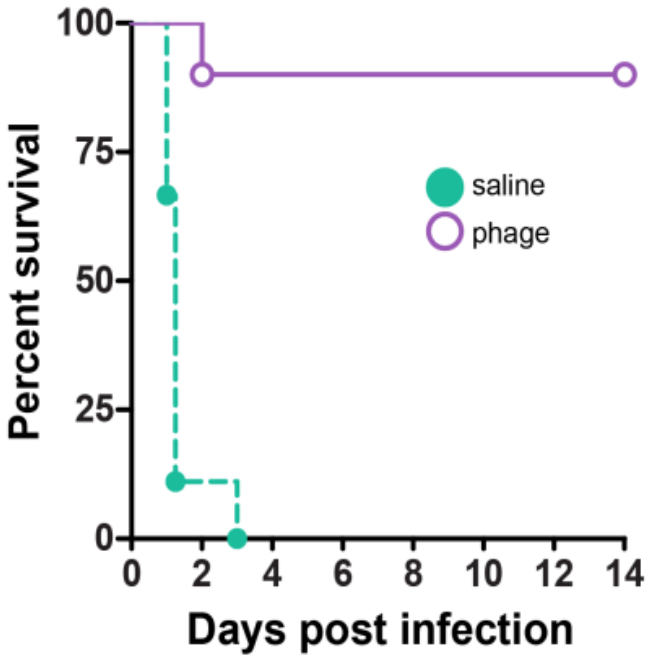
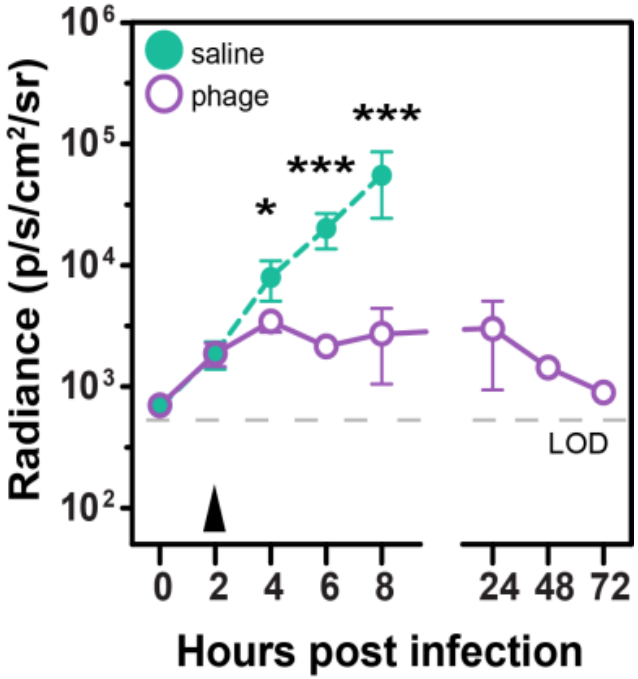
Phage therapy "works" in Rag2/Il2rg$^{-/-}$ mice

Rag2/Il2rg$^{-/-}$





Lymphocytes are not required for effective phage therapy

Roach *et al.*, Cell Host Microbe 2017

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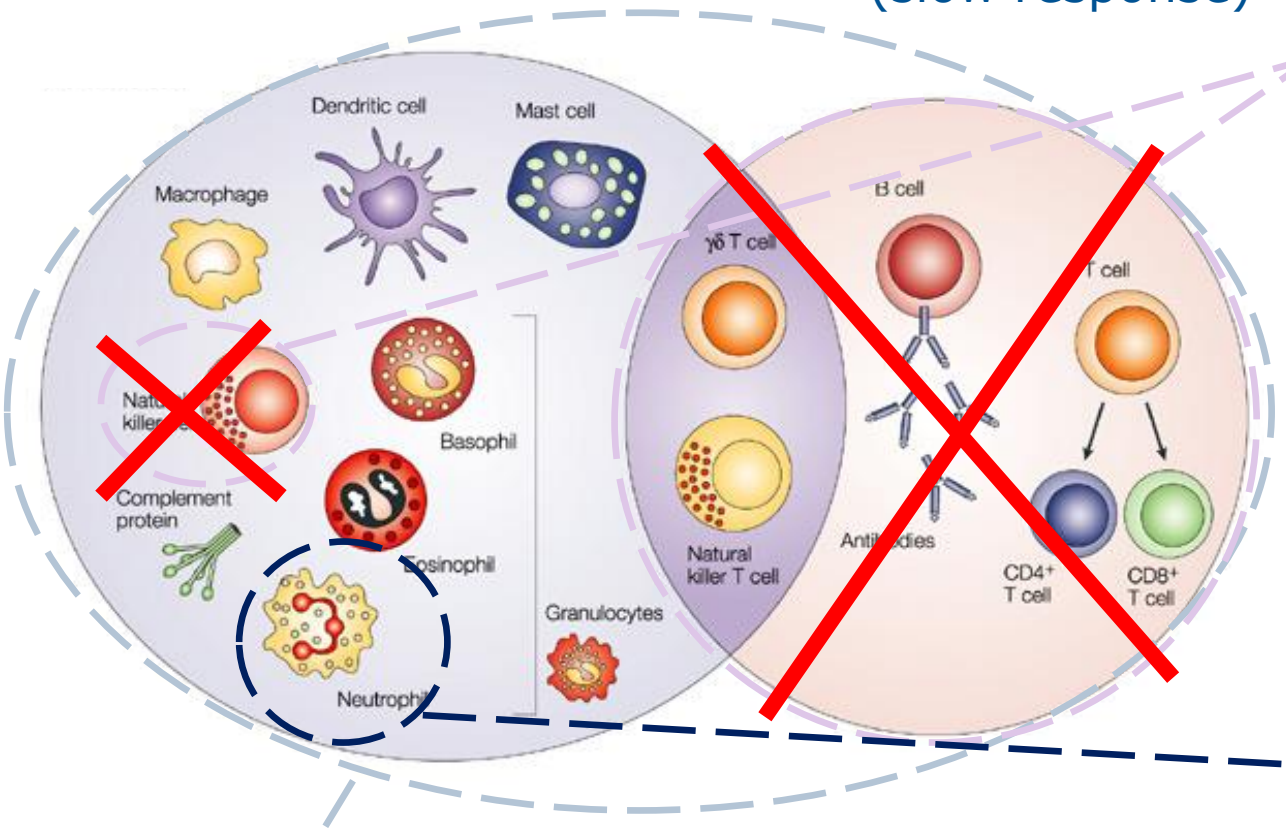
Role of innate cells, neutrophils ?

INNATE IMMUNITY
(rapid response)

ADAPTIVE IMMUNITY
(slow response)



2) lymphocyte deficiency (Rag2/Il2rg$^{-/-}$)

3) neutrophils

1) pathogen receptor signaling deficiency (MyD88$^{-/-}$)

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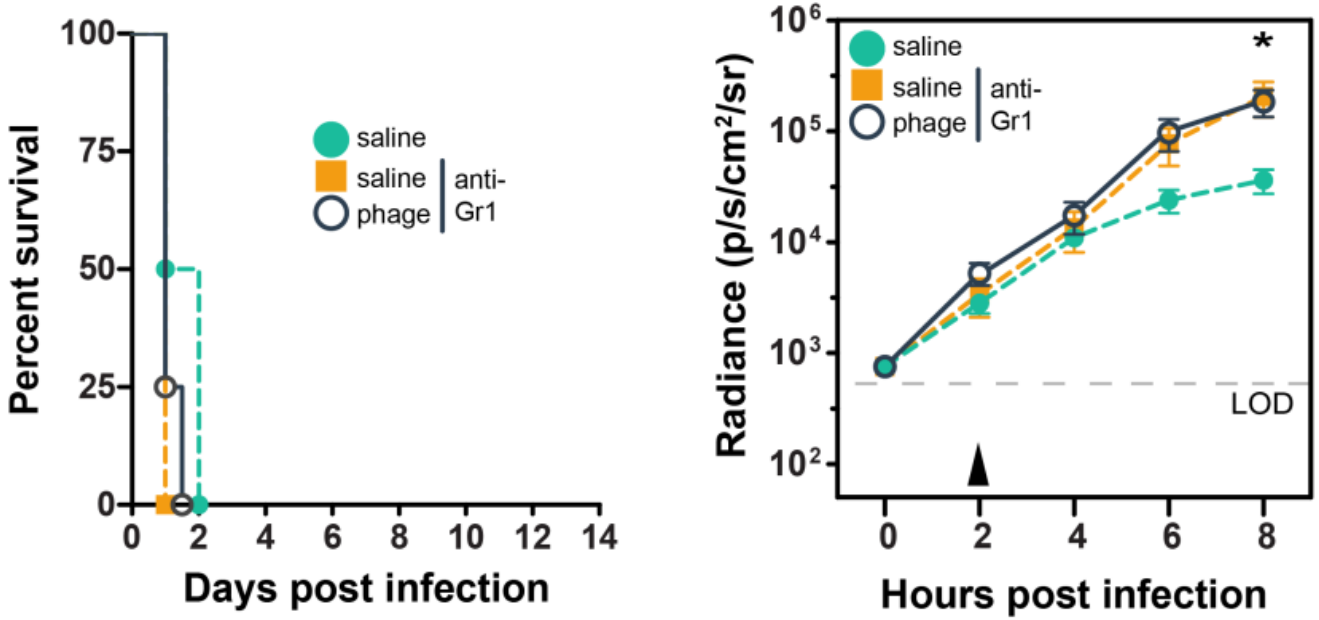
Phage therapy failed in neutropenic WT mice

Neutropenia (depletion with Ab) **in WT**



phage therapy failure without initial reduction of bacteria

Roach *et al.*, Cell Host Microbe 2017

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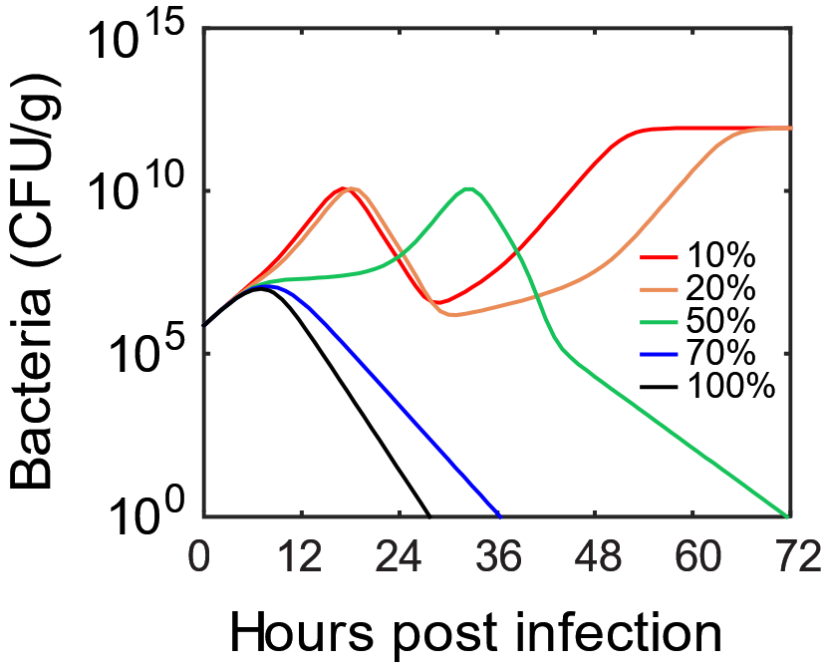
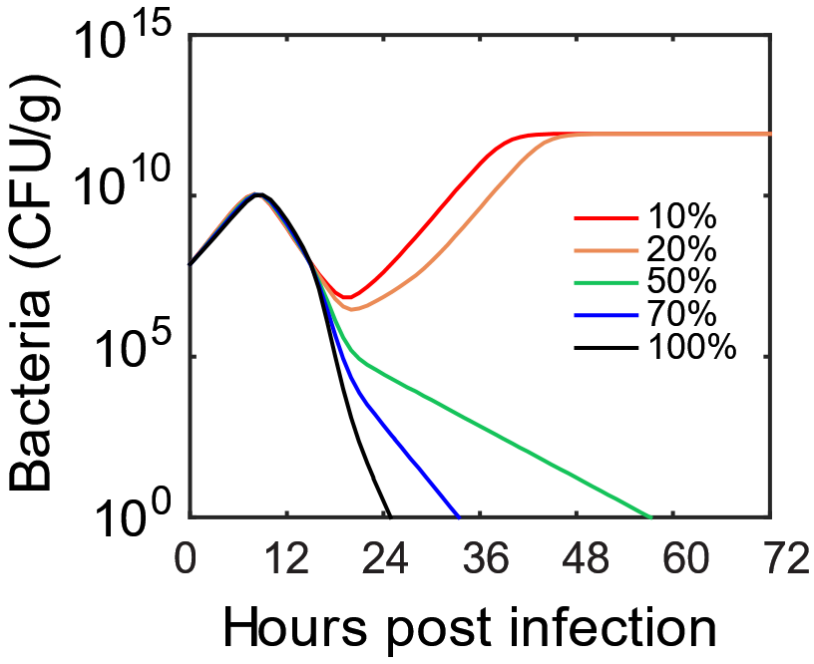
In silico simulations of variable immunodeficiencies

innate immune activation deficiency



neutropenia



simulations predict patients will need at least
50% healthy immune response

ImmunoPhage Synergy is needed for successful phage therapy

Roach *et al. Cell Host Microbe, 2017*

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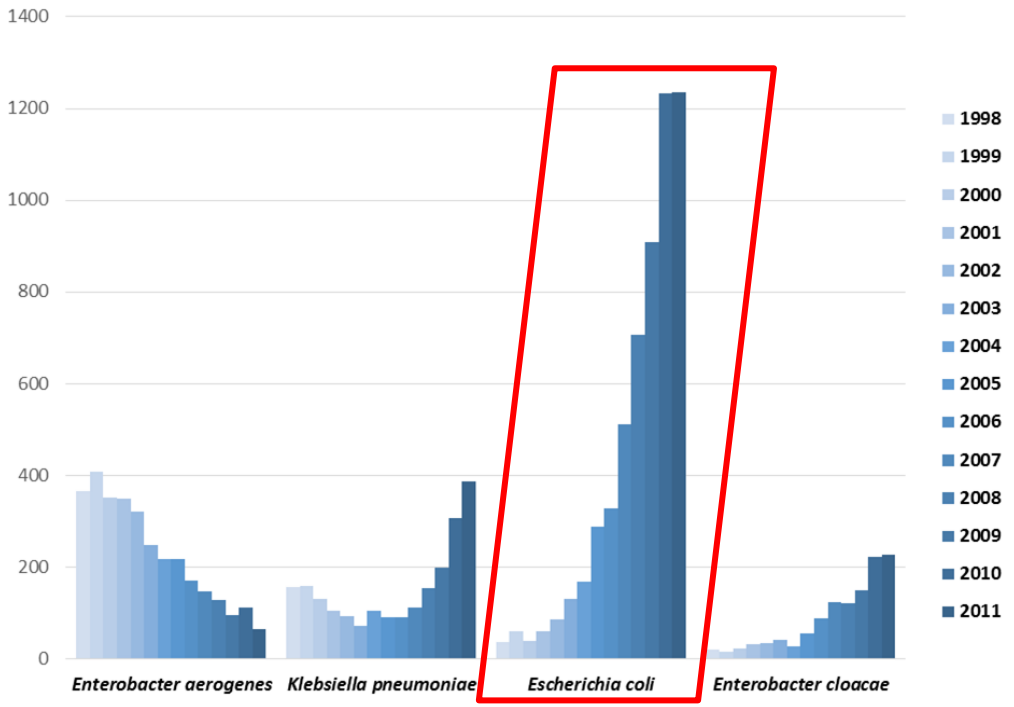
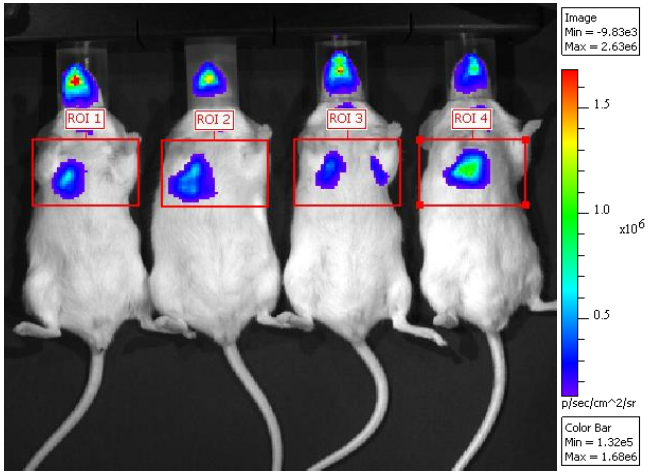
What about other pathogens ?

Ventilator-associated pneumonia patients (intensive care units)

Increase of *Escherichia coli* infections

Alarming rise of antibiotic resistance of *E. coli* strains





Strains producing β-LSE amongst *Enterobacteriaceae* from 1998 to 2011

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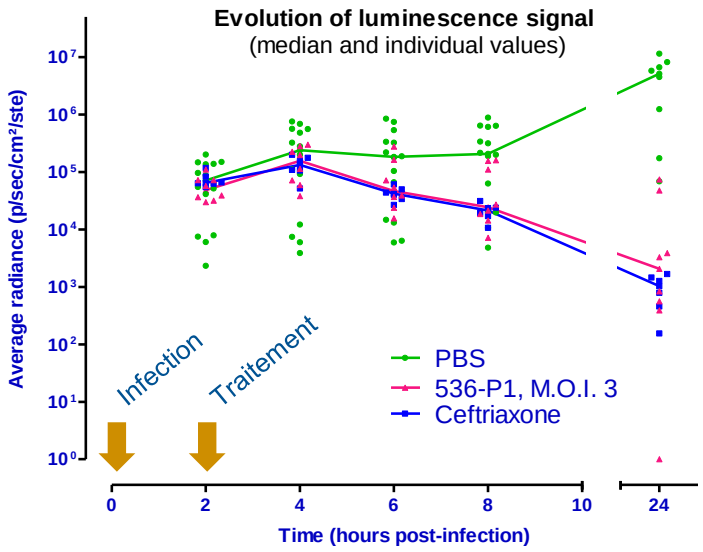
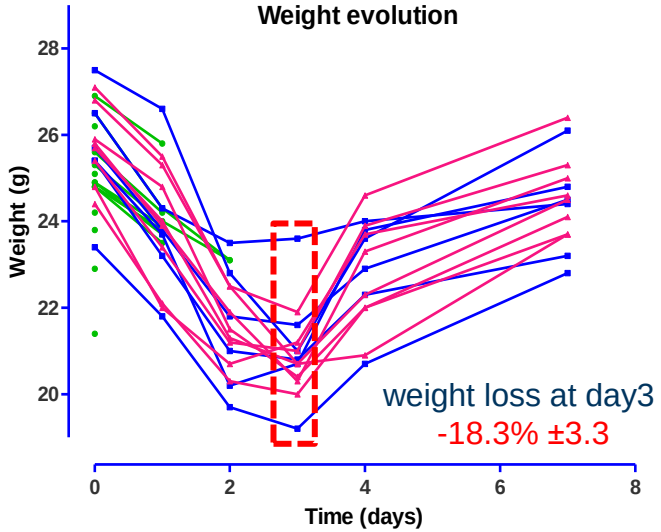
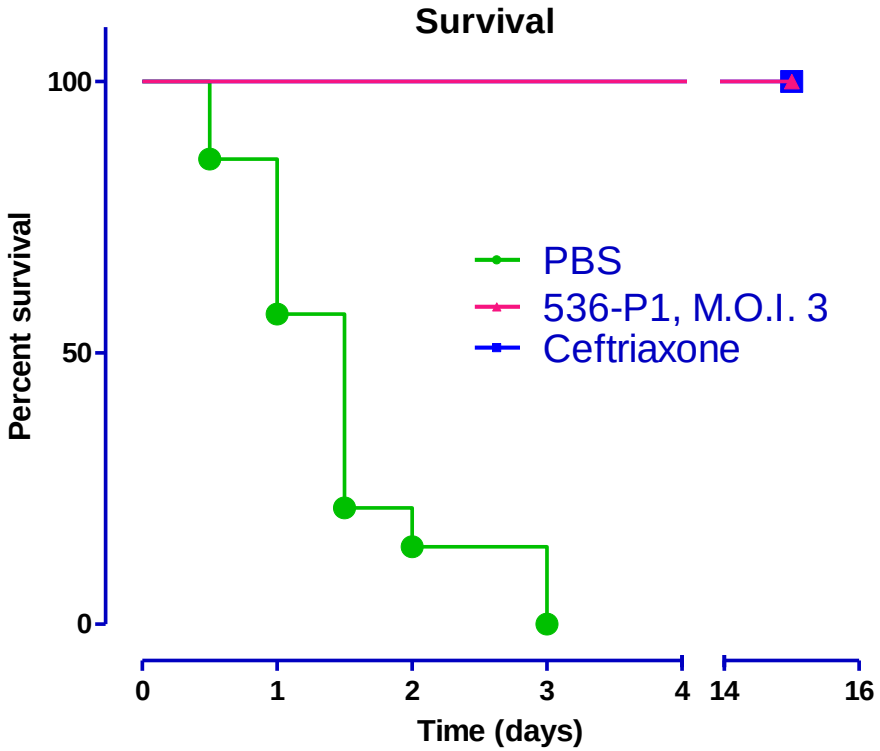
Efficacy: bacteriophages = antibiotics







Dufour *et al.*, Crit. Care Med. 2015

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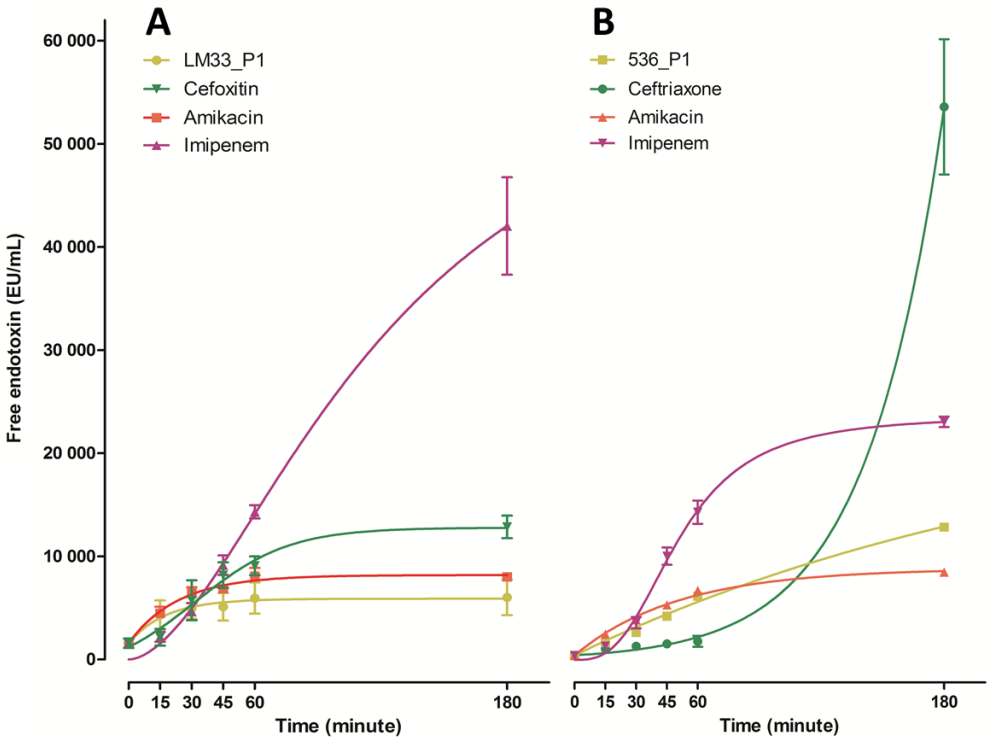
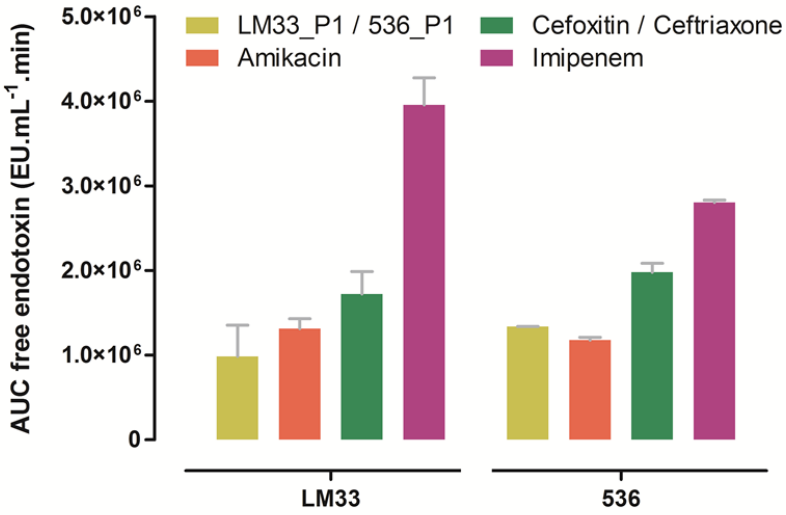
How much endotoxin is released: bacteriophages vs antibiotics

Endotoxin released





Endotoxin release:

Bacteriophages = Amikacin

Bacteriophages << β-lactams

Bacteriophages are as safe as Antibiotics

Dufour *et al.*, CID. 2017, *in press*

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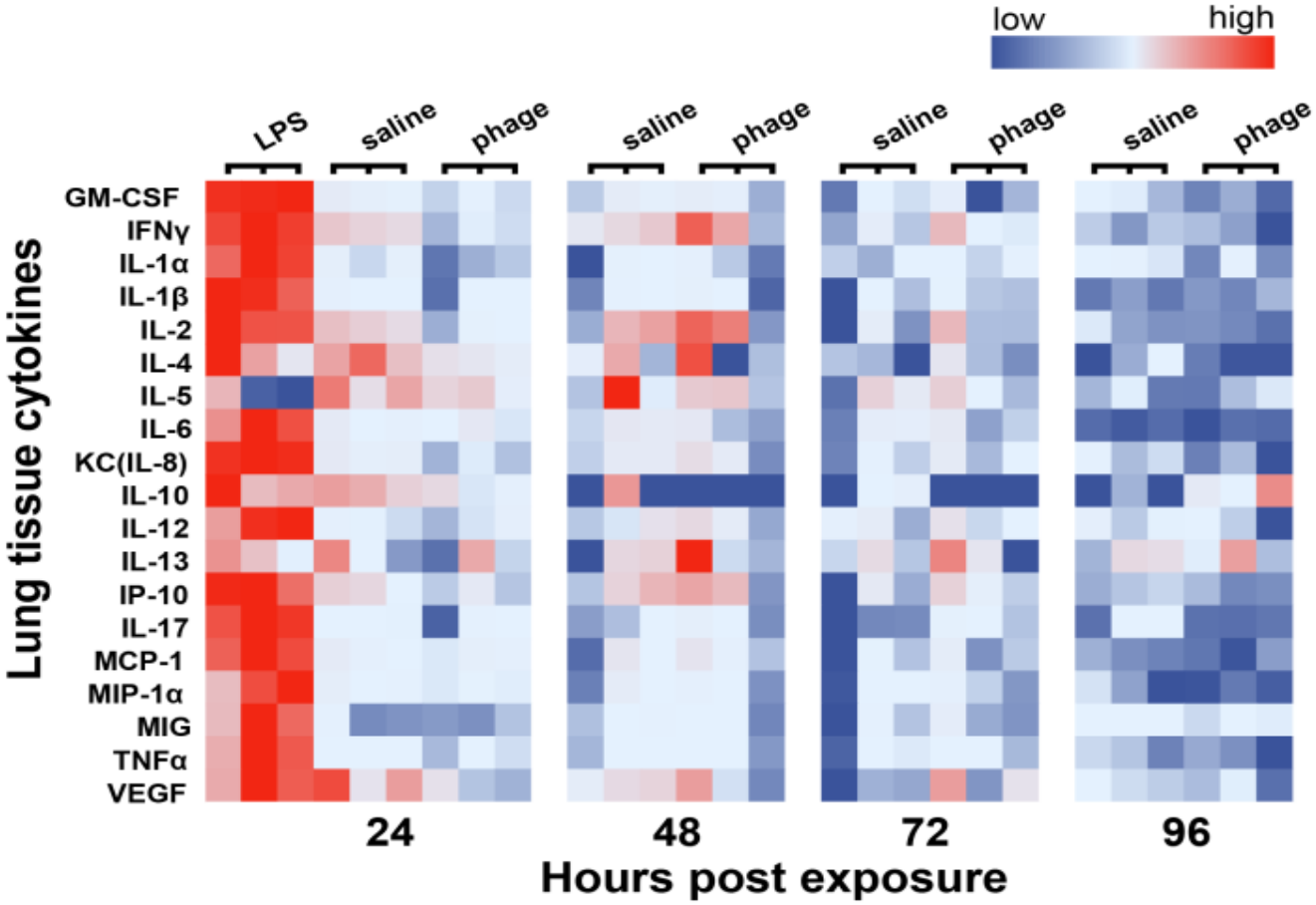
Bacteriophages do not stimulate cytokines



No induction of cytokines in lungs over time
(10 times higher dose, 10^9 pfu)

Roach *et al.*, Cell Host Microbe 2017

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Digestive tract, bacteria and bacteriophages

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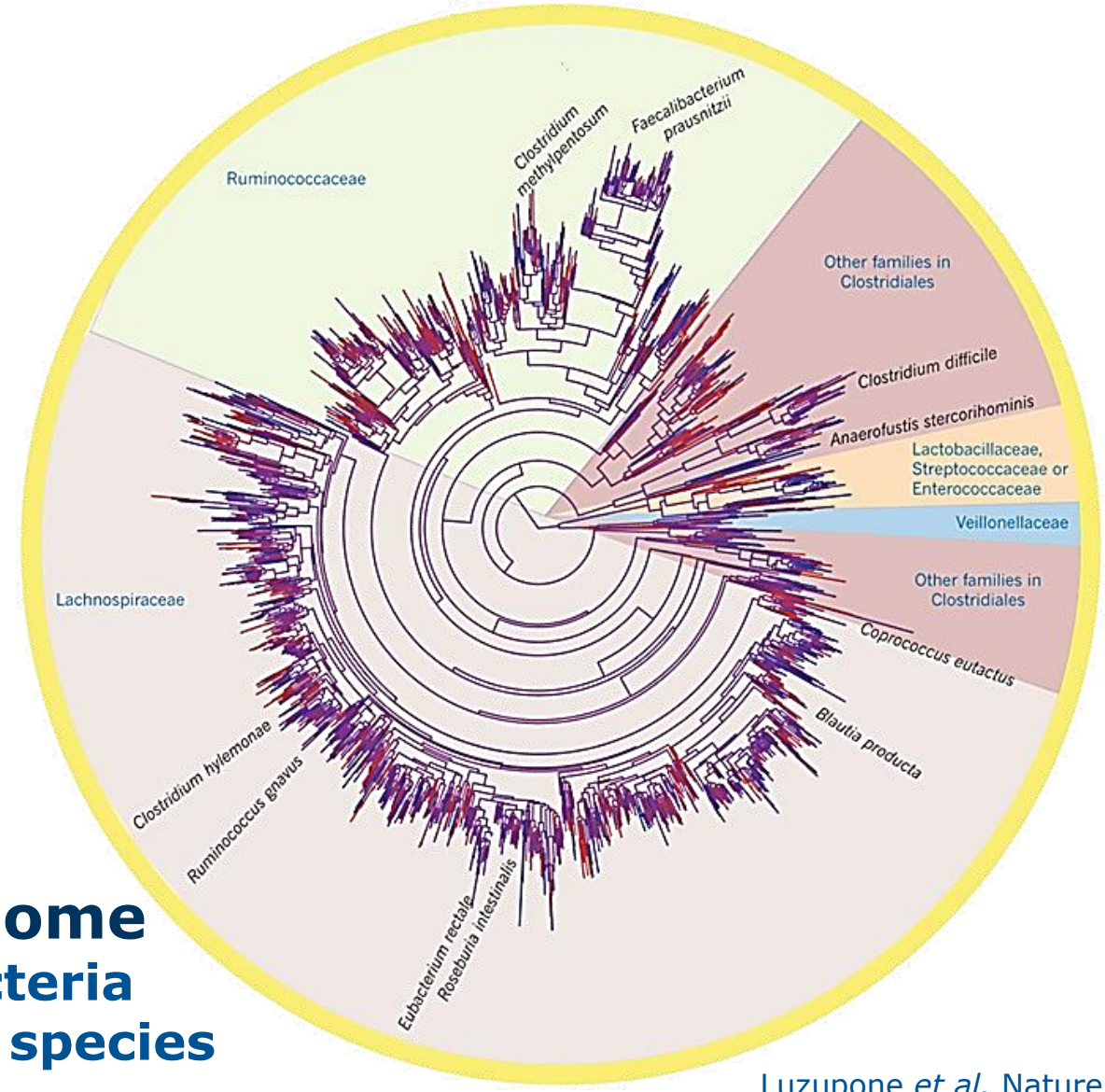
The gut microbiota

Bacteria, viruses, archaea, fungi



Microbiome

10^{13} bacteria

up to 1000 species

Luzupone *et al.* Nature 2012

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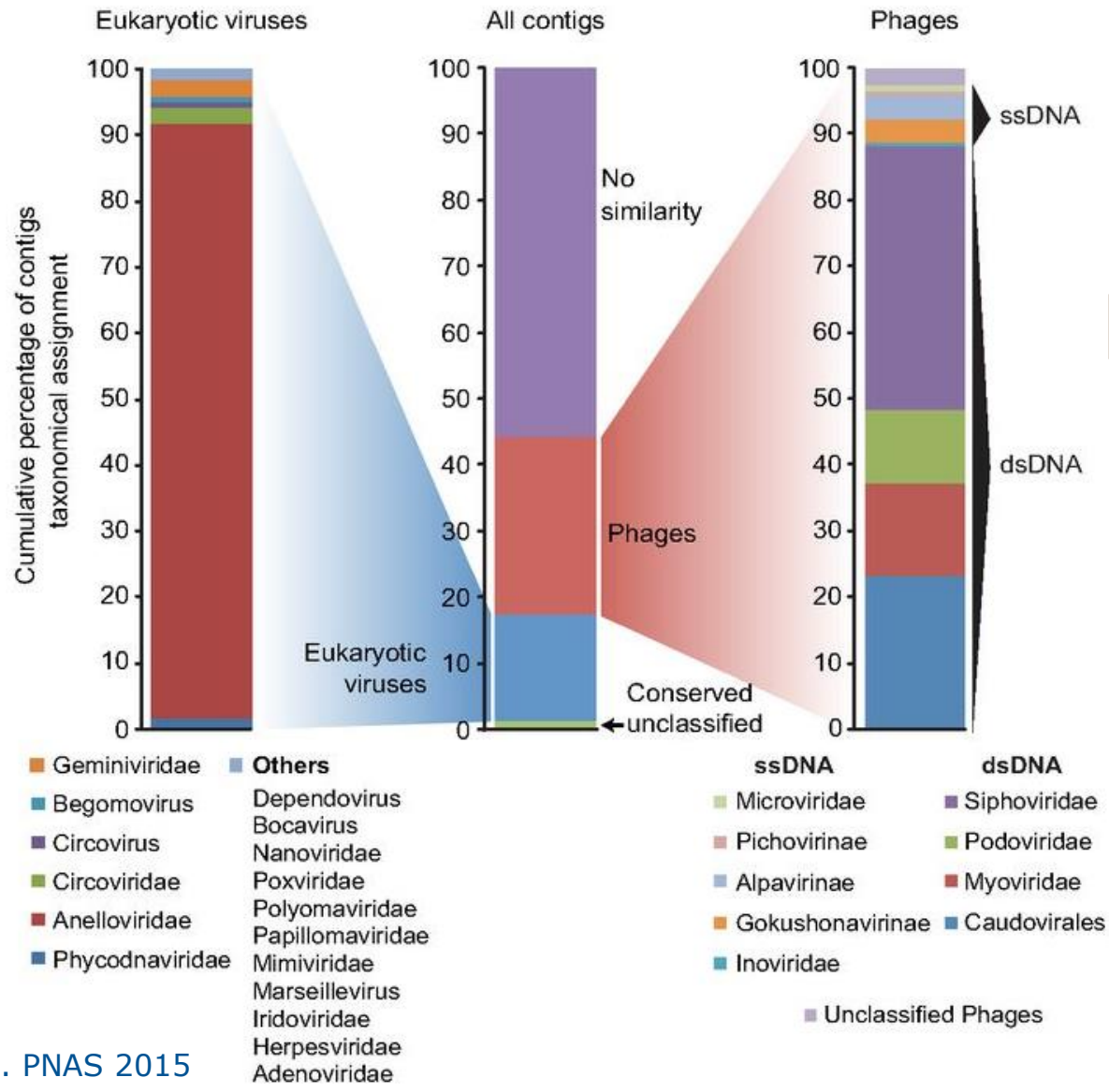
Virome: as abundant as bacteria



Phageome

Reyes et al. PNAS 2015

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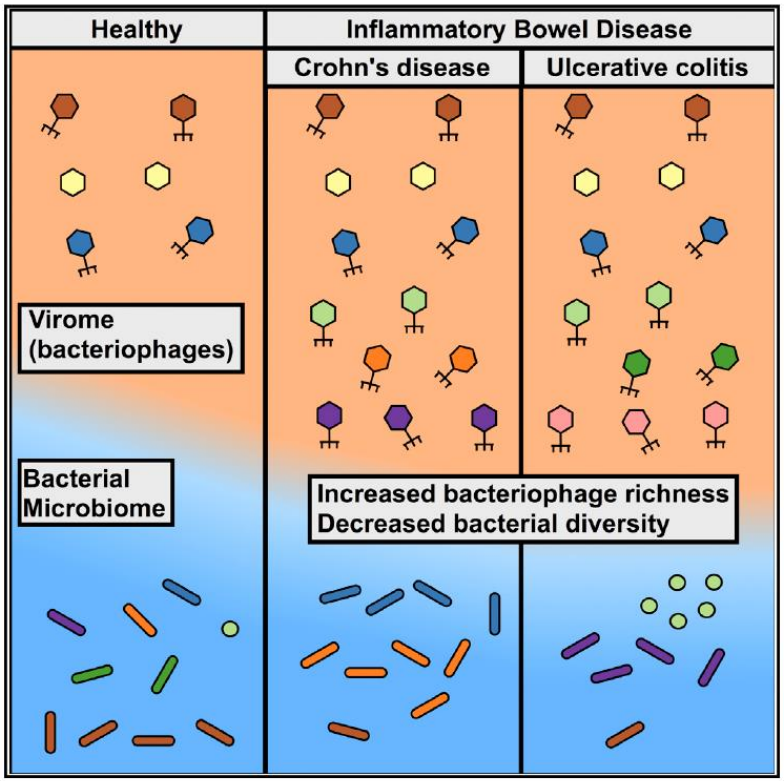
Link between bacteriophages and gut-related diseases ?

Article

Cell 2015 Jan 29;160(3):447-60

Disease-Specific Alterations in the Enteric Virome in Inflammatory Bowel Disease

Graphical Abstract



Authors
Jason M. Norman, Scott A. Handley, ...,
Miles Parkes, Herbert W. Virgin

In Brief

The enteric virome is abnormal in multiple cohorts of inflammatory bowel disease patients, exhibiting disease-specific features that are not explained by changes in bacterial diversity and richness.

PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Healthy human gut phageome

Pilar Manrique et al. PNAS 2016

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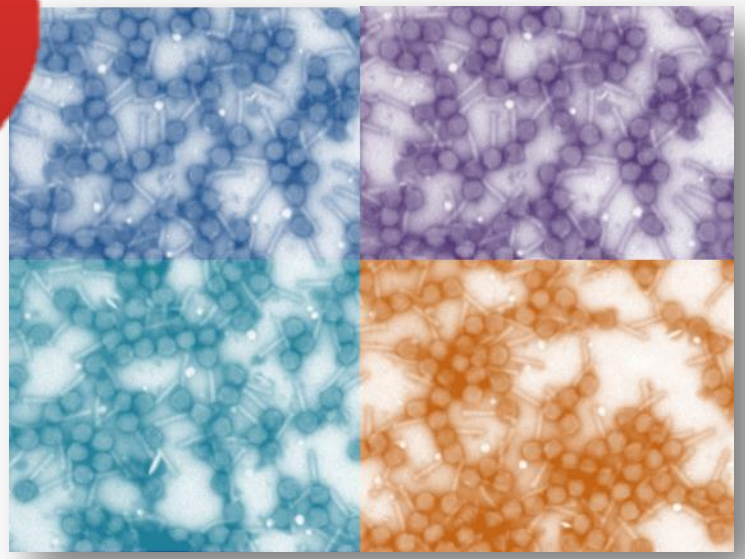
What is the role of bacteriophages ?



10^{10+}

bacterial cells

10^{10+}

bacterial viruses

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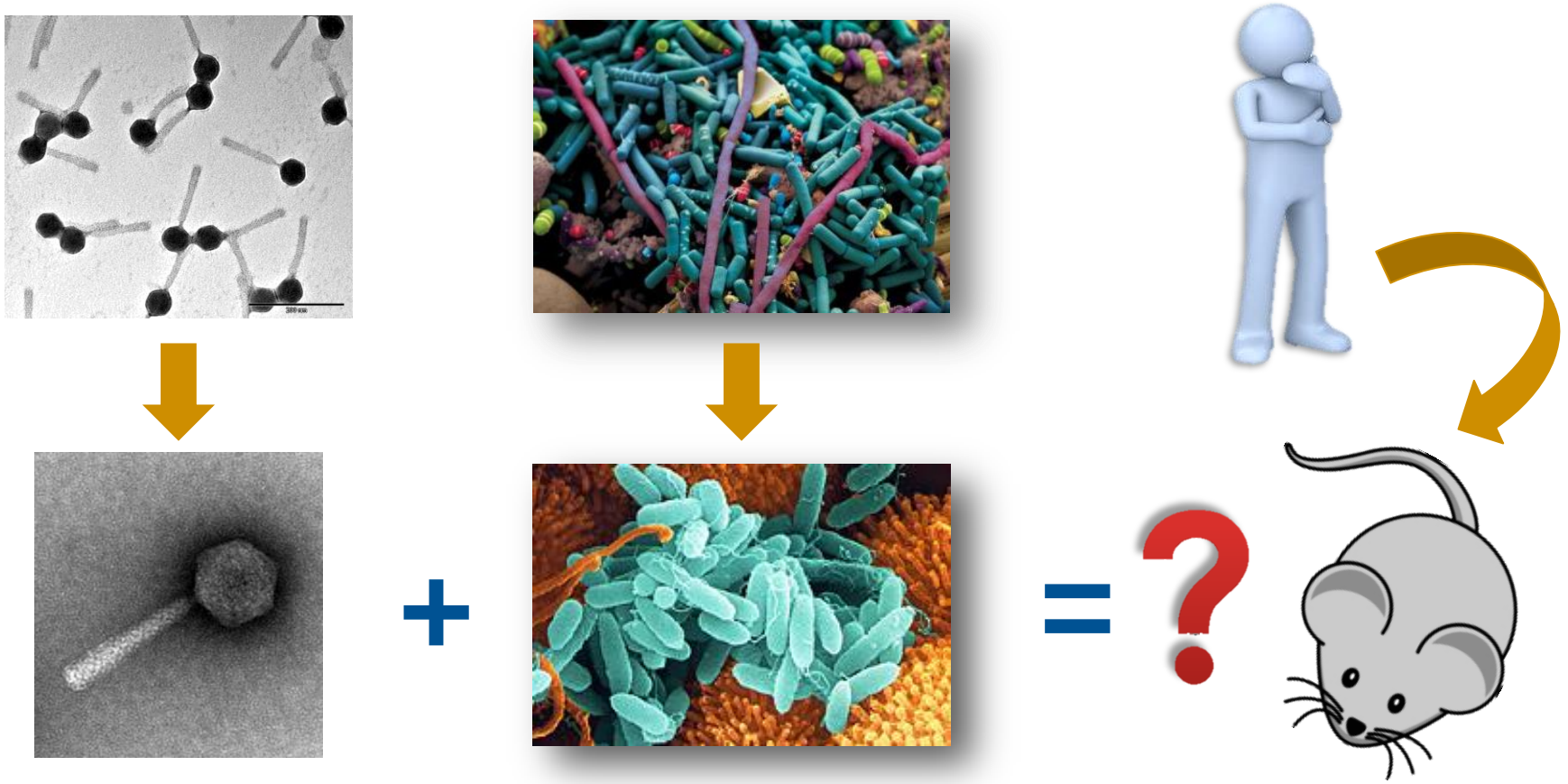
Reducing complexity



Virulent bacteriophages / *E. coli* / Mice gut

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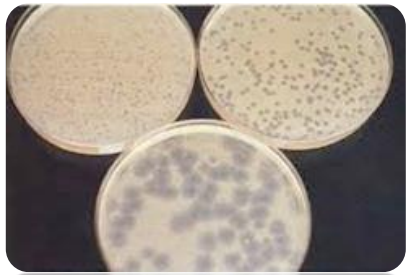
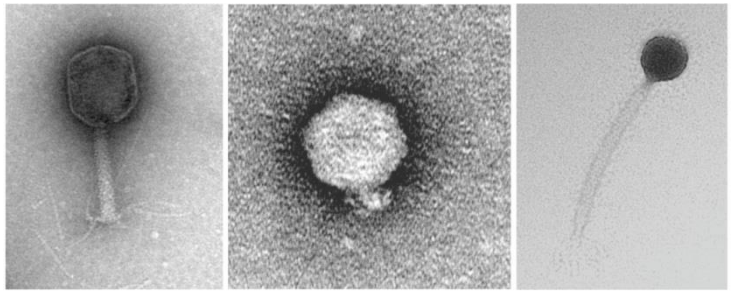
Virulent bacteriophages isolation

isolation

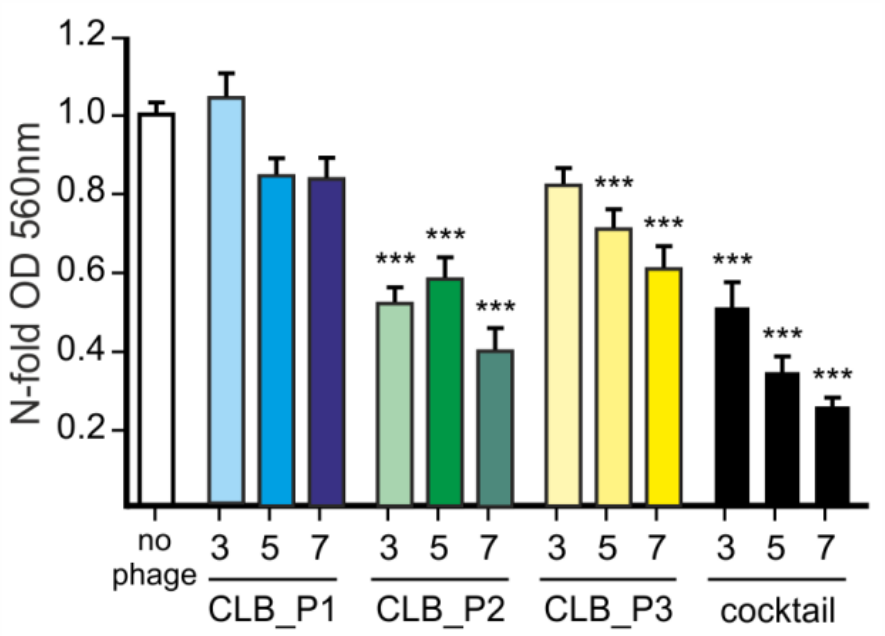
purification



characterization



formulation



Synergistic effect of the cocktail on biofilms

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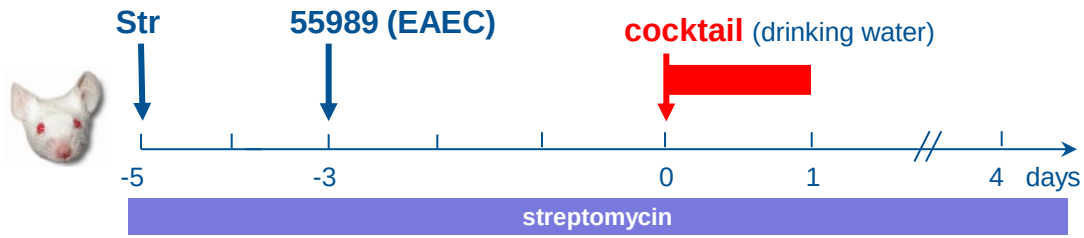
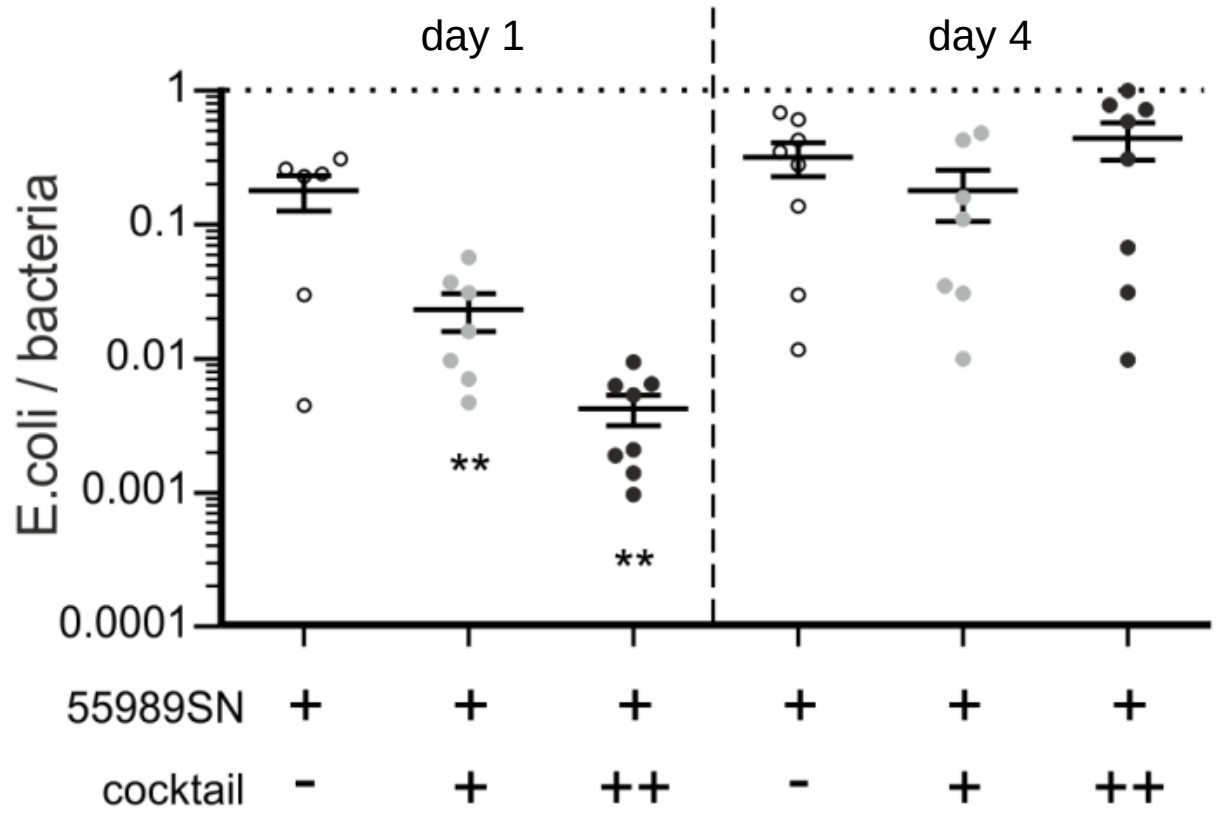
In vivo activity of the cocktail in colonized mice





Maura *et al.*, *AAC*, 2012

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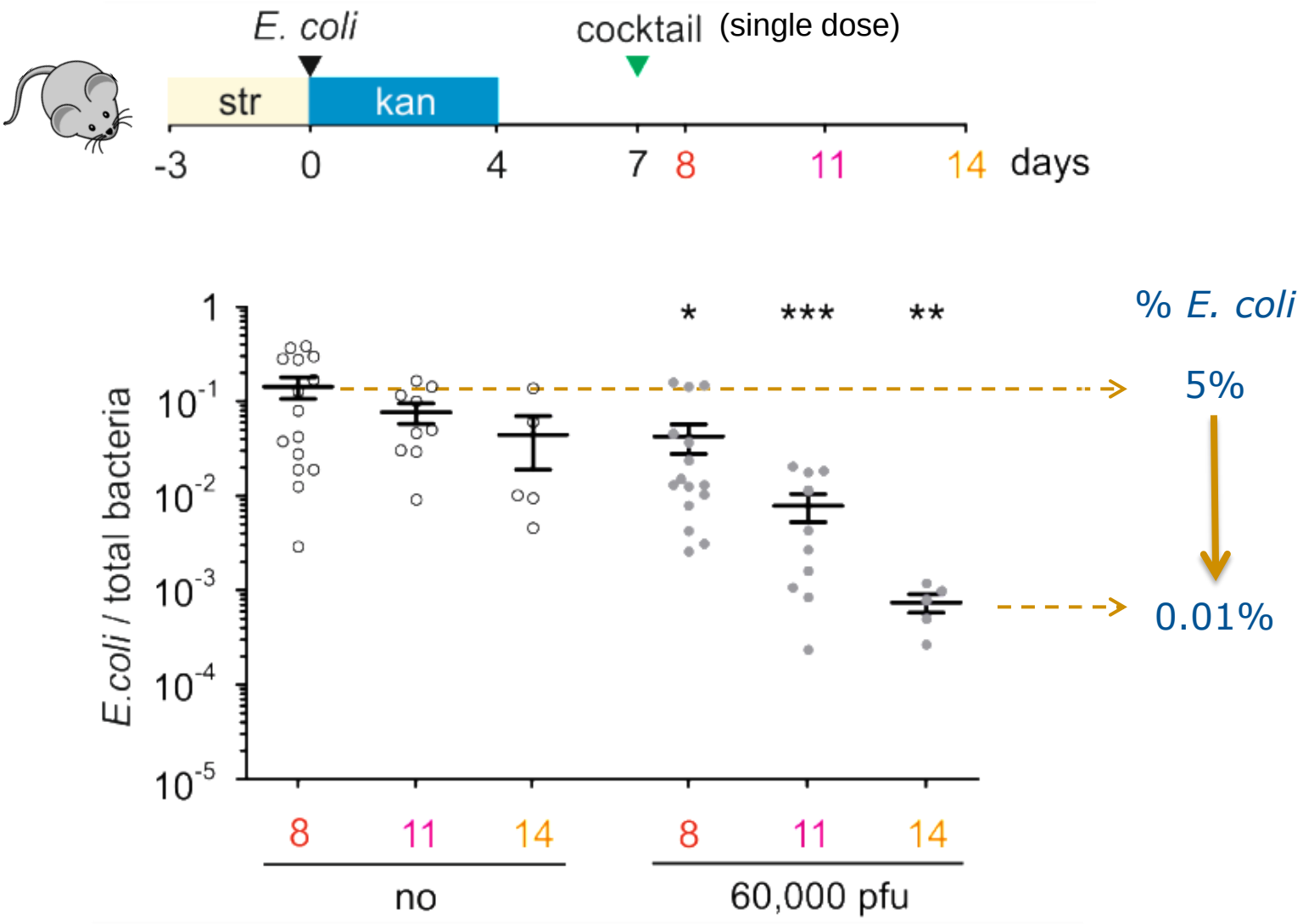
Gradual reduction of *UPEC* colonization



Galtier *et al.*, *Env. Microbiol*, 2016

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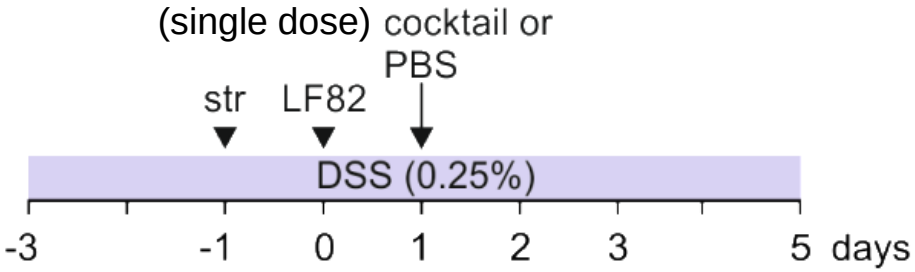
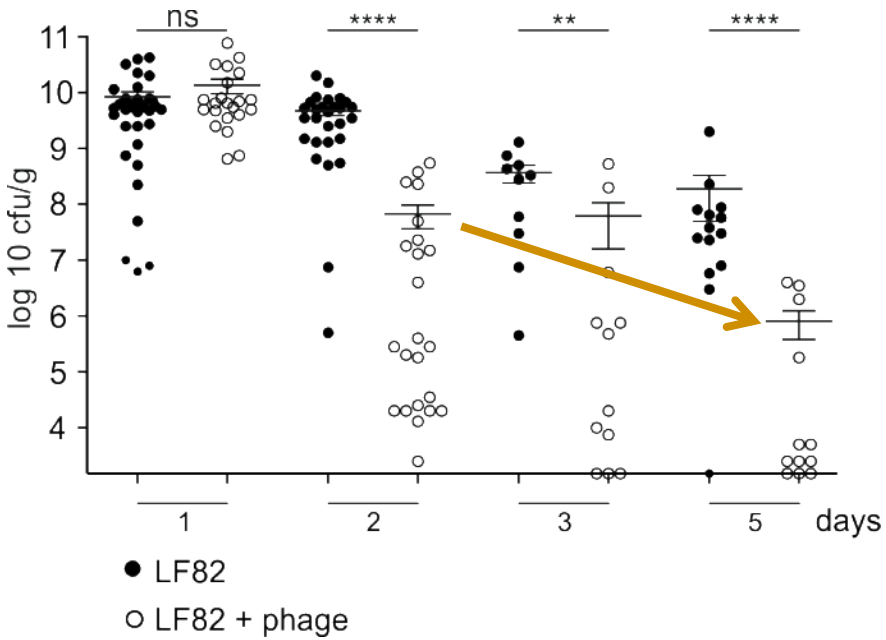
Targeting Adherent Invasive *E. coli* (AIEC)



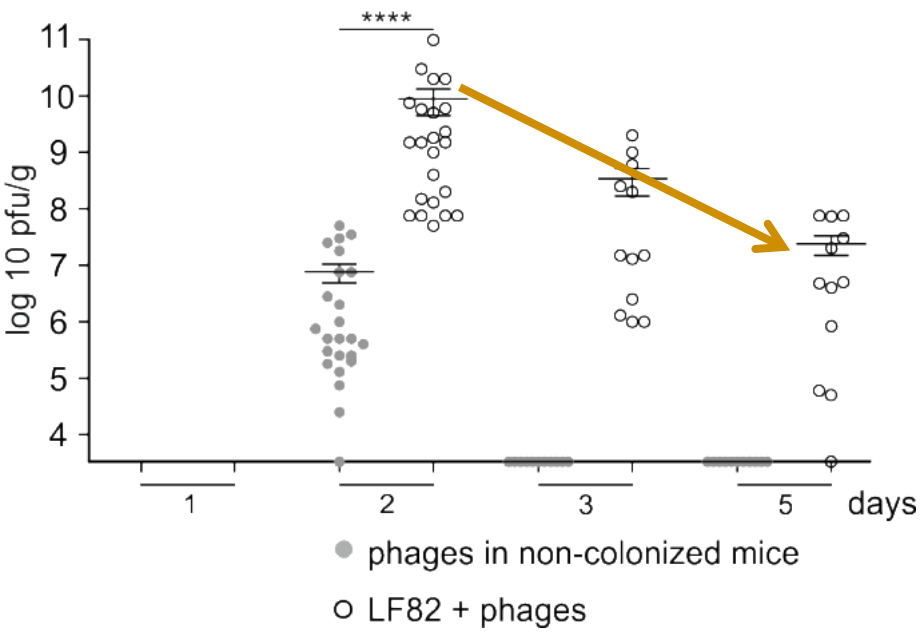
Transgenic mice expressing human receptor of strain LF82

Cocktail of 3 bacteriophages (single dose)

Strain LF82



Bacteriophages



Parallel reductions of host and viruses in fecal samples

Galtier *et al.*, *J. Crohn's Colitis*, 2017

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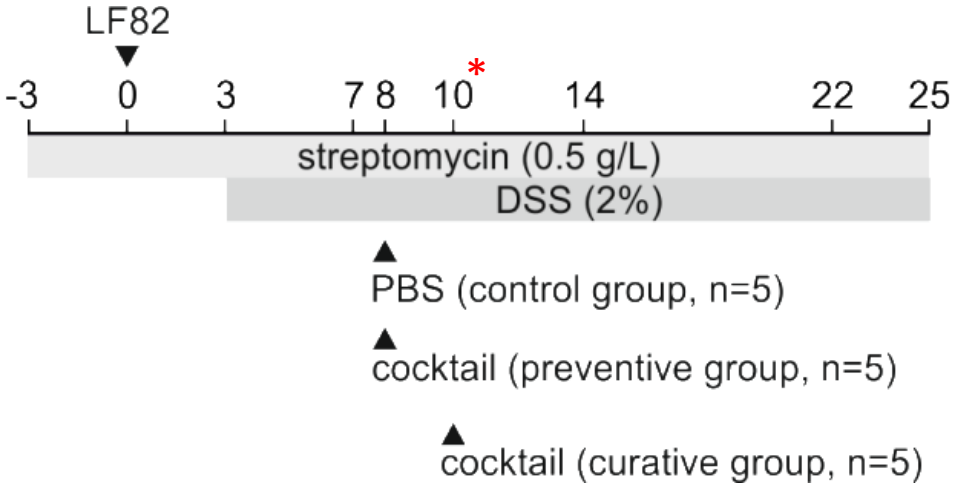
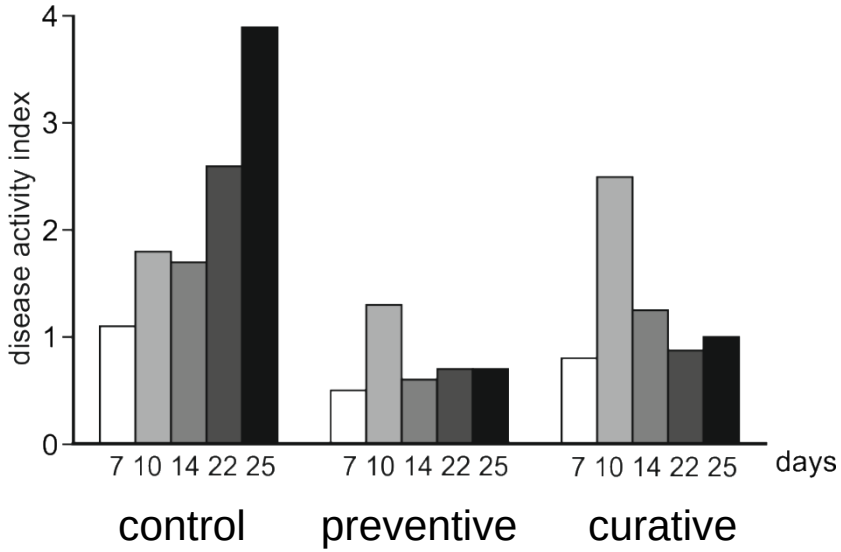
Targeting Adherent Invasive *E. coli* (AIEC)

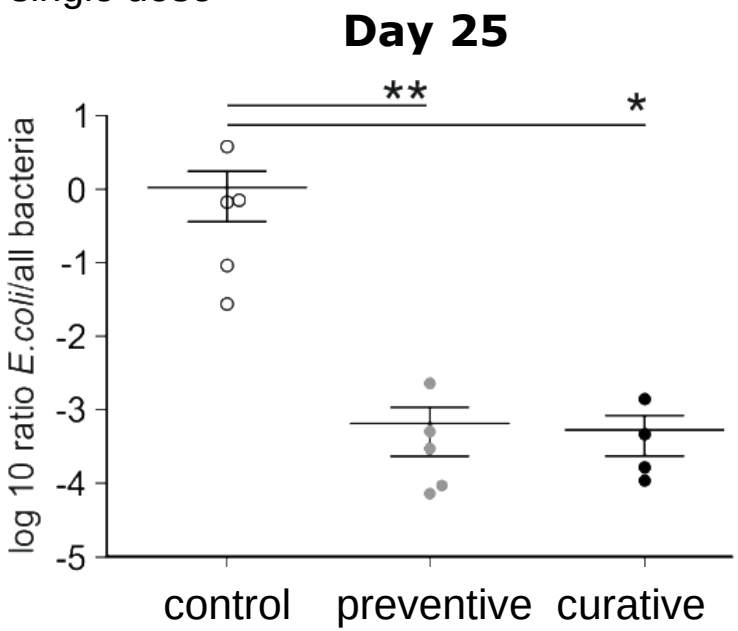


Conventional mice

* on day 10 colitis symptoms observed

single dose





Sustainable reduction of AIEC in both ileum and colon

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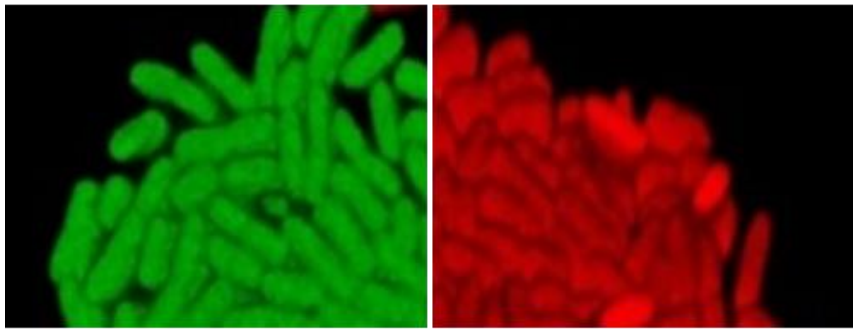
Galtier *et al.*, *J. Crohn's Colitis*, 2017, *in press*

Targeting the bad and ignoring the good

MG1655
(K12)

co-colonized mice



commensal pathogen

LF110_P1

LF82
(AIEC)

Bacteriophage LF110_P1 infects LF82 but not MG1655 (no affinity)

How this system will evolve over time ?

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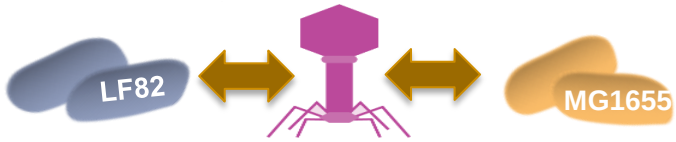
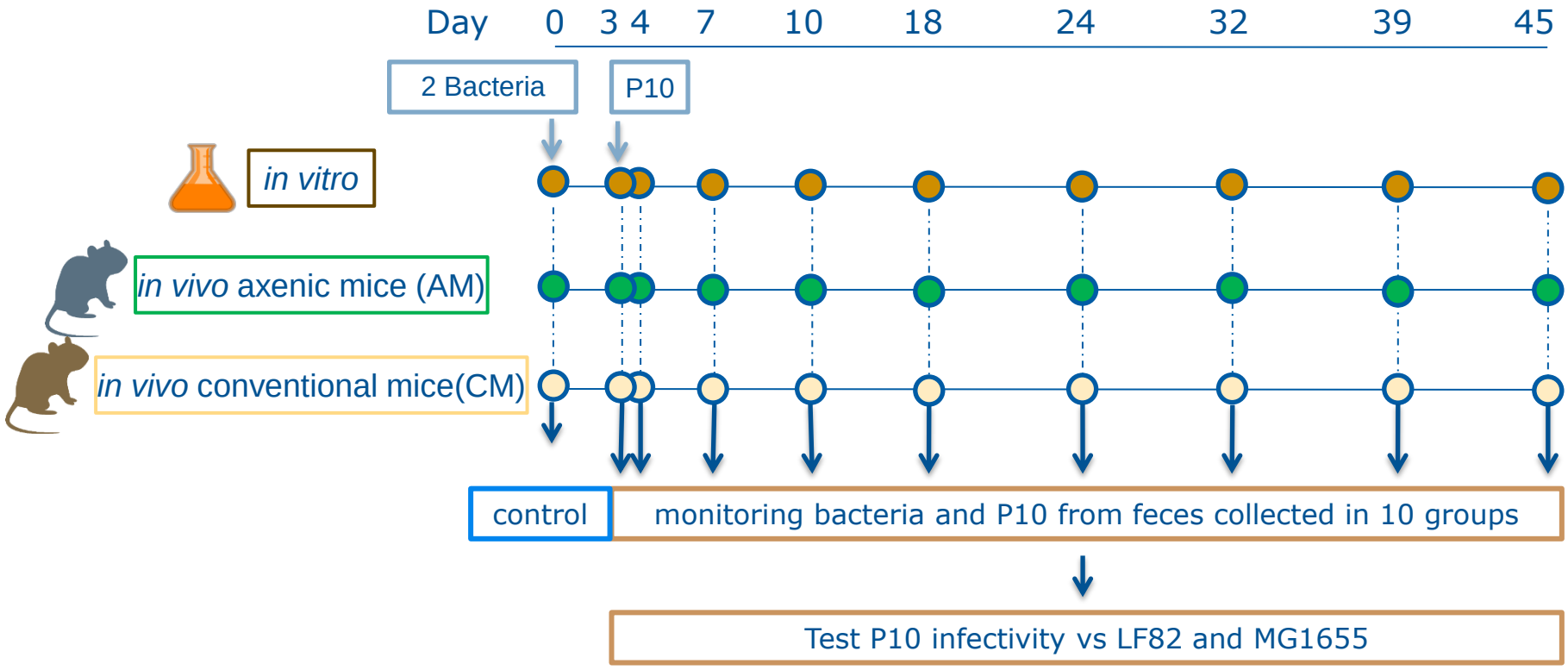
A model of coevolution

3 experimental settings





Host shift observed only in conventional mice

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Bacteriophage populations genomics

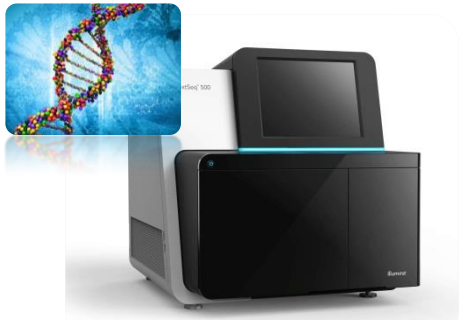
Whole evolving bacteriophage populations

In vitro

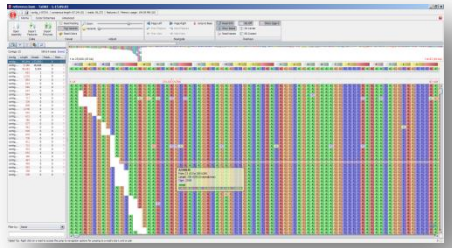
Axenic

Conventional
(adapted - and non adapted)

Phage isolation



DNA sequencing



Variant calling
versus the
ancestral P10

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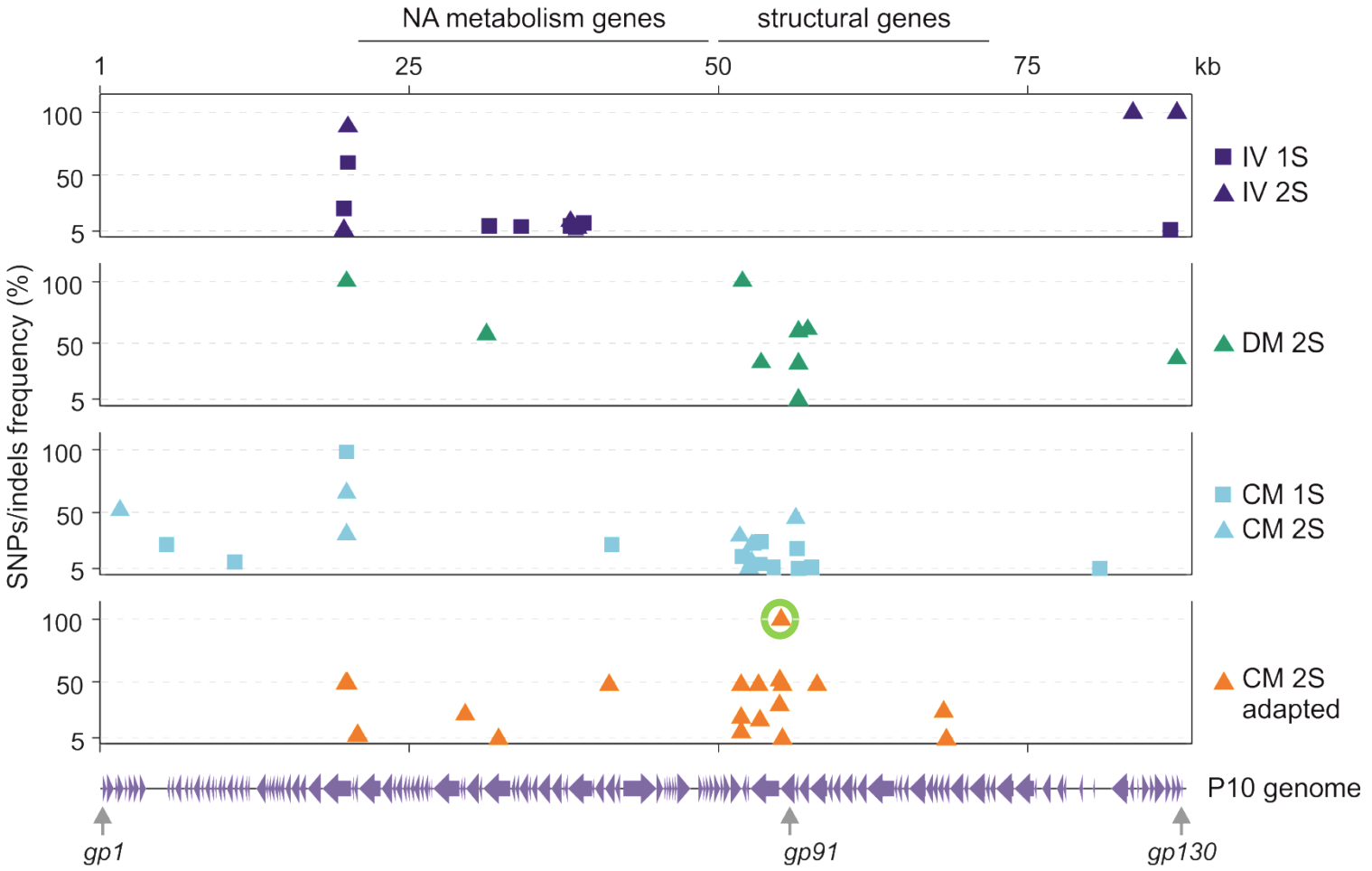
A unique SNP present only in conventional mice



Y284H mutation in GP91 is unique in conventional mice

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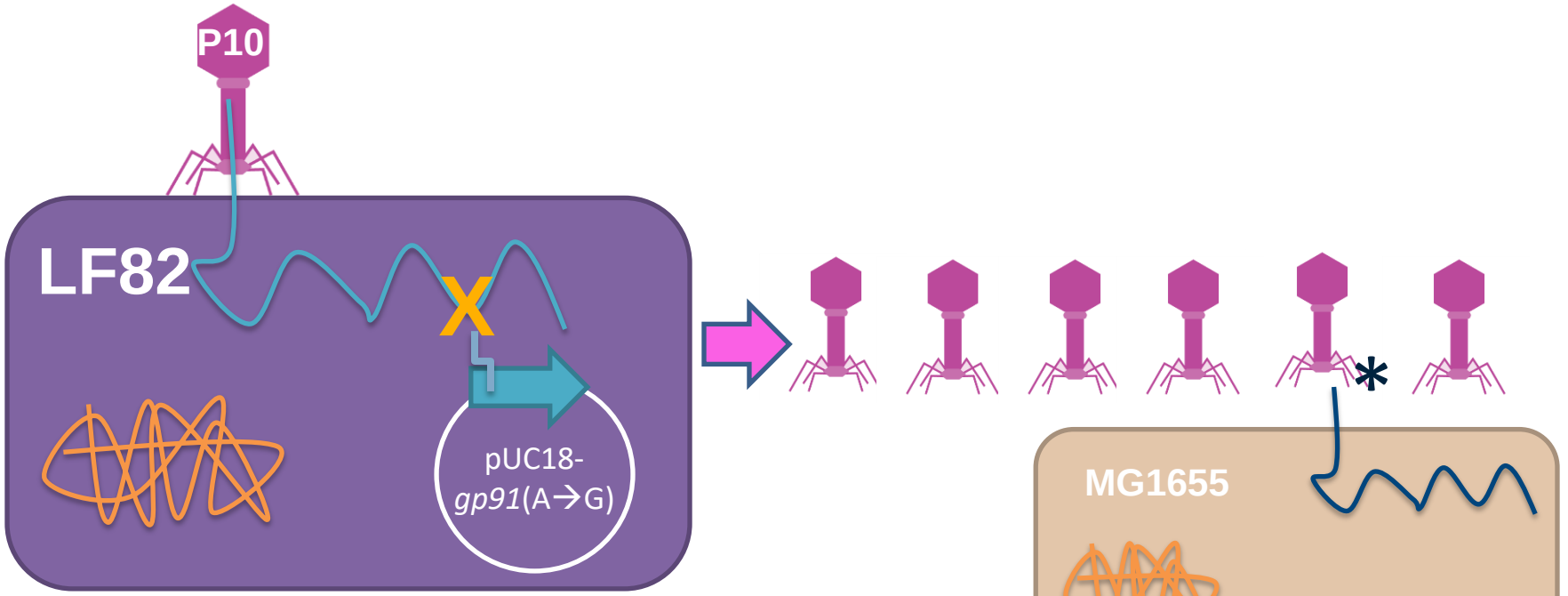
Can one mutation be enough for host-jump ?



All recombinant phages carry mutation Y284H

```
Recombinant 1  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 2  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 3  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 4  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 5  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Recombinant 6  CCGATAGGCCACCGCCTGCTTCATGTGTCAATCTGTGGTTATGAGCACC
Ancestral      CCGATAGGCCACCGCCTGCTTCATATGTCAATCTGTGGTTATGAGCACC
               ********************** ************************
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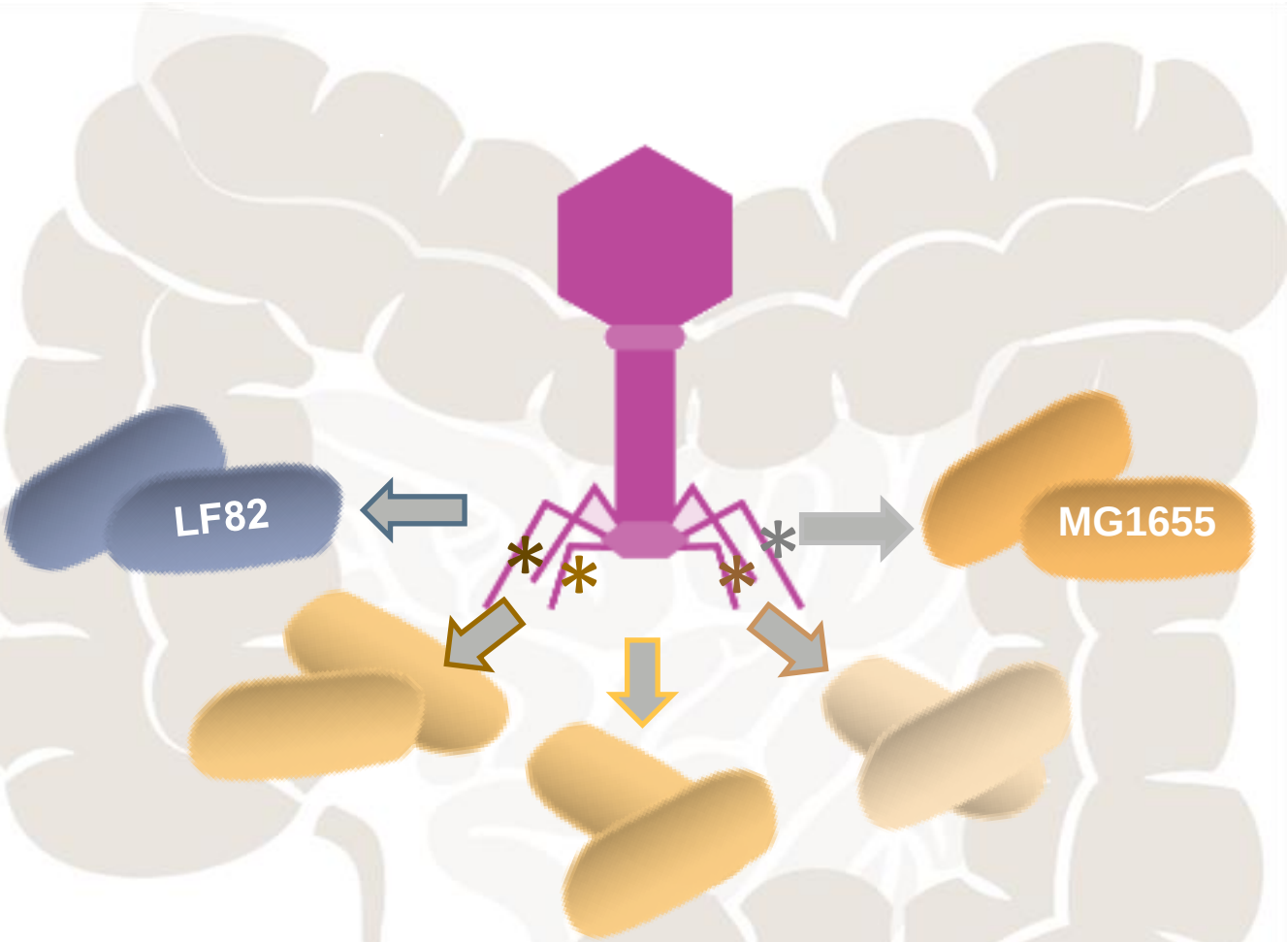
How viral evolution can be driven by microbiota?



Does it requires intermediate adaptation?

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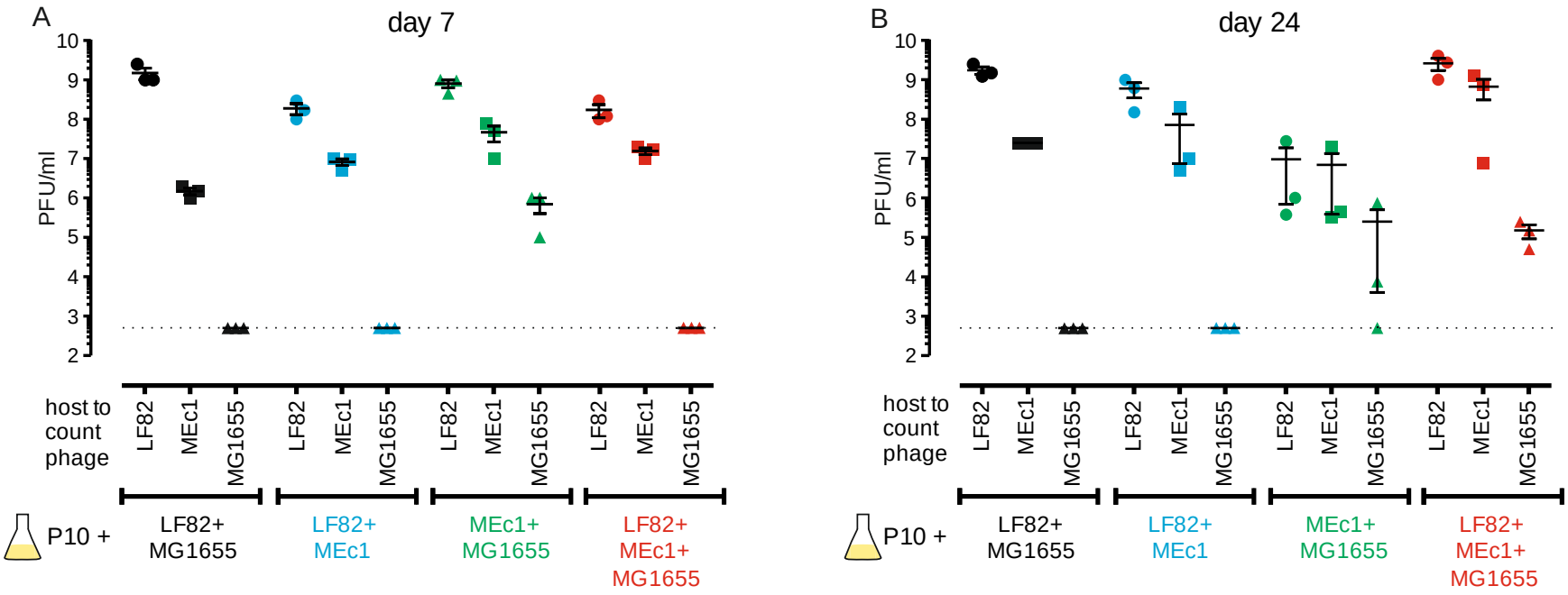
An intermediate host is required for host-jump

A murine *E. coli* strain sensitive to P10 was isolated from feces.



In vitro, the 3 *E. coli* strains can recapitulate the host jump.

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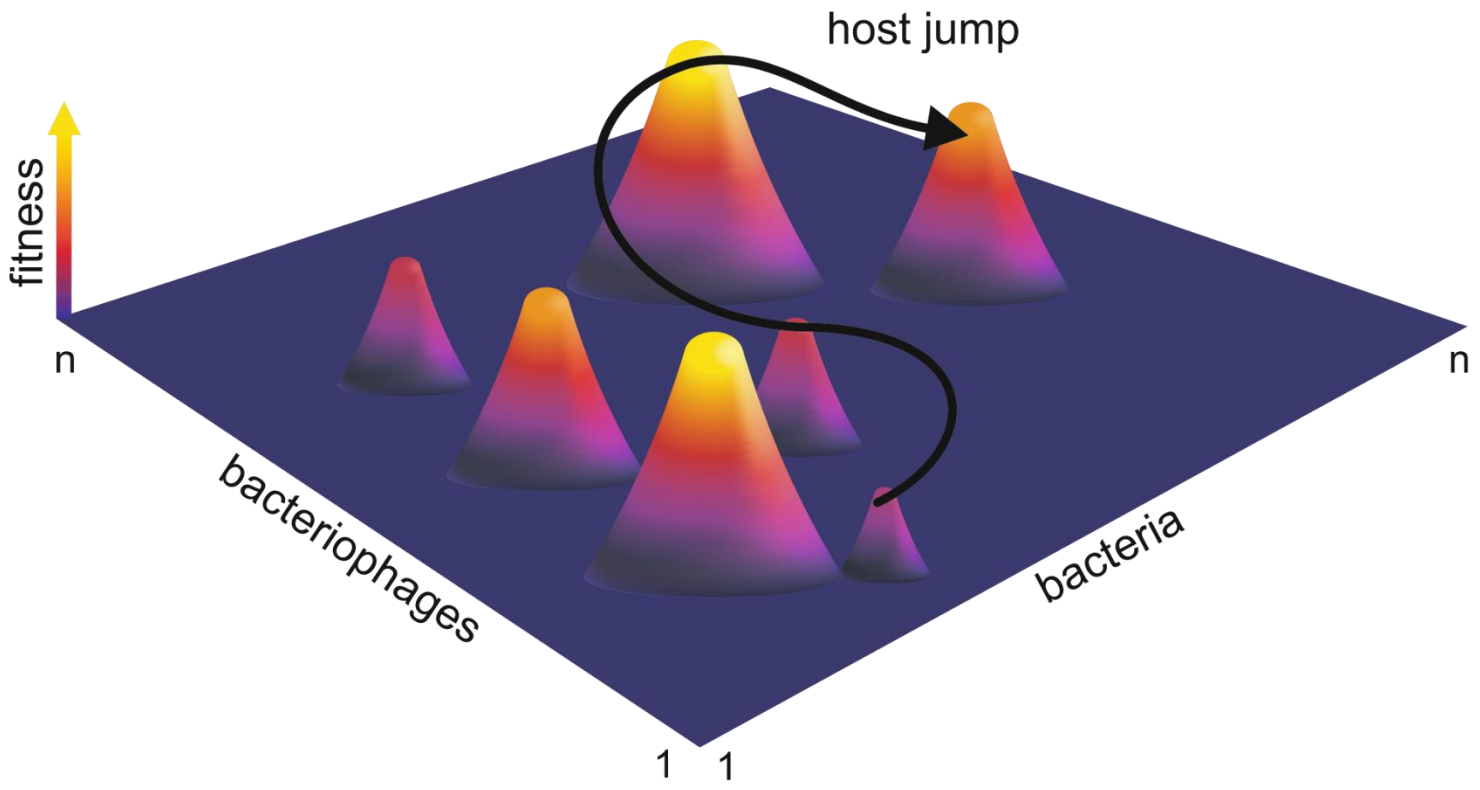
Microbiota is driving bacteriophage evolution leading to viral persistance



And vice et versa, bacteriophage evolution is driving microbiota diversity

De Sordi *et al.*, Cell Host Microbe, 2017, in revision

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How phage therapy will come back in clinics ?

1) Clinical trials

Problem: pharmaceutical companies not yet present

2) Regulations

Problem: not enough clinical data

3) Personalized vs generic treatments ?

Problem: economic sustainability vs ethical decision

4) Currently

National health agencies agree to treat only patients that are in urgent needs

Until when ?

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Interactions Bacteriophages Bacteria in Animals

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Raphaëlle DELATTRE (MD-PhD)
Nicolas DUFOUR (MD-PhD)
Luisa Di SORDI (Post-doc)
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Dwayne ROACH (Post-doc)

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