Natural World versus Laboratory World: Natural Gut Microbiota from Wild Mice Improve Host Fitness in Viral Infection and Carcinogenesis Models

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Advantages of the Laboratory Mouse Model

- Low costs
- Ease of genetic manipulation
- Wide variety of inbred strains
- High throughput
- Standardized environment

Discovery of basic immunological mechanisms:
- T cell receptor recognition, antibody diversification
- Identification of innate immune receptors and signaling pathways

Most Nobel prizes in immunology in the past 30 years have been awarded for work involving mouse models.
High rate of false positive results in preclinical studies

Results of
28 (37%) mouse studies replicated in human trials
14 (18%) were contradicted by randomized trials
34 (45%) remain untested.

Anti-TNF was developed for treatment of sepsis and protected mice in preclinical studies, but not humans in clinical studies.
Can Animal Models of Disease Reliably Inform Human Studies?

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The ability of animal studies to detect serious post marketing adverse events is limited

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Meta-Genome and Meta-Organism
Advantages of the Laboratory Mouse Model

Variation in the microbiome of the laboratory mouse

*Ivanov et al., Cell 2009*

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**OF MICE AND MICROBES**

The zoo of bacteria and viruses each lab animal harbors may confound experiments

*Servick, Science 2016*
Advantages of the laboratory mouse model


Reproducibility

WT: High IgA
WT: Low IgA

Vertical transmission of IgA phenotype
Reproducibility

WT: High IgA

WT: Low IgA

Vertical transmission of IgA phenotype

WT: High IgA

WT: Low IgA

Horizontal transmission of low IgA phenotype

Co-house mice

WT: High IgA

WT: Low IgA

Stappenbeck and Virgin,
Nature 2016
Reproducibility

**WT: High IgA**

- Vertical transmission of IgA phenotype

- Horizontal transmission of low IgA phenotype

**WT: Low IgA**

Co-house mice

**WT: High IgA**

**WT: Low IgA**

Demonstrate role of microbiome in phenotype

**WT: Low IgA**

Faecal transplant

**WT: High IgA**

**WT: Low IgA**

Demonstrate IgA degradation by commensal microbes

*Stappenbeck and Virgin, Nature 2016*
Required Information for Publication

Host genetics
- Specify strain using JAX or other commercial vendor nomenclature.
- Original source for purchased or shared mice used for breeders used to create colony.
- For mixed background, include data defining strain percentage (microsatellite analysis, number of markers).
- Define the method used to create the mutation (for example, homologous recombination in embryonic stem cells, transposon mutagenesis, chemical mutagenesis, Cas/CRISPR systems). Show data validating the altered allele.

Experimental methods within mouse facility
- Source of experimental and control mice (for example, bred in facility, purchased from specified vendor; for latter interval from arrival in facility to experiment).
- Control for microbiome effect (for example, littermates, multiple dams, co-housing, faecal transplant, gnotobiotic).
- Breeding scheme to generate experimental and control mice.
- Number of breeding pairs used to generate progeny for analysis.
- Number and gender of mice analysed per experiment.
- Number of experiments performed.
- Antibiotic exposure (type and duration) of breeders and progeny.

Husbandry details
- pH of drinking water.
- Diet source (vendor, nutrient composition), storage (temperature, duration) and treatment (irradiation, autoclave).
- Caging type (for example, ventilated, metabolic).
- Bedding amount per cage and type.
- Frequency and protocol for cage changing.
- Light–dark cycle of room.
- Temperature of room (include range).
- Pathogen screening (organisms tested for, methods, source of analysis in house versus commercial vendor).

Microbiome analysis
- Methods of sample collection, library preparation.
- Analytical pipeline including version and database dates.
- Methods of statistical analysis.
- Specify method used if corrections for multiple comparisons were performed.
Germfree or antibiotic treated

Lab mice in barrier facility

Wild mice *Mus musculus domesticus*
Advantages of the laboratory mouse model

Laboratory World
Normalized and Restrictive Environment
Natural World
Comparative Immunology of Lab and Wild Mice

Serum IgG

T cell differentiation

Abolins et al., Nat Commun 2016
Cytokine production after in vitro stimulation of splenocytes

**IL-12p40**

**IL-13**

Abolins et al., Nat Commun 2016
Hypothesis

Laboratory mice lack host-microbe interactions that are physiologically important and found in the natural world.

Question

Can we learn anything from a naturally co-evolved microbiome?
Mus Musculus Domesticus from Maryland are Close Relatives to Standard Laboratory Strains
Mus Musculus Domesticus from Maryland are Close Relatives to Standard Laboratory Strains
The Lab Mouse Gut Microbiome Differs from that of their Wild-Living Kin
The Lab Mouse Gut Microbiome Differs from that of their Wild-Living Kin

PC1 (19.9%)
PC2 (6.67%)

Wild
- 2014
- 2015

Lab
- Charles River
- Jackson
- Taconic
The Lab Mouse Gut Microbiome Differs from That of their Wild-Living Kin
## Bio-banking and Selection of Ileocecal Material

<table>
<thead>
<tr>
<th>Wild mice trapping location</th>
<th>A</th>
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<th>C</th>
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Transfer of Natural Gut Microbiota into Pregnant Germ-free Mice

Rosshart et al., Cell 2018
The Wild Mouse Gut Microbiome can be Maintained in a Laboratory Mouse Colony
Machine Learning: Indicator Species Analysis
Do Natural Microbiota Promote Host Fitness?

Virus

Toxins, Mutagens
Virus Model

Survival

Female mice

Male mice

Days post infection

Percent survival

Lab
LabR
WildR

****

****
The Wild Mouse Gut Microbiome Confers a Survival Advantage Upon Influenza Virus Infection

**Survival**

- Female mice
- Male mice

**Weight Loss**

- Female mice
- Male mice
The Wild Mouse Gut Microbiome Confers a Survival Advantage Upon Influenza Virus Infection

**Survival**

Female mice

Male mice

**Weight Loss**

Female mice

Male mice

**Virus Titer**

Female mice

Male mice
The Wild Mouse Gut Microbiome Confers Traits that Abrogate Excessive Inflammation

**Lung Histology**

Lab

LabR

WildR
The Wild Mouse Gut Microbiome Confers Traits that Abrogate Excessive Inflammation

**Lung Histology**

- Lab
- LabR
- WildR

**Lung Cytokines and Chemokines**

- G-CSF
- M-CSF
- GM-CSF
- CCL2 (MCP-1)
- CCL3 (MIP-1α)
- CCL4 (MIP-1β)
- CCL20 (MIP-3α)
- CXCL1 (GROα/KC)
- CXCL2 (MIP-2)
- TNF-α
- IL-6
- IL-10
Cancer Model

**Inflammation score**

LabLabR WildR

G

AOM

**AOM**

2% DSS 2.5% DSS 2.5% DSS

Day 12 to 55:

****P<0.0001 WildR versus Lab

***P<0.001 WildR versus LabR

Percent initial weight

0 6 12 18 24 30 36 42 48 54 60 66 Days post AOM injection

Inflammation score

0 1 2 3 4 Lab LabR WildR

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The Wild Mouse Gut Microbiome Protects from Colorectal Tumorigenesis

![Image showing comparison between Lab, LabR, and WildR in proximal colon and rectum with statistical data on number of tumors and tumor area/colon area.](image-url)
The Wild Mouse Gut Microbiome Protects from Colorectal Cancer

**H&E stain**

**Movat stain**

![Microscopic images of tissue sections stained with H&E and Movat](image)

**Invasiveness score**

![Box plot showing invasiveness scores for different groups](image)
Summary

Chimeric Meta-Organism

Fitness Promoting Microbes

Tractable Genetics
Animal models with natural microbiota should

• enable the discovery of protective mechanisms that are relevant in the natural world and absent in the laboratory.

• increase the predictive utility of laboratory mice for modeling complex diseases in the natural world.
Acknowledgements

**Immunology Section, Liver Diseases Branch, NIDDK**

- Stephan Rosshart
- Brian Vassallo
- Ashli Hunter

**Collaborators**

**NIAID**
- Davide Angeletti
- Heather Hickman
- Jon Yewdell

**NCI**
- Jonathan Badger
- Giorgio Trinchieri

**FDA**
- Kazuyo Takeda

**University of North Carolina**
- Andrew Morgan
- F. Pardo-Manuel de Villena

**Baylor College of Medicine**
- Diane Hutchinson
- Nadim Ajami

Disclosure: NIDDK license to Taconic