Resources & Considerations for Rigor & Reproducibility in Translational Research (in animals)

Cory Brayton, DVM, Diplomate, ACLAM, ACVP
Director, Phenotyping Core
Associate Professor, Molecular and Comparative Pathobiology
Johns Hopkins University, School of Medicine
725 North Broadway, BM 815
Baltimore, MD 21205

cbrayton@jhmi.edu

http://mcp.bs.jhmi.edu/me680712-phenotyping-functional-genetics

Disclosures

- No financial disclosures that I know of...
- All opinions expressed and implied in this presentation are solely those of Dr. Brayton.
- The content of the presentation does not represent or reflect the views of Johns Hopkins University or Johns Hopkins Health system, ILAR or NASEM.

DISCLAIMERS

- Biases (pet peeves): veterinarian, pathologist, >30 yr research in academia (medical schools).

RESOURCES: (Guidelines)

For REPORTING (publishing) Animal Research:
- ARRIVE guidelines – Kilkenny & al 2010 - NC3Rs
  https://www.nc3rs.org.uk/arrive
- ILAR/NAS guidance for reporting (NRC 2011)
  http://www.nap.edu/catalog.php?record_id=13241
- FASEB Recommendations 2016
- MIBBI Minimum Information for Biological and Biomedical Investigations
  https://www.nc3rs.org.uk/mibbi
- HARRP harmonized animal research reporting principles 2018 (ICLAS)

For EMERGING/NEW TECHNOLOGIES:
- https://mcp.bs.jhmi.edu/me680712

20 areas of a research manuscript (ARRIVE Guidelines)

- Title
  1. Accurate & concise description
  2. Methods
    - Ethical statement
    - Study design and rationale
    - Experimental procedures (animal, human, tissue)
    - Experimental animals (species, sex, weight)
    - Housing and husbandry
  3. Results
    - Baseline data
    - Numbers analyzed
    - Outcomes & estimation
    - Adverse events
  4. Discussion
    - Interpretations & implications
    - Generalizability and translation
    - Funding
  5. PROTOCOL
    - https://www.nc3rs.org.uk/arrive-guidelines

ILAR GUIDANCE (NRC 2011)


- Emphasis on Scientific justifications and references.

Examples of specific recommendations for Materials and Methods (M&M):

- Background strain:
  - *The use of shortened stock and strain designations (e.g., Sprague-Dawley or C57BL/6J mouse) instead of the fully defined genetic nomenclature is not appropriate in published animal descriptions...*
  - Microbial status:
  - *List of the pathogens excluded, reference to the pathogen exclusion list from the commercial supplier...*

ILAR Guide (NRC 2011)

‘THE Guide’ is a different document.
However, from the GuidePs: Key Concepts...

...The Guide is created by scientists and veterinarians for scientists and veterinarians to uphold the scientific rigor and integrity of biomedical research with laboratory animals as expected by their colleagues and society at large.

http://www.nap.edu/catalog/13240.html

ICLAS HARRP = Harmonization of Animal research reporting principles

1. Ethics: ...confirmation of ethical review ...mandatory ...
2. Funding and conflict of interests: ...must be reported.
3. Background and scientific objectives: ...and why the particular animal species and model are the most appropriate to address the scientific objectives.
4. Study design: ...must include sufficient detail ...must be included in publications.
5. Animal subjects: source and details of animal subjects ...must be included...
6. Experimental protocols: ...must include details of any procedures and materials related to the humane treatment and welfare of the animals.
7. Housing, husbandry and research environment: ...non-experimental research environmental factors ...must be reported.
8. Data availability...

Additional Guidance /Strategies

- Specialty group guidance
  - Minimum information etc recommendations:
    - e.g. MinPDX, MinPEPa (pathology), MinSC (stem cells)...
  - For study DESIGN: Smith et al. (2017). PREPARE:
    - guidelines for planning animal research and testing. Lab Anim als.
    - Trends in author/reviewer check lists
      - e.g. Nature, Gold Standard, etc.
    - PROTOCOL Resource/repository
      - https://www.protocols.io
      - Find, discuss, report, revise protocols, ‘publish’ your protocols with DOI (so you can cite it)

Which is better?

<table>
<thead>
<tr>
<th>Standardization (reductionist approach)</th>
<th>More heterogeneity / complexity in research animals and research conditions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple system</td>
<td>(NOT referring to randomization in design)</td>
</tr>
<tr>
<td>Fewer variables</td>
<td>More like real life?</td>
</tr>
<tr>
<td>Better defined</td>
<td>BUT Can you understand/define well enough to get to valid conclusions?</td>
</tr>
</tbody>
</table>

Based on 11 guidance documents.

Educational Use Only

20. Brayton 3 Reproducibility

cbrayton@jhmi.edu 2019
Which is better

- Standardization (reductionist approach)
  - Simple system
  - Fewer variables
  - More heterogeneity / complexity in research animals and research conditions?
  - More like real life

Either / both?
IF/When designed done reported well enough

Animal Factors

**METHODS**

---Male or female pet store mice were introduced into the cages of 6-8-week-old C57BL/6 mice of the same sex purchased from the National Cancer Institute. Cohousing occurred within a BSL-3 facility. Age-matched C57BL/6 laboratory mice maintained in SPF facilities served as controls...

ILAR Guidance - Background strain:
- The use of shortened stock and strain designations (e.g., Sprague-Dawley rat or C57BL mouse) instead of the fully defined genetic nomenclature is not appropriate in published animal descriptions.

Animal Factors

**METHODS**

---Male or female pet store mice were introduced into the cages of 6-8-week-old C57BL/6 mice of the same sex ...

ILAR Guidance - Background strain:
- The use of shortened stock and strain designations (e.g., Sprague-Dawley rat or C57BL mouse) instead of the fully defined genetic nomenclature is not appropriate in published animal descriptions.

Animal Factors

**METHODS**

---Specified pathogen-free C57BL/6 mice were obtained from Charles River Laboratories (Margate, UK), Harlan (UK) or B&K Universal (UK) and were housed in same-sex groups of five mice per cage, maintained in a 12 h light/12 h dark cycle with environmental enrichment and were fed on commercial rodent diet, ad libitum (EURodent diet 23%; PMI Nutrition International, LLC, Brentwood, MO, USA).

Immune Relevant Genotypes (a few):

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>MHC</th>
<th>c5</th>
<th>Afr</th>
<th>ApoA2</th>
<th>Tlr</th>
<th>Rmc</th>
<th>Slc11a2 (H2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>N2</td>
<td>A</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>A/J</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>Akr</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>Balb/c</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>C3H</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>C57Bl6</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>FVB/N</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
<tr>
<td>NOD</td>
<td>N2</td>
<td>N2</td>
<td>APOA2</td>
<td></td>
<td>TLR4</td>
<td>RMC</td>
<td>Slc11a2</td>
</tr>
</tbody>
</table>

**STRAIN MATTERS.**

Phenotypes in ‘Normal ‘wt’ (a few):

<table>
<thead>
<tr>
<th>Disease</th>
<th>Normalization Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teratomas</td>
<td>Tumor formation in tissues</td>
</tr>
<tr>
<td>Lung tumors</td>
<td>Malignant growth</td>
</tr>
<tr>
<td>Anoikis</td>
<td>Cell detachment</td>
</tr>
<tr>
<td>Amyloid</td>
<td>Protein aggregation</td>
</tr>
</tbody>
</table>

**Supported by:**

- https://www.nature.com/articles/nature17655
- https://www.nature.com/articles/natcomm7483

**ACKNOWLEDGMENTS:**

- The comparative immunology of wild and laboratory mice, Mus musculus domesticus. https://www.nature.com/articles/ncomms14831
Immune Variations, Strain Associated

<table>
<thead>
<tr>
<th>TH1 bias e.g B6</th>
<th>TH2 bias e.g. BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>(More) Innate</td>
<td>(More) Adaptive/Acquired</td>
</tr>
<tr>
<td>Hc cs</td>
<td>Soluble carrier family 11a member 1</td>
</tr>
<tr>
<td>StNl2clsolvent</td>
<td>Thy14 Tll1 receptor 4</td>
</tr>
<tr>
<td>Natural Killer Cell function - Variation in NK complex Kta (Ly49) Kth (Nkrp) etc</td>
<td>R2H 2:2; mutation 1 - Hypo-active variant of D-2 with decreased T cell activation</td>
</tr>
<tr>
<td>NAIP neuronal Apoptosis inhibitors proteins</td>
<td>TrkB8 T cell receptor beta variable 8</td>
</tr>
<tr>
<td>Sirp in NOD 8phagocytosis</td>
<td>Slam signaling lymphocyte activation molecule family (CD48) polymorphisms</td>
</tr>
<tr>
<td>Catherin E function</td>
<td>Dock2 - disruptor cytokinesis 2</td>
</tr>
</tbody>
</table>

B6 Genotypes (a few):

<table>
<thead>
<tr>
<th>Gene/allele info</th>
<th>System /phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>a/a</td>
<td>Black non agouti</td>
</tr>
<tr>
<td>H2-2</td>
<td>MHC haplotype</td>
</tr>
<tr>
<td>Ah/B3</td>
<td>MHC haplotype</td>
</tr>
<tr>
<td>Aplp2a</td>
<td>Apliprotein A2a allele</td>
</tr>
<tr>
<td>Cadl2a1</td>
<td>Cadherin 23 associated hearing loss 1</td>
</tr>
<tr>
<td>Scl2a1</td>
<td>solute carrier family 13 (Wrapjed) susceptible</td>
</tr>
</tbody>
</table>

B6 History and SUBstrains (a few):

- Expect genotype variations
- Which do you use?
- WHY?

Some Genes with known variations among J and N substrains:
Aamat: arylalkylamine N-acetyltransferase (eg: be melatonin) 
Dock2: dedicator of cyto-kinesis 2

Nlrp2: NLR protein 12

Nnt: nicotinamide nucleotide transhydrogenase deficient

B6 SUBstrains variations:

Dock2: dedicator of cyto-kinesis 2 - altered B cell development/migration, response to chemokines; lack M2 B cells; increased CD8+ memory cells; Dock2/JimHsd:

- Identified in GEM on C57BL/6N/Hsd background.
- Eliminated? From C57BL/6N/Hsd C28- still in GEM & ESC...

Nlrp2: NLR family, pyrin domain containing 22:

- Missense mutation Nlrp2/C57BL/6J (intrae immunity)
- Defective neutrophil recruitment, dendritic and myeloid cell migration
- Identified in C57BL/6J compared to C57BL/6N genome

B6 SUBstrains variations:

Dock2 - Envigo B6 Colonies 6/9/16

- US & EU colonies
- Occurred ~2008-9
- deletion in highly repetitive region of long arm (Yq)
- Impact: Mild Female bias sex ratio
  - Sperm abnormalities (bent heads)
  - C57BL/6N/Tac have a very slight male bias...
  - https://www.taconic.com/b6bom-fact-sheet/

B6 SUBstrain variations:

- Retinal degeneration B - single BP (frameshift) mutation in Crb2 gene
- Found in all C57BL/6N substrains (including C57BL/6BY)
- Similar to macular degeneration mutation in humans

Cryf2a1:

- G to A mutation occurred between 1961 and 1974 in C57BL/6N in cytoplasmic FMRI interacting protein 2
- Found in C57BL/6N substrains (NOT C57BL/6BY)
- Homozygotes for a dominant spontaneous mutation have impaired behavioral response to amphetamines, cocaine, fewer dendritic spines ....
- (targeted Homozygous knock-out → neonatal lethality → per MGI)

B6 SUBstrain variations:

- Y chromosome deletion C57BL/6JaomTac
- US & EU colonies
- Occurred ~2008-9
- deletion in highly repetitive region of long arm (Yq)
- Impact: Mild Female bias sex ratio
  - Sperm abnormalities (bent heads)
  - C57BL/6N/Tac have a very slight male bias...
  - https://www.taconic.com/b6bom-fact-sheet/

B6 SUBstrain variations:

Nlf: nicotinamide nucleotide transhydrogenase; encodes integral protein of inner mitochondrial membrane

Nnt/C57BL/6J: >1700 BP deletion! In C57BL/6J:
- Not C57BL/6JomTac (per tacnicom) arose after 1973...
- metabolic phenotypes – Altered insulin secretion glucose homeostasis...
- Fisher Wellman et al 2016

B6 SUBstrain variations:

Snca: alpha synuclein; protein in Lewy body inclusions, implicated in Parkinson’s Dz

Dcll1/Snca1Slab in C57BL/6Jo/het

- Deletion includes Mmmn + multiemin 1, a stored platelet and endothelial cell adhesive protein.
- Specht & Schoepfer 2004 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572725

Dock2 – Envigo B6 Colonies 6/9/16

- US & EU colonies
- Occurred ~2008-9
- deletion in highly repetitive region of long arm (Yq)
- Impact: Mild Female bias sex ratio
  - Sperm abnormalities (bent heads)
  - C57BL/6N/Tac have a very slight male bias...
  - https://www.taconic.com/b6bom-fact-sheet/
**Which C57BL/6 do you use?**

- C57BL/6J in original mouse genome project
- C57BL/6N (multiple substrains) in KKM IMPC
- C57BL/6J Aanat mutation (Roseboom & al 1998, etc)
- C57BL/6J Nnt mutation (Freeman & al 2006; Toye & al 2009)
- C57BL/6N rd8 mutation (MattaPallil & al 2012)
- C57BL/6N Nhsd Dock2 duplication mutation (Mahajan & al 2016)
- C57BL/6J Nr2f1 mutation (Ulland et al 2016)

**Thy1 (SFV/source) (Harley & al 2013; Denning & al 2011)**

- Alcohol preference; fetal alcohol syndrome (Anthony & al 2010)
- Simon & al. 2013. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains.

**Which C57BL/6?**

**IMPORTANT in YOUR RESEARCH?**

- Simon & al. 2013. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains.

**BALB/c History & SUBstrains**

(a few):

- Family tree (Pedigree) of Bagg’s albino mice
- Which do you use?
- Why?

**Female Bias in Infectious Dz Research?**

- ...Scott did not select or purposely breed BALB/c mice for aggressive behavior. ...In 1942 he notes that weaning BALB/c males could be housed 5 to a cage ... Further BALB/c albino became lethargic in warm weather ...
- ...at a loss to explain why BALB/CJ males are viciously aggressive when compared with their doable BALB/CJ.C57BL/6 ...  

From Potter 1985. History of the BALB/c Family

https://link.springer.com/chapter/10.1007978-3-642-707407_3

**Nomenclature: mission critical in scientific communication...**

- Correct strain names identify strain(s) of origin, and where it has been, and came from
- Correct mutant names tell a lot about the mice & mutations
- Incorrect names compromise communication, reproducibility, translational research

**INcompleteness of background strain reporting in recent publications**

<table>
<thead>
<tr>
<th>Background strain reporting in recent publications</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completely strain notation</td>
<td>54</td>
<td>56</td>
<td>49</td>
<td>52</td>
<td>43</td>
</tr>
<tr>
<td>Incompletely addressed</td>
<td>77</td>
<td>64</td>
<td>55</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>Incomplete due to C57BL/6</td>
<td>43</td>
<td>43</td>
<td>40</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Total number of articles</td>
<td>126</td>
<td>123</td>
<td>105</td>
<td>80</td>
<td>124</td>
</tr>
<tr>
<td>% incomplete total</td>
<td>61</td>
<td>52</td>
<td>52</td>
<td>62</td>
<td>64.5</td>
</tr>
<tr>
<td>% of incompletes due to C57BL/6</td>
<td>56</td>
<td>67</td>
<td>73</td>
<td>60.5</td>
<td>60</td>
</tr>
<tr>
<td>Average: 58.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average: 63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Background strain effects**

- A mouse is a mouse is a mouse? ✗
- B6 Represent ALL laboratory mice? ✗

**STRAIN MATTERS**

**SUBSTRAIN MATTERS**

**Nomenclature is mission critical to scientific communication**

- Those who don’t consider history (and nomenclature and pathology) may be condemned to? .... Retractions?
- Pathology is useful....

**cbrayton@jhmi.edu 2019**

Educational Use Only

Page 4 of 11
Xfactors EXTRA animal

AND:
- Microbial/microbiome
- Rx: interventions

4. VETERINARY CARE 105

... Preventive Medicine, 109
Animal Biosecurity, 110
Hematology and Stabilization, 110
Separation by Health Status and Species, 111
Surveillance, Diagnosis, Treatment, and Control of Disease, 112
Clinical Care and Management, 113

Temperature

- MOUSE Thermoneutral zone = 29.6-30.5°C
  - Large surface area → lose heat and moisture rapidly
  - Varies with strain and condition (nude, hairless)
  - Cold stress at housing temperatures?
    → Use more energy
    → Studies affected?
  - GUIDE RECOMMENDATION =20-26°C
  - Thermoregulation: mice can compensate from 20-35°C
  - Behavioral modifications in the wild
  - Non-shivering thermogenesis via brown Fat
  - Vasodilation/vasoconstriction to ears
  - Do not sweat
  - Neonatal mice are ectotherms until ~20 days

Diet Jargon

- CHOW is NOT sufficient description in publication ...
- Report the manufacturer and name/# at least
  - ‘Natural’ ingredient cereal or grain based
  - Open formula
  - Concentrations of all ingredients are published available
  - e.g. NIH 31 = fixed and open formula (soy alfalfa/bone, fish meal etc.)
- Closed formula → ingredients listed, % varies
  - E.g. ‘Constant Nutrition’ → Fixed nutrient composition BUT variable ingredients
  - ‘Certified’: representative sample tested for contaminants
  - Compressed/pelleted (= harder) than Extruded
  - Standard, irradiated, or autoclavable
  - Autoclavable diets are higher in heat labile nutrients
  - Purified/semipurified diet = fixed chemical composition

Barnd et al JAALAS 2009

- Mice Eat ~3-5 g of food/day (when they have WATER)
- Mice Drink ~6-7 mL water/day

Xfactors EXTRA animal

Does this list look familiar?
1. Microenvironment / Macroenvironment
2. Temperature / Humidity
3. Ventilation / Air Quality
4. Illumination / Light cycle
5. Noise / Vibration
6. Microenvironment
   - (Primary Enclosure) Caging, Environment etc
7. Behavioral / Social Management
8. Husbandry
   - Diet bedding water handling, etc.
9. Microbial/microbiome
10. Rx: interventions

Diet – lots of options

Natural ingredient
Cereal/grain based
- ingredients, vary w season cost etc.
- Environmental Contaminants
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMCC042877/

Purified
- AIN-93G = Growth
- AIN-93M = Maintenance
- Corn-Starch, Casein, Maltodextrin, Sucrose, Soybean Oil, Cellulose, Mineral Mix, Vitamin Mix, L-Cystine, Choline Bitartrate, BHT
- Reeves et al 1993. AIN-93 purified diets for laboratory rodents: final report

<table>
<thead>
<tr>
<th>INGREDIENT COMPONENT</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td></td>
</tr>
<tr>
<td>Alphaffa meal</td>
<td></td>
</tr>
<tr>
<td>Chinchilla</td>
<td></td>
</tr>
<tr>
<td>Fish meal, Meat/Bone meal</td>
<td>Nitrosoamines, Potential carcinogen</td>
</tr>
</tbody>
</table>

390.0 % 391.0 %

Protein 18.1 18.6
Fat 7.1 4.3
Fiber 4.8 4.8
Ash 2.2 2.2
Moisture <10 <10
Carbohydrate 50.3 67.5
Energy 354 316

DIET

OPTIONS
CONSIDERATIONS

Fat/Prot/Carb/Fiber
% & Sources
Alphaffa, soy etc
Phytosources
Animals products
Nitrosamines

Special Diets
Special handling, Loss of nutrients, Palatability, Prefer bedding

Restricted vs ad lib

BEDDING

CHARACTERISTICS
CONSIDERATIONS

$5$ Any study
Dust/allergens
Asthma/resp studies
Palatability
Restricted or special diets
Absorptance
Bioburden ...
Humidity
Contaminants
Many studies?
Endocrine disrupters
Many studies?
My peeves and biases

Animal Factors
- Species/ subspecies
- Strain /substrain
- Age
- Sex
- Social life / parity

Xfactors EXTRA animal
- Rx: interventions
- Unintended Consequences of antimicrobials and common experimental interventions
- Microbial/microbiome;

Antimicrobials – other effects
- Clavamox
- Dysfunction in maturation and transitional ameloblasts, resulting in hypomineralized enamel
- Enrofloxacin
- Cartilage effects in growing animals
- Trimethoprim Sulfamethoxazole
- Thyroid effect
- Fenbendazole
- Immunomodulatory
- Anticancer?

Antimicrobials....
- Effects on microbiome?
- Other immunomodulation

Unintended Consequences: RX

<table>
<thead>
<tr>
<th>OPTIONS</th>
<th>CONSIDERATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto Water Bottles</td>
<td>Dehydration, Can they reach it? Drowning $$$</td>
</tr>
<tr>
<td>Municipal RO Acid/Cl</td>
<td>Contaminants Chemical, Microbial Palatability</td>
</tr>
<tr>
<td>Medication</td>
<td>Activity, suspensions</td>
</tr>
</tbody>
</table>

Unintended Consequences: RX

- Which is normal?
- Diagnoses?
- Causes?
- Human relevance?

- Enamel Hypomineralization amoxicillin/clavulanic acid Rx
- Amoxicillin Rx in early childhood may be associated with enamel hypomineralization

Antimicrobials – other effects
- Clavamox
- Dysfunction in maturation and transitional ameloblasts, resulting in hypomineralized enamel
- Enrofloxacin
- Cartilage effects in growing animals
- Trimethoprim Sulfamethoxazole
- Thyroid effect
- Fenbendazole
- Immunomodulatory
- Anticancer?

Unintended Consequences: RX

- NODscid
- Mortality post irradiation
- Not just an incidental finding

- Alpha irradiation

Unintended Consequences: RX

- NON estrogenized
- Bearing Breast Ca Cell line
- ESTROGENIZED
- Bearing Breast Ca Cell line

Unintended Consequences: RX

- Inoculated with Breast cancer cell line
- Found dead
- Some Pale with Watery blood
- Decalcified Tibia

(C57BL/6 NOS)
Mihalaş et al.
http://journals.sagepub.com/doi/abs/10.1177/0192623315610822

Unintended Consequences: RX

- Larsen & al. 2006.
- cbrayton@jhmi.edu 2019

Educational Use Only

Page 6 of 11
Rx interventions (therapeutic/experimental)

- YES THEY MAY AFFECT HEALTH AND DATA
  - And have unintended effects
- PATHOLOGY can identify unexpected effects

Microbial Implications for Animal Health and Welfare:

- In immune sufficient mice, rats
  - Sick animals are unusual today,
    - 'Detection' is more common than Disease.
- In immune DEFicient mice, rats,
  - Sick or dead animals are not so unusual;
    - Beware of pet store & wild rodents.
    - Beware of biological materials.

Microbial Implications for Human Health:

- Be aware (BEWARE) of immune deficient or humanized animals as reservoirs of potential pathogens for humans or non human animals.
- Beware of pet store & wild rodents.

Micro Jargon

- SPF = Specific Pathogen Free
- Defined by the Exclusion list...
- (= tested/excluded agents)
- Gnotobiotic = defined flora
- ASF = altered Schaedler's flora
- Axenic = germ free
- Autochthonous flora (indigenous flora)
- Microbiota microflora
- Microbiome = genetic material of all microbes...
- Virus, bacteria, protists, fungi... i.e. not just fecal bacteria
- Allochthonous flora (transient flora).

Micro Jargon

- Commensal
  - living on or within another organism, deriving benefit without harm or benefit to the host
- Symbiont
  - Organism in symbiotic relationship with host
- Opportunist
  - May infect (allochthonous) or normally live in harmony with the host (autochthonous), except when the system is disturbed
- Subclinical
  - Infects, no clinical signs of disease
- Pathogen
  - Often or usually causes disease

What is/are 'normal' flora?

- What is in your GEM?
- What is in your control mice?
- Does it matter?
- Commensal or Opportunist?
  - No problem in competent or control...
  - Problem in immuno weird or other mutant
  - Some may enhance immunity & survival (SFB)

What is/are 'defined' flora?

- Schaedler's flora
  - various recipes
    - 8 bacterial spp e.g.
      - lactobacilli
      - clostridia
      - Bacteroides
      - Eubacterium
      - ASF#’s
- No fungi
- No protozoa
- No SFB

THUS you should NOT see these in gnotobiotic, or in clean immunodeficient rodents from isolators...

You should not see protozoa in clean rederived rodents...

Wymore et al 2015 ILARJ
Common ‘friends’? ‘frenemies’?

?? significance

Stomach - yeasts

Small Intestine - Segmented Filamentous Bacteria

Large intestine – Flagellates + Entamoebae

You should NOT see these in gnotobiotic, or in clean immunodecient mice from isolators...

You should not see protozoa/protists in clean rederived mice.

Most Vendors test for & exclude the protozoa/protists

Esophagus

Yeasts

Lactobacillus sp

Streptococcus / enterococcus – like

Chains of cocci

NOT in ASF

Gastric Yeasts


Torulopsis pintolopesii - ON surface

SFB & Th17 & B6 sources

Ivanov & al. 2009. (Tac vs J B6)

Figure 2. SFB in Th17 Cell-Sufficient and Th17 Cell-Deficient Mice

(A)qPCR for SFB Bacterial (EUB) 16S rRNA in Tac & B6 feces,

(B) SEM & TEM ileum 8wo Jax & Tac B6 after cohousing

(C)qPCR 14d postcohousing Jax & Tac B6 (Jax-Coh)

(D) SFB colonization of Jax B6 14 d postcohousing


SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

Cecum, colon

B6, 129, FVB

Diagnosis?

Significance?

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.

SFB & Th17 & B6 sources

Denning & al. 2011. (C57BL/6 vs J B6)

Ability of DC and macrophage subsets to induce Foxp3(+)/T(reg) cells versus Th17 cells was strikingly dependent on Mouse source:

- DCs from C57BL/6 mice from CRL (with SFB) that robustly induce Th17 cells in situ were more efficient at inducing Th17 cells and less efficient at inducing Foxp3(+)/T(reg) cells than DCs from B6 from TGL (- SFB).

- Only CD11c(+)/CD11b(+)/CD209(+) DCs efficiently induced Th17 cells

- Distribution correlates with Th17 cells: duodenum > jejunum > ileum > colon.

- Conversely, CD11c(+)/CD11b(-)/CD209(-) DCs, macrophages, and Foxp3(+)/T(reg) cells were most abundant in colon & scarce in duodenum.
Trichomonas musculi
Immunomodulation

- IL-17, TH1 effects
- Anti-microbial
- Pro Cancer?
- Related protist is prevalent in healthy humans


Helicobacters – ‘Normal’ flora?
Research Relevance?

- Experimental model phenotypes
  - Model helicobacter infection
  - IBD inflammatory bowel disease
  - Helicobacter influence on enterohepatic tumors
  - Strain and sex influences on disease
- Research interference?
  - Immunomodulation
  - Enterohepatic inflammation
  - Enterohepatic and other tumors
  - Zoonotic concern? H bilis, ganmani, hepaticus?

Human/rodent helicobacters...

- Helicobacter pylori,
- Helicobacter bilis,
- Helicobacter hepaticus,
- Helicobacter ganmani.
- Trend of a higher presence of Helicobacter spp. in patients with biliary tract cancers compared with normal controls or those with benign biliary diseases...

Zhou et al 2011, Shen & al 2014, etc

Is CLEANEST BEST?
for the mice? for the project?

- ‘Barrier’ defined by the agents excluded, and the exclusion practices
- ‘SPF’ defined by the agents excluded, and the exclusion practices
- ‘Clean’ moving target, as new issues/pathogens emerge or are rediscovered

Environmental/Microbial ‘challenge’ may expose an important phenotype.
Rederivation may lose it....

DO YOU know YOUR Microbial Status ??

Is CLEANEST BEST?
for immunodeficient mice?

- ‘Barrier’ defined by the agents excluded, and the exclusion practices
- ‘SPF’ defined by the agents excluded, and the exclusion practices
- ‘Clean’ moving target, as new issues/pathogens emerge or are rediscovered

Probably, usually, YES.

Microbial/Microbiome

- How heterogeneous do you really want ...
- Let’s get Dirty!
- Fun for pathologists 😊
- Most of our mice are not quite as clean as in Abolins & Beura papers, fresh from vendors..
- You want pet store and wild flora? REALLY?

cbrayton@jhmi.edu 2019

Early Aging?

- Early aging phenotype
- Failure to thrive
- Weight loss
- Hunched
- Frail
- MUD

Tm (GEM)

19 Brayton 3 Reproducibility

SPF

N O T a
Super Power.
Must be defined in reporting...

Mighty Mouse

Early Aging?

Educational Use Only
Table 1. Incidents/outbreaks identified by the review, by zoonotic agent

<table>
<thead>
<tr>
<th>Zoonotic agent</th>
<th>Virus type</th>
<th>Sample type</th>
<th>Family or genus</th>
<th>Species</th>
<th>Symptoms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis – rat urine contaminated water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica typhimurium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBF = rat bite fever due to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirillum minus – spirillary RBF, sodoku</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptobacillus moniliformis – Streptobacillary RBF, Haverhill fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia pestis ? – black plague Via rat fleas ?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H bilis? hepaticis? gammapi?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycoplasma CABacillus, Helicobacters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williams et al. 2018 (NYC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5904411/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5904411/</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etc...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fatal Rat-Bite Fever in a Child
San Diego County, California, 2013

- CDC.gov
  - Healthy 10yo boy → rigors, fevers, vomiting, headaches, leg pains, death
  - PCR Pos Streptobacillus moniliformis (Lung, Liver, epiglottis)
  - owned 2 pet rats; 1 PCR Pos oropharyngeal swab
  - Streptobacillus moniliformis

- Nearly all domestic and wild rats carry S. moniliformis. Elliot 2007
- 17% of NYC R norvegicus... Firth& al 2014

- BEWARE OF PET rodents
  - https://www.cdc.gov/mmwr/volumes/67/wr/mm6704a5.htm

Hantavirus, Bunyaviridae

- Seoul virus
  - Rats: R norvegicus – subclinical seroconversion
  - Human: Hemorrhagic fever with renal syndrome (HFRS)
  - 150,000 human cases/an; most in China
  - 2017 - Seoul virus infection identified in rat breeders and owners in US.
  - 31 facilities in 11 states with human and/or rat Seoul virus infections; 6 facilities exchanging rats with Canadian ratteries.

- BEWARE OF PET rodents
  - https://www.cdc.gov/mmwr/volumes/67/wr/mm6704a5.htm

Cowpox

- Lesions on rats and humans in Germany, 2009.

A. Pet rat with lesions on right hind limb;
B. Neck lesions of a girl without previous vaccinia virus (VV) vaccination.
C. Neck lesion of the girl's grandmother with a history of VV vaccination.
Campe & al 2009

Monkeypox

- PET Giant Gambian Pouched rat
  - Cricetomys gambianus

- Source of 2003 US monkeypox outbreak
  - Subclinical infection in rats
  - Clinical in humans

Wild rodents & viruses

- NYC >30 viruses, multiple new viruses
- Pathogens
- Chapparrovirus, MHV, MNV etc
- Williams et al. 2018 (NYC)
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5044411/
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5044411/
  - Firth et al 2014
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4205793/

Wild (& pet) Rodents near us...

Zoonotic: Gi tract Histo Fecal
Capillaria hepatica – 9.5% – 4
Rodentolopex nana (small intestine) 14.3% 7.1% 20
NON Zoonotic: Rodent Specific
Heterakis spumosa (large intestine) 76.2% 2.4% 11.9% 1*
Trichosomoides crassicauda
*bladder) – 31% –
Mastophorus musis (stomach) 2.4% 11.9% 1*

*Eggs of ingested T. crassicauda in stomach wall in 2 samples
McGarry et al. 2015, UK urban rats

From http://online.library.wiley.com/doi/10.1113/pth.223166/full
31106 Rat
- Calodium hepaticum
  - (Capillaria hepatica)

31106 Rat
- Calodium hepaticum
  - (Capillaria hepatica)
- Capillarid eggs
- Bipolar plugs

37572 Pet rat: R nana
- Zoonotic...

Who is it?
- A gift of globalization...
- Transmission to humans is by eating intermediate hosts, a tiny translucent slug ...
- Imperceptible on lettuce ...
- Slime from an infected slug is a transmission risk.
- Eating raw or undercooked freshwater prawns, crabs, and frogs is also a risk factor....
- But OK to eat rats...

PET STORE & WILD RODENTS
Better for research??

Headlines:
- We CAN do better.