THE LABORATORY MOUSE

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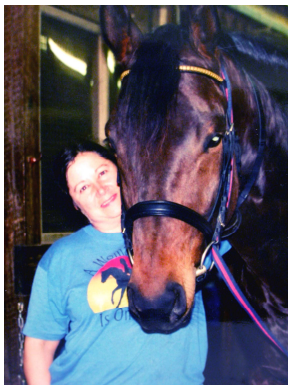
- **All information is deemed reliable and correct**
  - No warranty for accuracy
- **No information presented is known to be specifically included in the ACLAM Board Certification Exam**

"Horse people are crazy……"



"Horse people are crazy……"

There is something about the outside of a horse that is good for the inside of a man. ~Winston Churchill

Vets Dealing with "Mousers"

- Talk the Talk …..
- Walk the Walk ….

Vets Dealing with "Mousers"

- Talk the Talk …..
  - – Mouse genetics vocabulary
  - – Scientific vocabulary
- Walk the Walk ….
  - – Husbandry/colony management
  - – Strain specific idiosyncrasies

Dealing with “Mousers”

- Talk the Talk …..
  - Scientific vocabulary
    - Genotyping methods
      - PCR, Real Time-PCR
      - Single nucleotide polymorphism (SNP) mapping
    - Imaging technologies
      - IVIS®, DEXA, MRI, microCT
    - “Humanized” mice

Vets Dealing with “Mousers”

- Talk the Talk …..
  - Mouse genetics vocabulary
    - Strain types, names/designations
    - Genetic alteration methods and technologies
    - Breeding strategies, problems, issues

Talk the Talk…..

- http://www.informatics.jax.org/mgihome/nomen/gene.shtml#genenom
- Guidelines for Nomenclature of Mouse and Rat Strains
- Quick Guide to Nomenclature for Genes
- Quick Guide to Nomenclature for Alleles and Mutations

Nomenclature

- Outbred Strain
- Inbred Strain
- Substrain
- F1 Hybrid
- Recombinant Inbred
- Congenic Strain & Coisogenic Strain

Outbred Strain

- Genetically undefined; no two mice alike
- Bred to maintain maximum heterozygosity
- Long lived, resistant to disease, highly fertile
- Name = holding institution:strain name
  - Tac:ICR = ICR strain held by Taconic Farms

Inbred Strain

- Mice within strain are genetically identical
- Requires minimum of 20 generations of brother x sister mating
- Name designated by capital letters and/or numbers: CBA, 129, C3H
- Generations of inbreeding: CBA (F87)

Related Inbred Strain

- Related inbred strains have a common origin but were genetically separated before F20
- Their names reflect this relationship: NZB, NZO

Inbred Strains: Abbreviations

• C57BL/6	B6
• BALB/c	C
• C3H	C3
• DBA/1	D1
• C57L/J	L
• SWR	SW

Substrain

- Branches of an inbred strain with known or probable genetic differences
- Separated by 20 generations from a common ancestor
- Name designated by parent stock followed by "/" and substrain symbol
- Example: DBA/1 & DBA/2

F1 Hybrid

- Offspring of crossing two different inbred strains
- Female parent listed first, then male parent; abbreviations are often used
- B6D2/F1 = offspring of C57BL/6 female and DBA/2 male

Recombinant Inbred Strains

- Cross mice from two progenitor inbred strains, followed by 20 generations of brother x sister matings
- Named by combining strain abbreviation symbols (female parent first) linked by capital "X"
- CXB = RI strain from BALB/c x C57BL/6

Alleles and Mutations

- Genes and alleles are italicized
- Spontaneous & Induced Alleles
  - Superscripted letters and numbers
  - Lowercase start = recessive (*Wnt3a*^{vt})
  - Capital start = dominant (*Atp7a*^{Mo})

Coisogenic Strain

- Formed by mutation within an inbred strain
- Mice are genetically identical EXCEPT for the mutated gene
- Name is a compound symbol of: parent strain - *gene symbol*
- Example: MRL/MpJ - *Fas^{lpr}/J*

Congenic Strain

- Generated by repeated backcrosses to an inbred strain with selection of a particular marker from the donor strain
- Requires a minimum of 10 backcross generations
- Number of backcrosses: N(15)

Congenic Strain

- Name is a compound symbol = host strain.donor strain - *gene symbol*
- C57BL/6.AKR – *H2^{k}*
- Congenic mice are genetically similar to the host strain

CONGENIC ≠ COISOGENIC!

- Congenic strains differ from the host strain by a short chromosomal segment, **NOT A SINGLE GENE**
- N5 congenic is >95% of host genetic background
- N10 is > 99% of host background

"Speed Congenics"

- Marker assisted selection breeding produces the equivalent of 10 backcross generations in as few as 5 generations
- Donor strain contribution unlinked to the selected locus is < 0.01
- Need careful selection and genomic spacing of markers

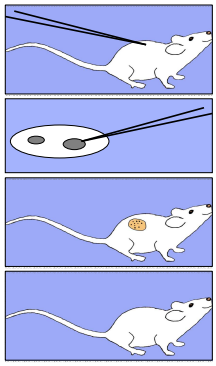
Creating Transgenic Mice

- Four types of mice are needed:
  - Donor females for zygote production
  - Fertile stud males
  - Sterile (vasectomized) males to create pseudopregnancies
  - Pseudopregmant females to serve as zygote recipients

Construct for Transgenic Mice

- Gene of interest
- Promotor
  - endogenous
  - ubiquitous
  - tissue specific
  - inducible
  - developmentally regulated

Transgenic Mice



Superovulate donor females and harvest embryos

Microinject pronucleus of mouse embryos with transgene construct

Culture and implant transgenic embryos in pseudopregnant females

Birth of transgenic mice

Inbred Mice for Zygote Donors

- Pro:
  - Eggs of FVB strain have large pronuclei that facilitate microinjection
  - FVB strain has good reproductive performance
  - Transgene is placed in a defined genetic background

Inbred Mice for Zygote Donors

- Con
  - Most inbred mice reproduce poorly
    - females may respond poorly to superovulation
    - males may be inefficient breeders
  - Inbred mice are more expensive to buy/breed
  - Inbred mice may have strain-specific differences that interact deleteriously with the transgene

Hybrid Mice as Zygote Donors

- Pro:
  - Good reproductive performance
  - High egg survival rate
  - Eggs efficiently retain microinjected DNA

Hybrid Mice as Zygote Donors

- Con:
  - Mixed genetic background
  - Creating a congenic strain will require minimum of 10 generations of backcrossing (3-4 years)

Transgenic strains

- Tg stock names spell out the features of the Tg manipulation
- On inbred background, designation is parent strain - Tg symbol
  - C57BL/6 - TgR(ROSA26)26Sor
- On mixed background
  - B6,129 - TgR(ROSA26)26Sor

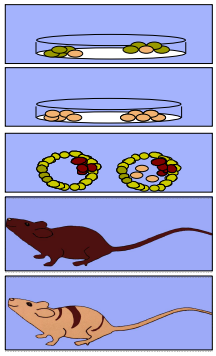
Transgenic Alleles

- Tg(YYYY)#Zzzz
  - YYYY = inserted gene
  - # = serial number
  - Zzzz = lab code
  - Example: Tg(Hoxa1)1Chm

Creating Knockout Mice

- Knockout construct with selectable marker
- Embryonic stem cell cultures (ES)
  - totipotent cells from inner cell mass of mouse blastocysts
  - 129 strain is source of most ES cell lines
- Blastocysts from B6 mice
- Pseudopregnant females

Gene Targeted (KO) Mice



Introduce homologous recombination in cultured ES cells

Select ES cells containing targeted gene construct

Inject transfected ES cells into normal blastocyts

Implant chimeric blastocysts into surrogate female

Birth of chimeric mice

Breeding Scheme for KO Mice

Chimeric (+/+; KO/KO) X **129 Coisogenic (+/+)**

↓

N1(+/KO?) X **N1(+/KO?)**

↓

N1F1(+/+; +/KO; KO/KO)

Breeding Scheme for KO Mice

C57BL/6 (+/+) X **Chimeric (+/+; KO/KO)**

↓

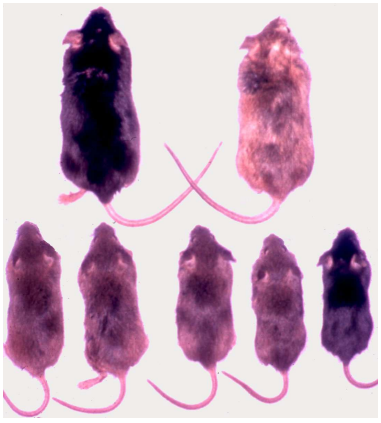
Agouti (+/KO) X **Agouti (+/KO)**

↓

N1F1(+/+; +/KO; KO/KO)

↓

B6,129 segregating —N10→ **C57BL/6 Congenic**



Targeted (KO) Alleles

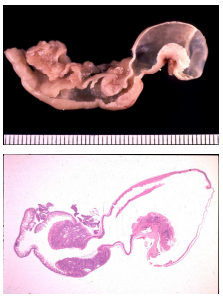
- tm#Zzzz
  - tm = targeted mutation
  - # = serial number
  - Zzzz = lab code
  - Example: *Egfr*^{tm1Mag}

Limitations of Mouse Models

- Mice ain't people….
  - Same mutation → different phenotype
    - HPRT – Lesch-Nyhan syndrome in humans, no disease in KO mice
  - Same mutation → "similar" phenotype
    - Apc (^{Min}) – colon cancer in people, SI polyps in mice
  - Accentuation of mouse lesions
    - p53 – carcinomas in people, sarcomas in mice

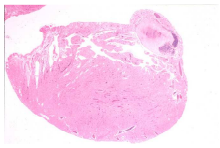
Accentuation of Mouse Lesions

- Gastric/duodenal polyps

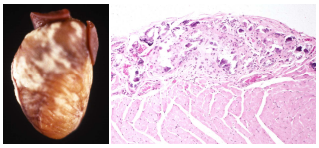


Accentuation of Mouse Lesions

- Atrial thrombus



- Epicardial mineralization



Current Issues with GE Mice

- Lack of resources for "phenotyping"
  - Trained veterinary personnel
    - Pathologists, clinicians
  - Specialized equipment
    - Faxitron, Micro-CT, rodent behavior, DEXA
  - Core labs
    - Histology, clinical pathology

Current Issues with GE Mice

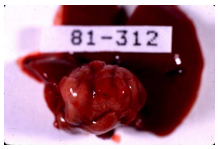
- Separating phenotype from:
  - Strain-specific lesions
  - Infectious disease
- Opportunistic infections in “clean” mice
- Humane considerations in lethal models
  - Endpoints
  - Standards of care

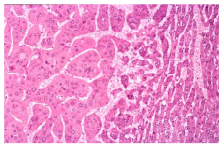
Case Study 1

- Unexpected liver tumors seen at necropsy in mice transgenic for a clotting factor
- Lesions noted only in males > 1 yr. of age
- Some non-Tg control males also have the tumors

Strain-specific Variations

- Liver tumors seen at necropsy in mice transgenic for a clotting factor
- Origin of the strain is B6xC3/F1 hybrid; C3H males are predisposed to liver tumors



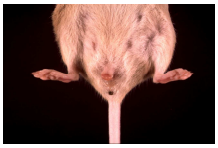


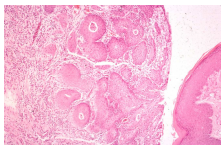
Case Study 2

- “Sores” noted on genitalia of female KO mice
- PI complains that females with “sores” are not breeding

Strain-specific Variations

- “Sores” noted on genitalia of female KO mice
- Mice are coisogenic on the 129 strain; females are prediposed to vulvar squamous cell carcinomas

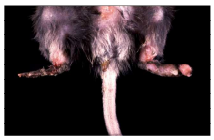




Infectious Disease Issues

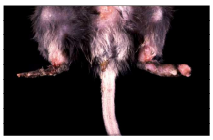
- Atypical presentations of classic diseases
- Cross-breeding immune deficient mice
- Colony/experiment specific problems
  - Improper husbandry
  - Improper experimental technique

Case Study 3



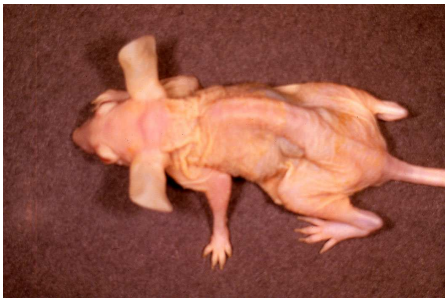
- Ordinary B6 inbred mice arrive from a vendor
- Within 1 week, all of the shipment develops lesions on their paws
- Do You Panic?

Atypical Disease Presentation



- Beta-hemolytic *Staph. aureus* cultured from paws
- No serologic evidence of Ectromelia infection
- Sigh of relief!

Phenotype vrs Infectious Disease



Wasting from……

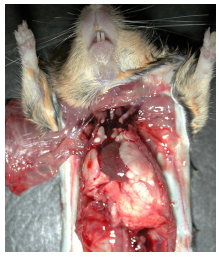
- *Pneumocystis murina* pneumonia
- *Helicobacter*- induced colitis
- Cystitis/pyelonephritis
- Tumor-induced cachexia
- MHV infection

Case Study 4

- GE breeder mice are purchased from commercial vendor
- Mice presented for necropsy because of poor reproduction and numerous early deaths

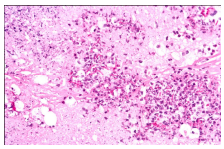
Case Study 4

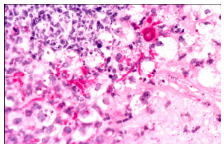
- Mice are emaciated, dyspneic, lethargic
- Lungs do not collapse when thorax is opened, appear white, dry and leathery



Case Study 4

- Histopathology reveals pyogranulomatous pneumonia with regional infarction.
- Fungal hyphae found on histopathology, *Aspergillus terreus* cultured from lungs





B6.129S-*Cybb*^{tm1Din}/J Mice

- "PHOX -/-" mice are a model for chronic granulomatous disease in humans
- Phagocytic cells are incapable of creating a respiratory burst, fail to kill bacteria
- Vendor recommended sterile housing; PI requested conventional housing on the order

Improper Husbandry

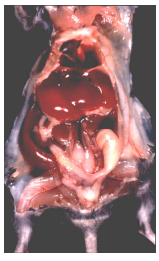
- Strain name ""B6.129S6-*Cybb*^{tm1Din}/J" was unfamiliar to animal husbandry; no recognition as immune-deficient
- *A. terreus* grows beautifully on damp, non-autoclaved corn cob bedding!

Case Study 5

- Complaint of poor reproductive performance in breeder mice
  - Infertile pairs
  - High incidence of prepucial gland abscesses

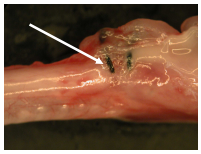
Poor Experimental Technique

- Complaint of poor reproductive performance
  - Pyometra due to *Pasteurella pneumotrophica*
  - Mice are mildly immune deficient
  - Estrus monitor used for multiple mice without cleaning
  - Breeders more than 1 year old



Poor Experimental Technique

- Induction models - Tamoxifen/ER
  - Gavage errors
  - Sclerosing aseptic peritonitis



Know Your Mouse Genetics?

GE mice dying unexpectedly soon

Hi Dr. Godfrey,
Here is the genotyping info on those mice you dissected:

#192665 (08-030): K18eGT121+/-;Xpten+/m;PbCre4+/- DOB:10/2/07

#193345 (08-031): K18eGT121+/-;Xpten+/m;PbCre4+/- DOB: 10/9/07

#191933 (08-032): APT121+/-; p53f/f; FSPCre+/- DOB: 9/19/07

#197207 (08-033): p53f/f; FSPCre+/- DOB: 11/28/07

#195974 (08-034): APT121+/-; p53f/+; FSPCre+/- DOB: 11/13/07

#193121 (08-035): K5eGT121+/-;PbCre4+/- DOB: 10/3/07

Hi Dr. Godfrey, Sorry for not explaining it well……

K18eGT121 = BAC transgenic. K18 promoter (whole K18 gene in a BAC) drives floxed eGFP stop T121. Without Cre, eGFP is expressed. With Cre, T121 will be activated.

K5eGT121 = BAC transgenic. K5 promoter (whole K5 gene in a BAC) drives floxed eGFP stop T121. Without Cre, eGFP is expressed. With Cre, T121 will be activated.

Xpten+/m = floxed Pten allele (or mutant allele, conditional KO mice). Only one allele is floxed, the other allele is wildtype.

PbCre4 = Probasin promoter drives Cre. This line intended to be used for prostate specific deletion. However, it is not prostate specific. Thymus has very low expression of Cre.

FSP Cre = FSP (fibroblast specific protein) promoter is driving Cre. This intends to be FSP specific. However, I have evidence that epithelial cells also express Cre in this line.

p53f/f = floxed p53 (conditional p53 KO mice). Both alleles are floxed.

APT121 = probasin promoter drive T121 directly (transgenic).

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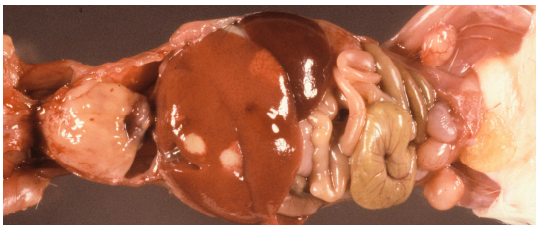
Thymus has low expression….



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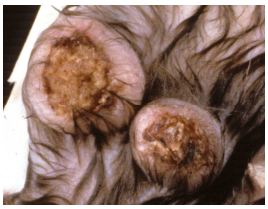
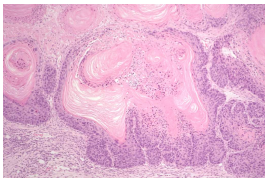
Epithelium expresses cre…



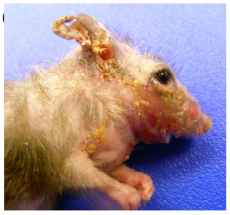


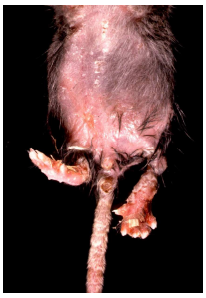
Humane Issues with GEM

- Lethal mutations
  - What is the “pain and distress” category?
  - Where do you set the endpoints?
    - When should the mouse be referred for vet care
    - When should euthanasia be mandated
  - What level of supportive care is required?

Humane Endpoints

- Autoimmune mice with chronic





Humane Endpoints

- Leukemia/lymphoma models