Fragile Superheroes:
Caring for Humanized Mice

Peggy J. Danneman
Topics

- Immune competent vs. immune deficient
- Infectious disease problems in severely immune deficient mice
- Preventing and treating infectious diseases
- Health monitoring considerations
- Other health and breeding problems
- Issues related to humanization
A Spectrum of Immune Deficiency

Immune competent

Severely immune deficient
(Mostly) Immune Competent

- DBA/2 (defect in tumor necrosis factor alpha production) susceptible to lung infection with *Pseudomonas*

- C3H/HeJ (homozygous for the defective LPS response allele $Tlr4^{Lps-d}$)
  - Gram neg infections
  - Also C57BL/10ScN and C57BL/10ScNJ (deletion of the $Tlr4$ gene)
NOD

- Multiple immune abnormalities, incl.
  - defective NK cell function
  - defective cytokine production from macrophages
  - lack hemolytic complement C5
- No evidence of unusual susceptibility to infectious agents
‘More’ Immune Deficient

- These strains are more susceptible to a wider range of infectious agents, but can be maintained in a typical barrier without unusual precautions

- Beige ($\text{Lyst}^{bg}$)
  - Granulocytes show reduced bactericidal activity
  - NK cells exhibit decreased cytotoxic activity

- XID ($\text{Btk}^{\text{xid}}$)
  - X-linked defect in humoral immunity, characterized by:
    - Impaired maturation of B-cells
    - Diminished immunoglobulin production
Severely Immune Deficient
Nude \((Foxn1^{nu})\)

- Homozygotes lack a thymus → T-cell precursors cannot develop into mature lymphocytes
  - Occasional development of functional mature T cells, esp in adults

- Susceptible to a wide range of infectious agents, including pathogenic and opportunistic viruses and bacteria
  - Normal or increased resistance to some agents, incl. \(E.\ coli,\ Pseudomonas, C.\ piliforme\)
  - Outbred nudes are hardier than those on inbred backgrounds
Scid

- Protein kinase, DNA activated, catalytic polypeptide (prkdc) is essential for the generation of antigen receptors (Ig and TCR molecules)

- Homozygotes for a mutant allele of this gene ($Prkdc^{scid}$) are characterized by:
  - Absence of functional T cells and B cells
  - High levels of innate immunity, including normal antigen-presenting cell, myeloid, and NK cell functions

- Some mice develop partial immune reactivity ("leaky")
  - Highly strain dependent, increases with age, and is higher in mice housed under non-SPF conditions

- Cannot effectively fight most infections
Simultaneous activation of Rag1 and Rag2 genes is required to initiate V(D)J rearrangement / recombination to generate of antigen receptors (Ig and TCR molecules)

Homozygotes for null mutations of either Rag1 or Rag2 (e.g., Rag1tm1Mom or Rag2tm1Fwa) produce no mature T or B cells

- “Non-leaky” scid - B and T cell maturation is blocked earlier and more completely than with the scid mutation
- High levels of NK activity

Cannot effectively fight most infections
Many Combinations, e.g.,

- Scid beige
  - Defective NK cells vs. ↑ NK activity in scid
- Beige nude xid
  - NK (beige), T cell (nude), and B cell (xid) deficiencies
- NOD scid
  - Reduced “leakiness” vs. other inbred backgrounds
- NOD scid $B2m^{null}$
  - Further reduced innate immunity vs. NOD scid
- NOD $rag^{null}$
  - Greater radioresistance and a complete absence of serum Ig vs. NOD scid
NSG, NRG, and NOG

- Lack of functional T and B cells and NK cells from Prkdc<sup>scid</sup> or Rag mutation and Il2rg inactivation
- Reduced innate immunity from the NOD background
  - macrophage dysfunction
  - defect of C5 hemolytic activity
- No B or T cell leakiness with aging
- Extreme sensitivity to a wide range of pathogenic and opportunistic microorganisms
Infectious Disease Problems in NSG (and likely NRG & NOG)

- Few reports in the LAS/LAM literature
- Primary sources of information
  - Personal communications from users (1° NSG users)
  - JAX pathology records
Common Lesions

- Infected skin wounds, cellulitis
- Abscesses involving skin and internal organs
- Otitis media, conjunctivitis, panophthalmitis
- Localized and widespread infections involving liver, heart, lungs, uterus, accessory sex glands, etc.
- Most common – UTI
  - Cystitis, nephritis, pyelonephritis
    - Acute, chronic, occasionally resolved (fibrosis only)
  - Females diagnosed twice as often as males
Other Problems

- Non-specific clinical problems,
  - Unthriftiness, diarrhea, wasting, sickliness
  - Weakness, lethargy
  - Acute and/or premature death

- Breeding problems, including
  - Embryonic deaths
  - Small litters
  - Small, weak, and/or sickly pups
  - Pup mortality
Diagnosed Etiologies

**S. aureus**

Coagulase-negative

*Staphylococcus* spp.

*Pneumocystis murina*

*Pseudomonas*

*Klebsiella* spp.

**Enterococcus**

Most common etiology at JAX

- *Enterobacter*
- *Citrobacter*
- *Proteus*
- *C. bovis*
Circumstances

- Etiologies include pathogens, opportunists, commensals
- Problems have occurred in mice housed under “loose” barrier conditions, strict barrier conditions, even isolators
- Problems are most often seen after mice have been in the facility a while
- Problems often appear to be an individual animal vs. colony issue
  - Often sporadic
  - Sick and healthy animals in the same cage not uncommon
Contributing Factors

- Ascending infections involving kidney
- Vascular spread to many organs from
  - Skin wounds, e.g., needle punctures, bacterial dermatitis
  - Molar gingival sulcus
- Hormonal influences likely, esp UTI
  - Increase in bladder infections in mice treated with estrogen to facilitate mammary tumor growth
- Clinical problems appear to be more common in breeding females, often lactating females
Barrier Maintenance – How Clean is Clean Enough?

- Barrier practices adequate to maintain nude, or even scid, mice may not be adequate for NSG
- Recommend
  - Microisolator / IVC housing
  - Sterilize everything that comes into contact with the mice
  - Strict microisolator technique
- Under maximum barrier conditions at JAX, bacterial disease in NSG are uncommon – less than 1% of mice >200 days
Additional Suggestions

- Rederive to eliminate opportunists from colony
- Chlorination or acidification of water to control *Pseudomonas*
- More frequent bedding changes might be helpful – reduce the chance of a minimal inoculating dose of opportunistic or commensal bacteria
Treatments

- Baytril* has been effective in treating individual mice; also other antibiotics (e.g., amoxicillin, cephalexin) on occasion
  - No evidence of effectiveness for prophylaxis
- Trimethoprim-sulfa to control *Pneumocystis*
  - Incorporate in feed or water
    - 50 mg/kg/day trimethoprim + 250 mg/kg/day sulfamethoxazole
  - Significant decrease in mortality
    - Fungi may or may not be detectable by histopath, but continue to be detectable by PCR

* 5-20 mg/kg SC or PO BID for 7 days; in drinking water – 100-200 mg/liter for 14 days
Health Monitoring

- Expanded health surveillance to detect all organisms excluded from barrier, including opportunistic bacteria
  - Shedding often intermittent
  - ↑ Non-lethal monitoring, e.g., fecal or oropharyngeal swab culture, of colony mice desirable
- Immune competent mice may be transient carriers of opportunists that cause significant disease in immune deficient, e.g., *Corynebacterium, Pneumocystis*
  - Testing may fail to detect because of transient nature
  - Test immune deficient mice directly
- Direct tests (e.g., culture, PCR), not serology, for severely immune deficient
Thymic Lymphomas

- Scid – starting ~ 3 mo; higher incidence in males vs. females; mean lifespan ~ 8-9 mo
- NOD scid - starting ~ 5 mo; higher incidence in females vs. males
- Lower incidence in NOG
- NSG highly resistant, even after sublethal irradiation; mean lifespan 22 mo
Breeding

- NOD background - good breeders, but breeding life may be shortened by diabetes
  - Not an issue in NOD scid, NSG, NRG, or NOG
  - If diabetes is a concern, single injection of CFA (50 µl) @ 4-10 wk will prolong breeding life

- Scids on BALB/c background (not NOD, B6 or outbred) are challenging breeders

- Nude – Female homozygotes are not effective breeders
  - Ovulation starts late at 2.5 months and ends early at 4 months
  - Many/most do not produce adequate milk for a litter
  - Heterozygote females are exceptionally good breeders
    - Breed to homozygous males (also good breeders)
Issues Related to Humanization

- Possible human pathogens require ABSL2 housing & BSL2 laboratory
- Possible mouse pathogens from human donor, e.g., LCMV
  - Test tissues
- Graft vs. host disease – severely immune deficient mice “attacked” by human tissue / cell transplants
  - Hunched posture, ruffled fur, reduced mobility, tachypnea, diarrhea, weight loss and/or hair loss / skin lesions
  - Time course (days to weeks) depends on strain of mouse, irradiation preconditioning, and type(s) and numbers of human cells injected
Acknowledgements

- Lenny Shultz
- Oded Foreman
- Bonnie Lyons
- Jim Fahey
- Stephen Griffey