Tips and Tricks for the Care of NSG Mice

Dominique Kagele, Ph.D.

Technical Information Services

The Jackson Laboratory

Leading the search for tomorrow's cures

The Jackson Laboratory's Mission

Performing Research

Investigating genetics and biology of human disease

Providing Resources

JAX® Mice Clinical & Research Services, bioinformatics data, technical publications and more…

Educating Scientists

World-class courses, internships and other programs

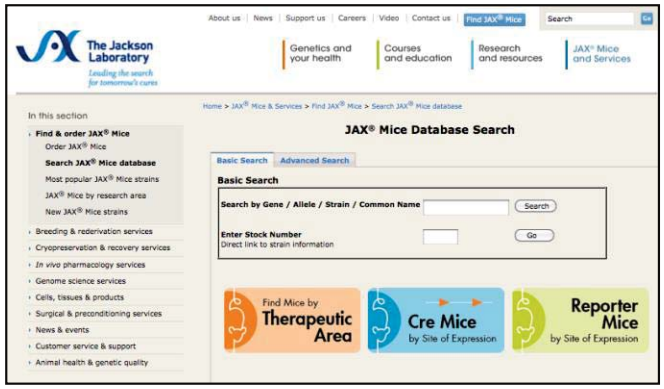
JAX® Mice

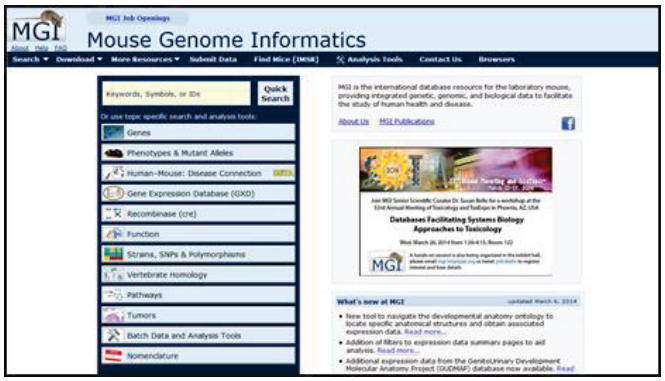
The *Gold Standard* for Biomedical Research

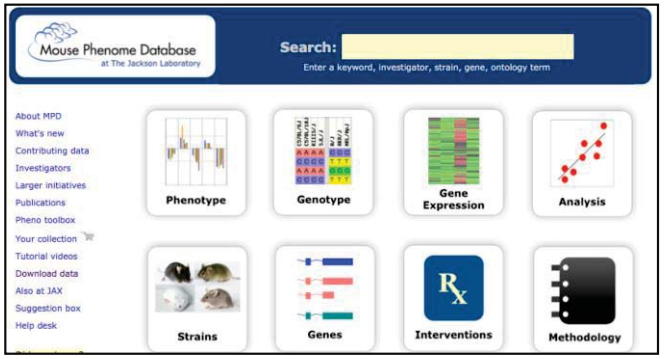
- NIH funded resource
- >7,500 strains and growing
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources

Online Resources to Expedite Research

- JAX® Mice Database
  www.jax.org/jaxmice
- Mouse Genome Informatics
  www.informatics.jax.org
- Mouse Phenome Database
  www.jax.org/phenome
- And many more unique resources







Presentation Overview

Handling and Care of the NSG Mouse

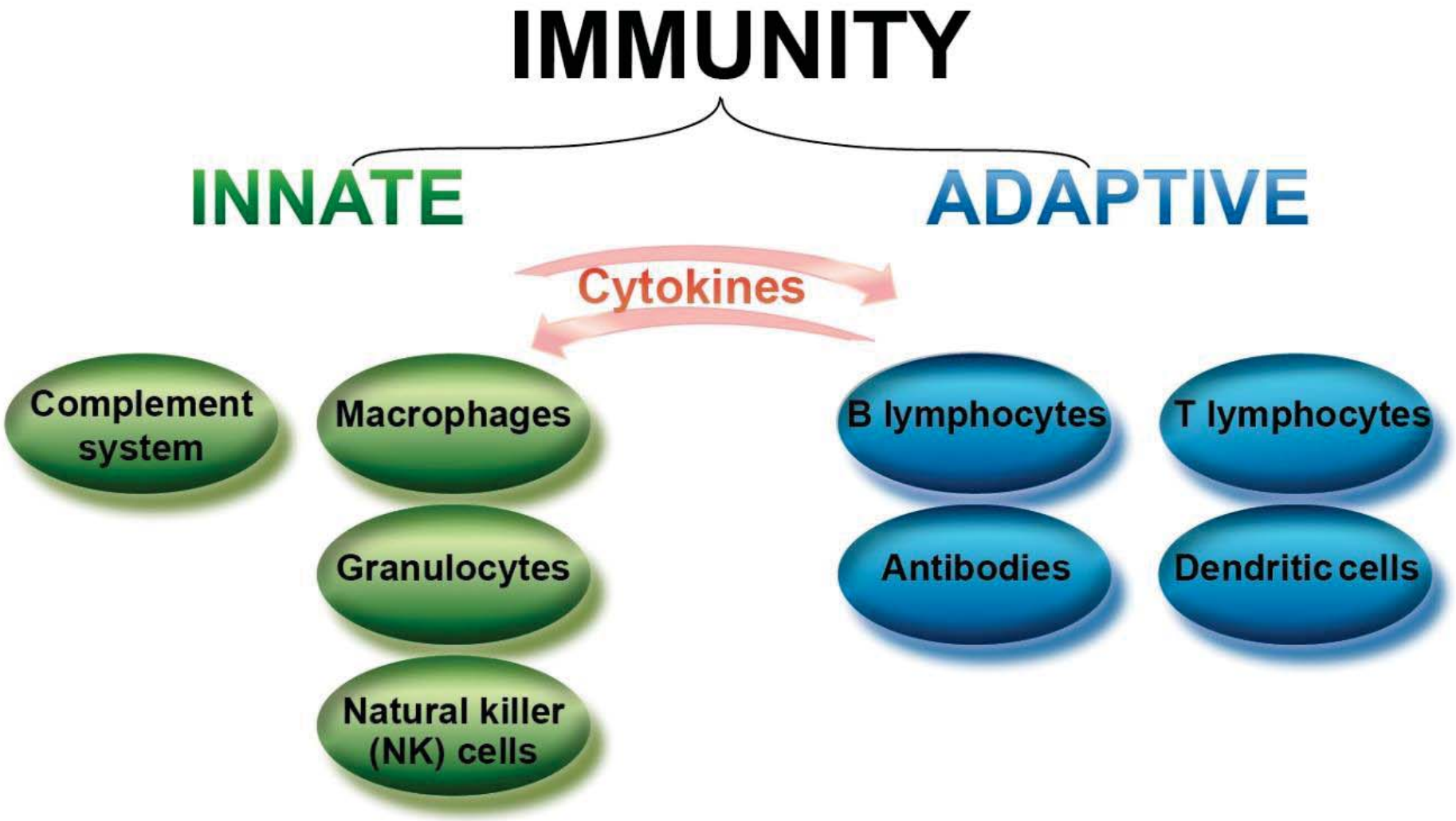
Presentation Overview
Learning Goals

- Explain features and uses of NSG mice
- Identify the types and signs of common infections in NSG mice
- Describe the processes and caging recommendations for successful care of NSG mice

Presentation Overview
Learning Goals

- Explain features and uses of NSG mice
- Identify the types and signs of common infections in NSG mice
- Describe the processes and caging recommendations for successful care of NSG mice

Immune System Components



A Spectrum of Immune Deficiency

Immune deficient

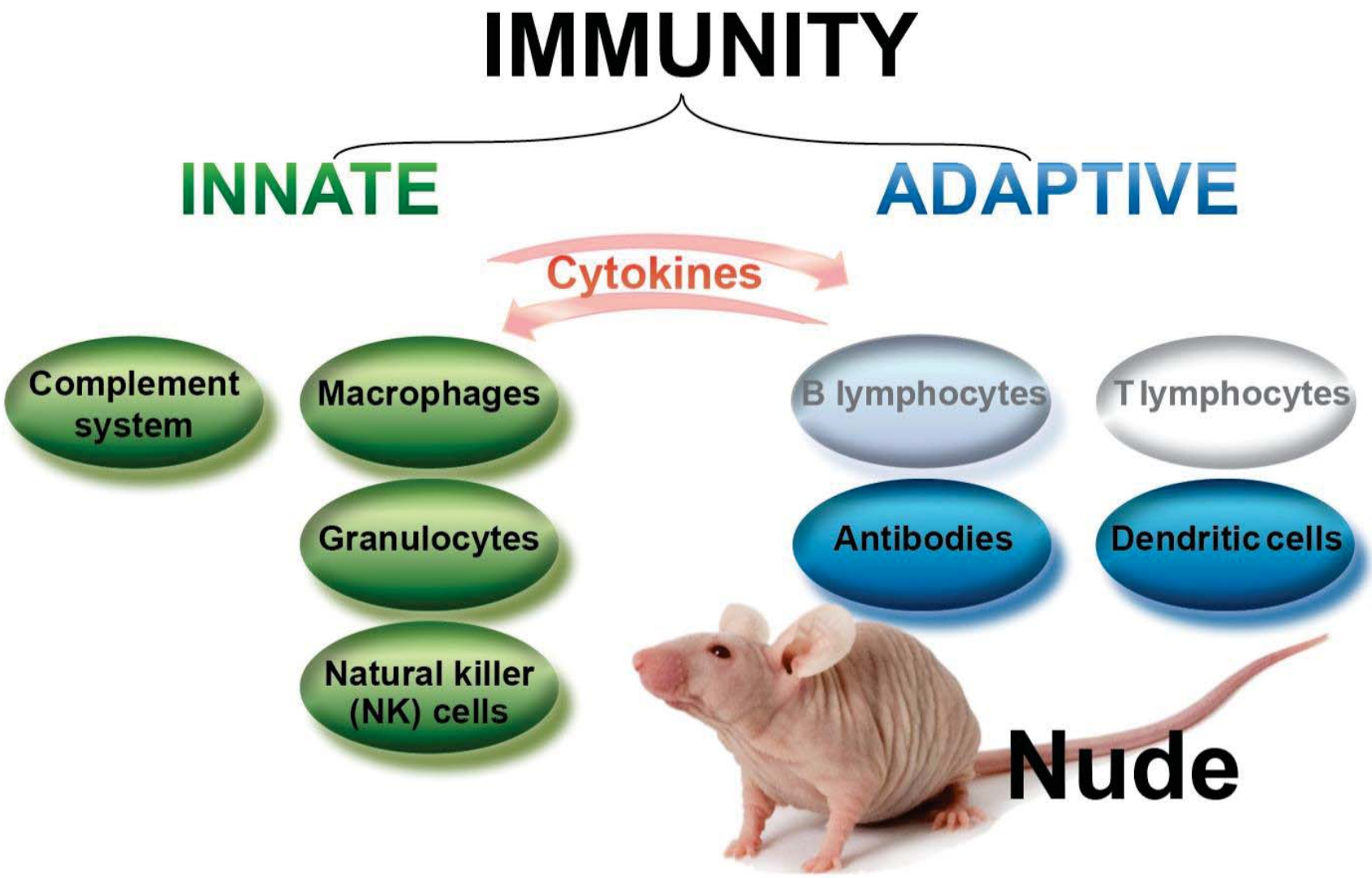
Immune competent

http://jinavie.tumblr.com/post/23052923021/three-little-pigs#.URU70WdL35w

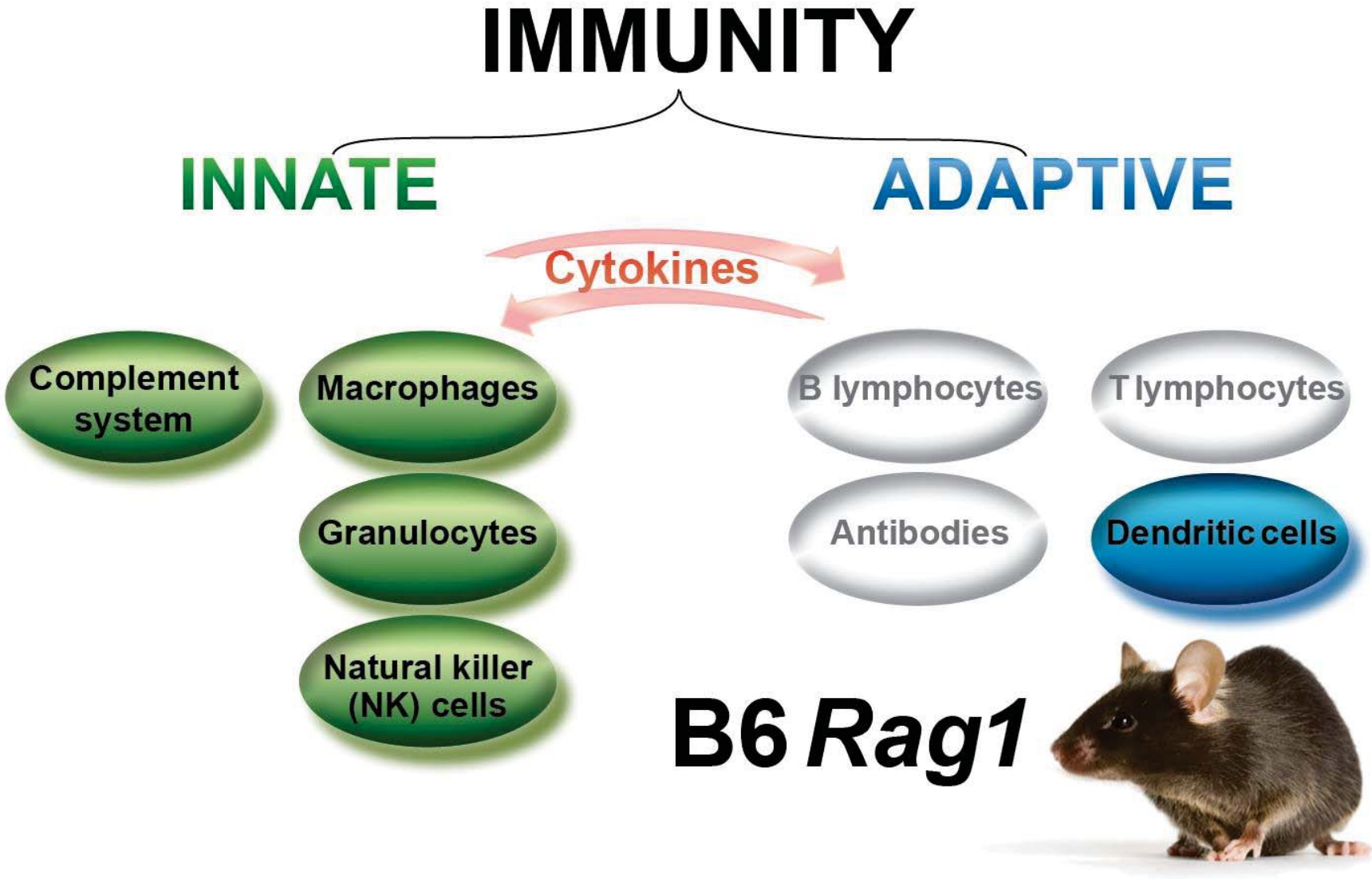
Severely Immune Deficient Strains
"Fragile Superheroes"

- Nude
- *Rag1*/*Rag2*
- *scid*
- NOD *scid* gamma (NSG)

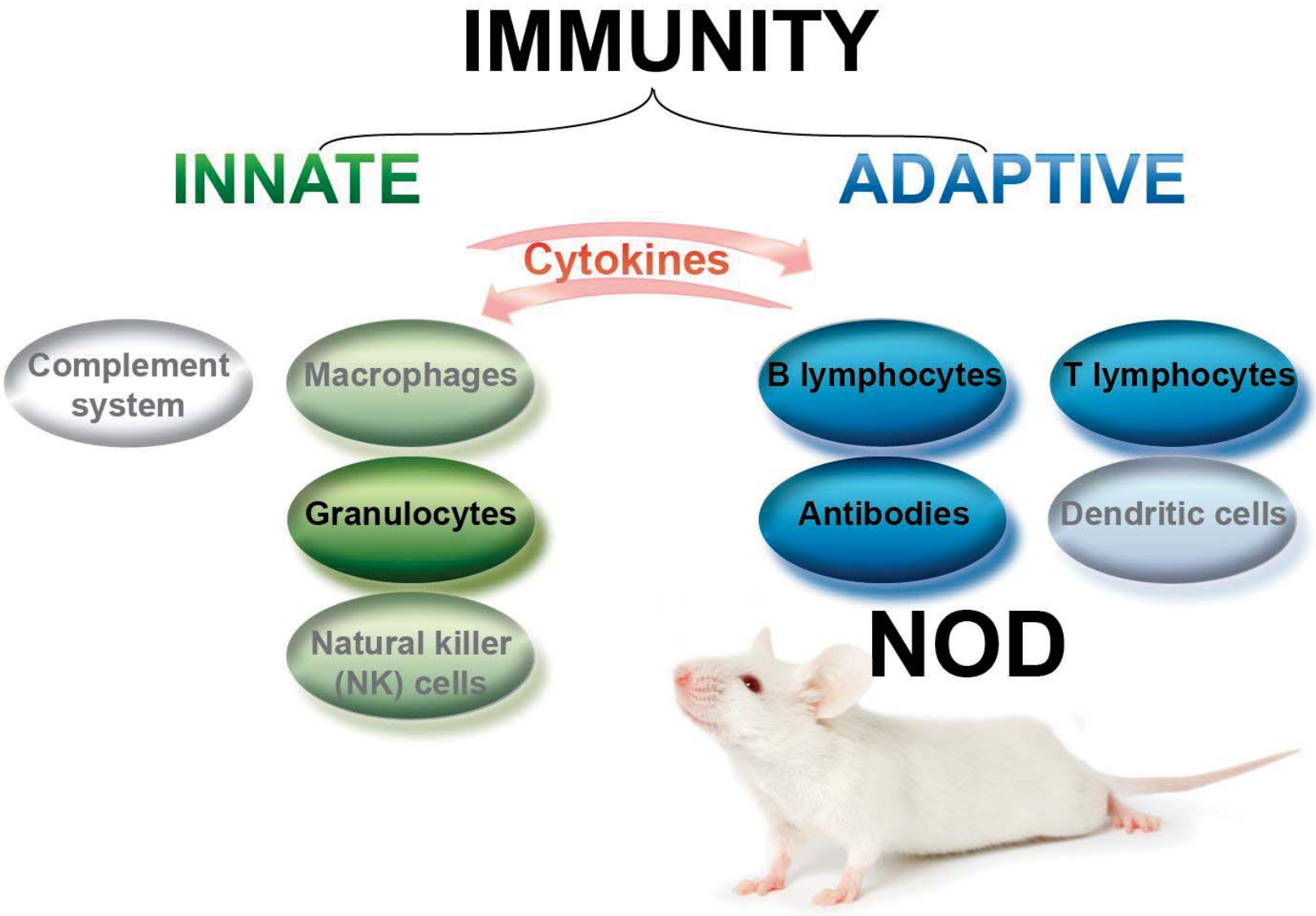
Immune System Components



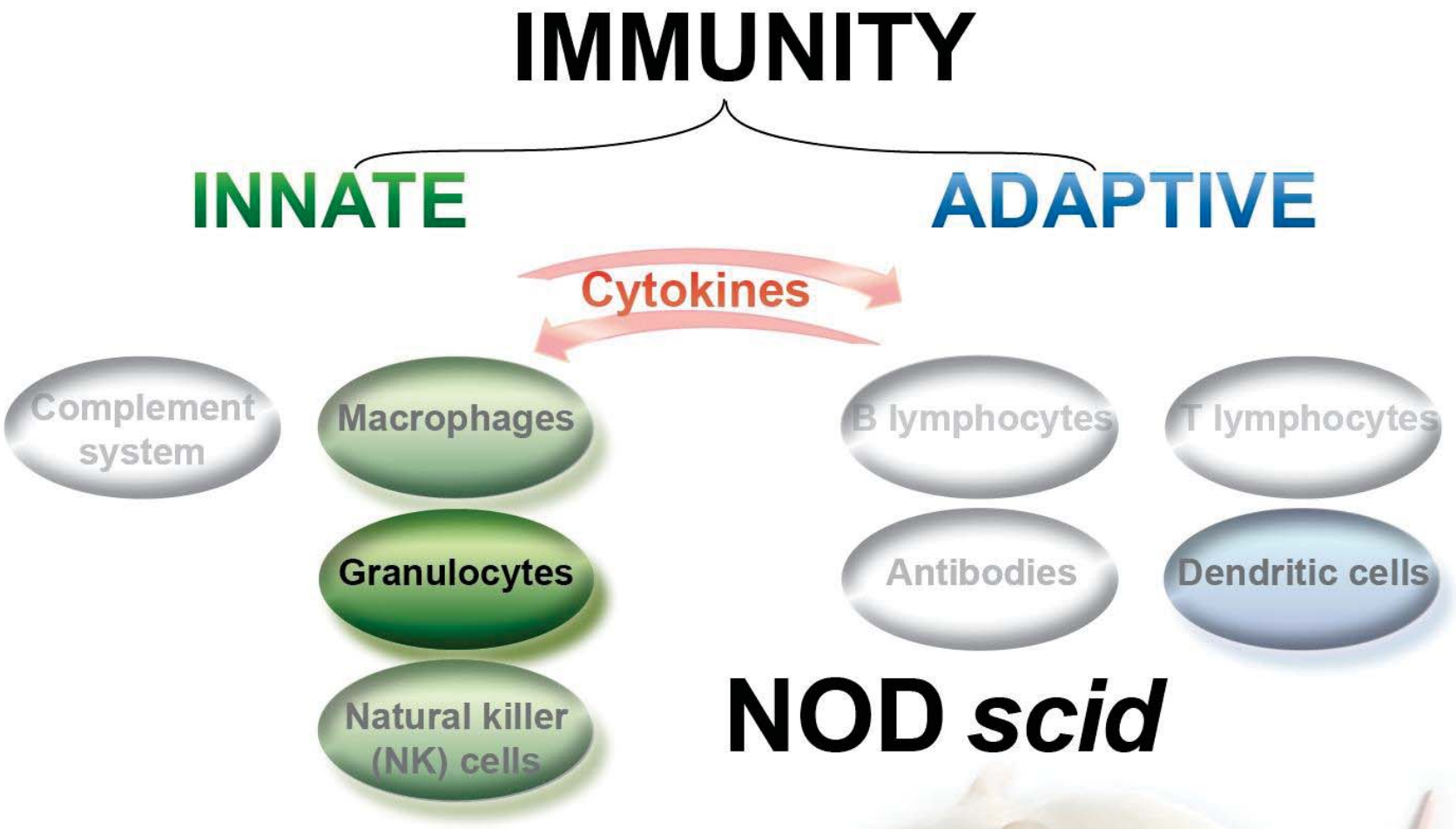
Immune System Components



Immune System Components

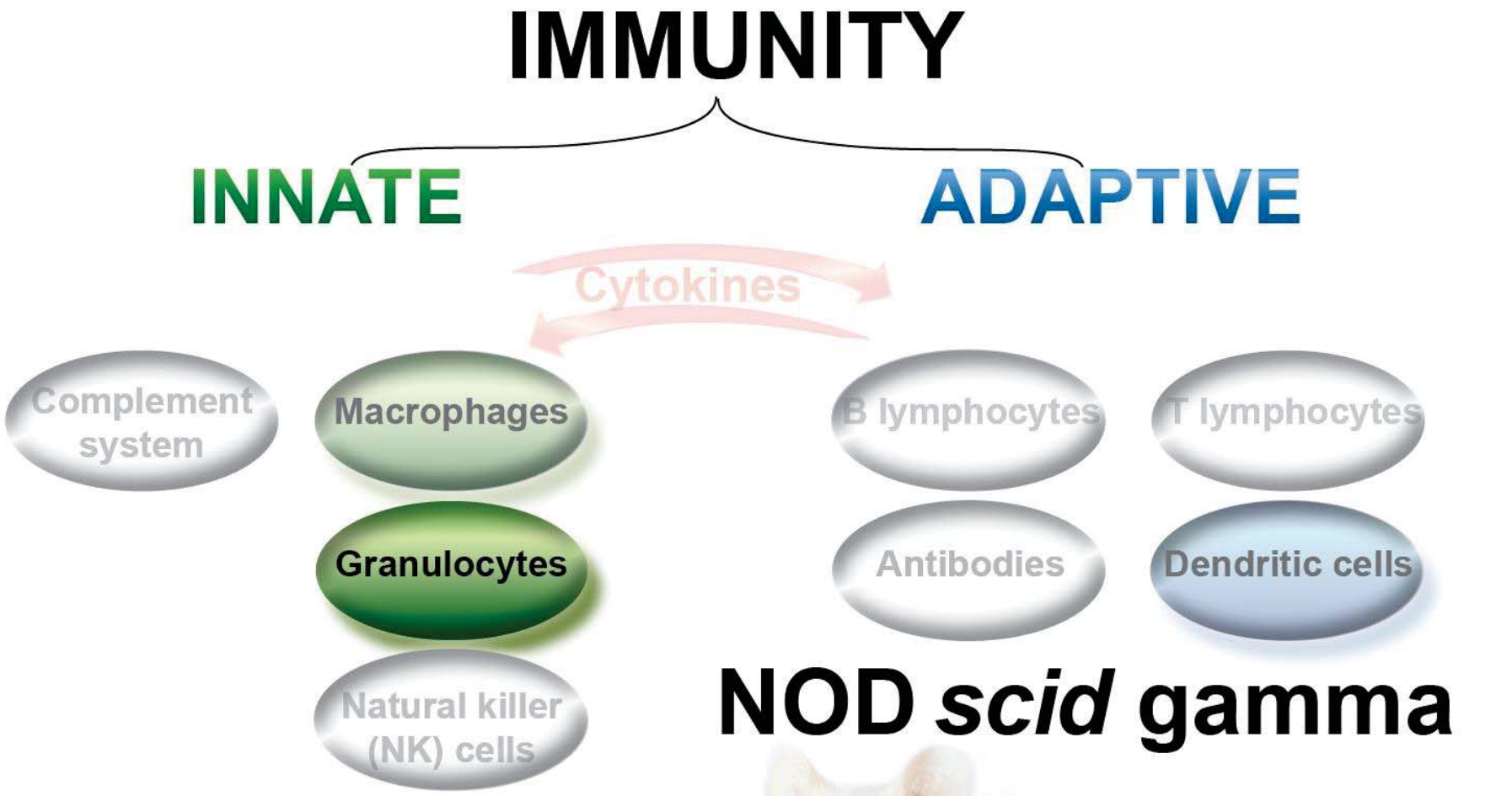


Immune System Components



NOD *scid*

Immune System Components



NSG, NOD *scid* gamma:
Features

Official nomenclature: NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (005557)

Many Immunological Deficiencies

- **NOD** background contributes innate immune deficiencies
  - Macrophages, dendritic cells defective
  - No complement system
  - Bone marrow readily colonized by human hematopoietic stem cells
- ***scid*** mutation prevents development of mature T and B cells
- ***Il2rg*** gene knockout blocks signaling from 6 distinct interleukins and blocks NK cell development

Benefits:

- No *scid* leakiness
- Longer lifespan than NOD *scid* (mean lifespan ~22 months)
- Highly resistant to thymic lymphoma development, as compared to other *scid* mutant strains

To find NSG information online, visit
www.jax.org/jaxmice/research/immunology/005557

NSG Research Applications

An incredibly useful and versatile immunodeficient mouse model

Research applications:

- Primary tumor engraftment
- Human hematopoiesis
- Humanized mice
- Infectious disease
- Regenerative medicine

To find NSG information online, visit
www.jax.org/jaxmice/research/immunology/005557

Presentation Overview
Learning Goals

- Explain features and uses of NSG mice
- Identify the types and signs of common infections in NSG mice
- Describe the processes and caging recommendations for successful care of NSG mice

NSG mice

Infectious Disease Concerns

High level of immunodeficiency results in extreme susceptibility to:

- Pathogenic microorganisms:
  - Infectious agents that typically causes disease in immunocompetent hosts
- Opportunistic microorganisms:
  - Potentially infectious agents that rarely cause disease in immunocompetent hosts
- Commensal microorganisms:
  - Potentially infectious agents that reside in normal host tissues without causing disease

Foreman O, et al. 2011. *Vet Pathol*. [PMID: 20817888]

Pathogenic and Commensal Microbes

Common Threats to NSG mice

- *C. bovis*
- *Citrobacter*
- *Enterobacter*
- *Enterococcus* spp.
- *Klebsiella* spp.
- *Proteus*
- *Pneumocystis murina*
- *Pseudomonas*
- *S. aureus*
- Coagulase-negative *Staphylococcus* spp.

Infectious Disease Concerns

Opportunistic Infections

- **Opportunistic microorganisms normally present in gut flora of healthy mice can become pathogenic in NSG mice, most commonly:**
  - *Klebsiella oxytoca*
  - *Enterococcus spp.*

- Clinical Signs:
  - Hunched posture, scruffy coat
  - Females (twice as likely to be infected)

Foreman O, et al. 2011. *Vet Pathol*. [PMID: 20817888]

Contributing Factors to Mortality

Opportunistic Infections

- Urinary tract infections (UTIs)
  - Normal intestinal flora
  - Estrogen supplementation (increased risk)
- Ascending renal infection and pathology
- Secondary infection after primary insult
  - Skin wounds (ie needle punctures, bacterial dermatitis)
  - Molar gingival sulcus
- Breeding (often lactating) females more susceptible

Signs of Infection

Commonly Observed in NSG mice

- Infected skin wounds, cellulitis
- Abscesses (skin and internal organs)
- Otitis media, conjunctivitis, panophthalmitis
- Localized and widespread infections involving liver, heart, lungs, uterus, accessory sex glands, etc.

Forman et al. 2011.*Vet Pathol.* 48(2):495-9. PMID:20817888

Infections in NSG mice

Contributing Factors

- Can occur in mice housed under less strict barrier conditions, strict barrier conditions, and isolators
- Most often seen after mice have been in the facility a while
- Often appear to be an individual animal vs. colony issue
  - Sporadic
  - Sick and healthy (or infected/non-infected) animals commonly in the same cage

Presentation Overview
Learning Goals

- Explain features and uses of NSG mice
- Identify the types and signs of common infections in NSG mice
- Describe husbandry and caging recommendations for successful care of NSG mice

How Clean is Clean Enough?

Decontamination

Decontamination

Sanitization:

- Reduction of microbial organisms from inanimate surfaces

Disinfection:

- Destruction or inactivation of most microbial organisms from inanimate surfaces

Sterilization:

- Complete destruction or inactivation of all microbial organisms from inanimate surfaces

How Clean is Clean Enough?
Housing Conditions

- Barrier practices adequate to maintain nude, or even *scid* mice may not be adequate for NSG

- Recommend:
  - Sanitize hands before gloving (washing)
  - Disinfect surfaces (laminar flow hoods, experimental equipment, floors, walls)
  - Sterilize tools (forceps, scissors, ear punches, etc), bedding, and cages
  - More frequently change cages
  - Monitor for pathogens frequently (swabbing)

Care and Handling of NSG mice:
Personal Protective Equipment

- Personal Protective Equipment (PPE)
  - Sterile scrubs, gloves, dedicated shoes and shoe covers
  - Mask, goggles, hair/beard bonnet
  - Sterile smock
  - PAPR (Powered air purifying respirator): if necessary

Environmental Conditions:
Suggestions

- Entry room/space (anteroom or dedicated hallway)
  - Clearly marked with tape and/or signage
  - Limited entry
  - Air shower (if available)

Environmental Conditions

- Sterilize or disinfect anything that may come into contact with the mice (autoclave or vaporized hydrogen peroxide (VHP))
- Use laminar flow hoods (or biosafety cabinet working with human pathogens) whenever possible
- Micro-isolator/ individually ventilated cages (IVCs)
  - HEPA (High-efficiency particulate absorption) filtered
- Cage Changes:
  - More frequent
  - Perform in disinfected laminar flow hood
  - Disinfect gloved hands and anything goes into the hood
  - Disinfect forceps (Wescodyne) between cages
  - If hands leave hood, disinfect again

NSG Mice at JAX:
Barrier Conditions

Maximum barrier at JAX:

- Sterilized individually ventilated caging
- Sterilized feed and drinking water
- Air shower entry
- Change into clean room processed scrubs, smock and shoes
- Gloves, air hat or mask, cap and face shield
- Under maximum barrier conditions at JAX, bacterial disease in NSG are uncommon – less than 1% of mice >200 days

Overview of barrier levels at JAX: http://jaxmice.jax.org/health/barrier.html

Experimental Procedures and Transport

- Disinfect experimental equipment and environment (especially shared)
- Sterilize smaller tools (by autoclaving)
- MAP test and/or culture material prior to implantation to ensure sterility
- Use secondary containment during transportation
  - Plastic bag
  - Sterile smock

Breeding NSG mice

NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (005557)

- Homozygote/homozygote female x homozygote/hemizygote male
- No need to genotype pups, but genotype breeders
- Rotate every 8-9 months
- Replace if no litters in 60 days or if appear sick

NSG Mice

Care and Husbandry Suggestions

- Food
  - Autoclaved and/or irradiated
- Water
  - Acidified to pH 2.5 - 3.0 with HCl (or chlorinated) to control for *Pseudomonas* spp.
  - Autoclaved
  - ***No antibiotics added routinely***
- Pathogens monitored
  - Directly (infected mice)
  - Indirectly (sentinel mice)

Pathogen monitoring

- Sentinel mice are those used for the detection of pathogens present in the room, and include:
  - Dirty-bedding (mostly fecal-oral)
  - Cage-contact (direct contact, aerosol, urine, fecal-oral)
  - Exhaust air (aerosolized)

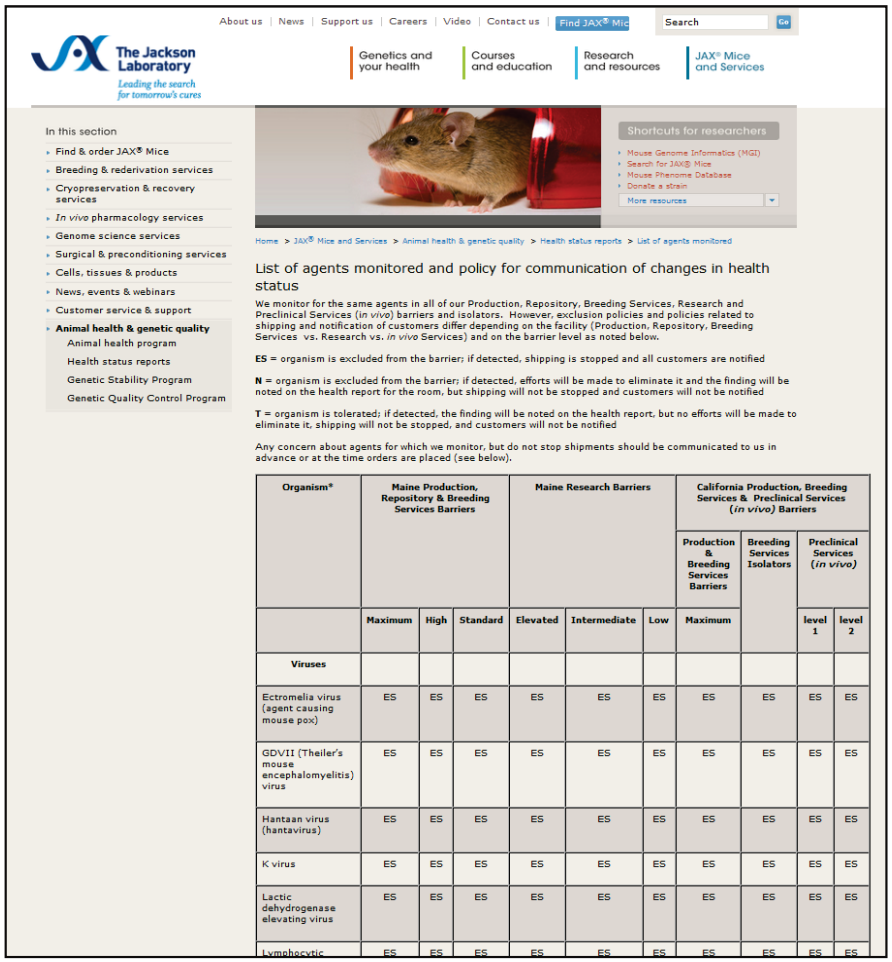
- Use both immunocompetent and immunodeficient (including NSG periodically) as sentinels

For more information, please visit http://jaxmice.jax.org/jaxnotes/archive/497j.html

Health Monitoring Considerations

- 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 due transient nature
  - Test immunodeficient mice directly
- Direct tests (e,g., culture, PCR), not serology, for severely immune deficient

Immunodeficient Mice at JAX:
Animal Health Reports



About us | News | Support us | Careers | Video | Contact us | Find JAX® Mice | Search

The Jackson Laboratory
Leading the search for tomorrow's cures

Genetics and your health | Courses and education | Research and resources | JAX® Mice and Services

In this section

- Find & order JAX® Mice
- Breeding & rederivation services
- Cryopreservation & recovery services
- *In vivo* pharmacology services
- Genome science services
- Surgical & preconditioning services
- Cells, tissues & products
- News, events & webinars
- Customer service & support
- **Animal health & genetic quality**
  - Animal health program
  - Health status reports
  - Genetic Stability Program
  - Genetic Quality Control Program

Shortcuts for researchers
- Mouse Genome Informatics (MGI)
- Search for JAX® Mice
- Mouse Phenome Database
- Donate a strain

More resources

Home > JAX® Mice and Services > Animal health & genetic quality > Health status reports > List of agents monitored

List of agents monitored and policy for communication of changes in health status

We monitor for the same agents in all of our Production, Repository, Breeding Services, Research and Preclinical Services (*in vivo*) barriers and isolators. However, exclusion policies and policies related to shipping and notification of customers differ depending on the facility (Production, Repository, Breeding Services vs. Research vs. *in vivo* Services) and on the barrier level as noted below.

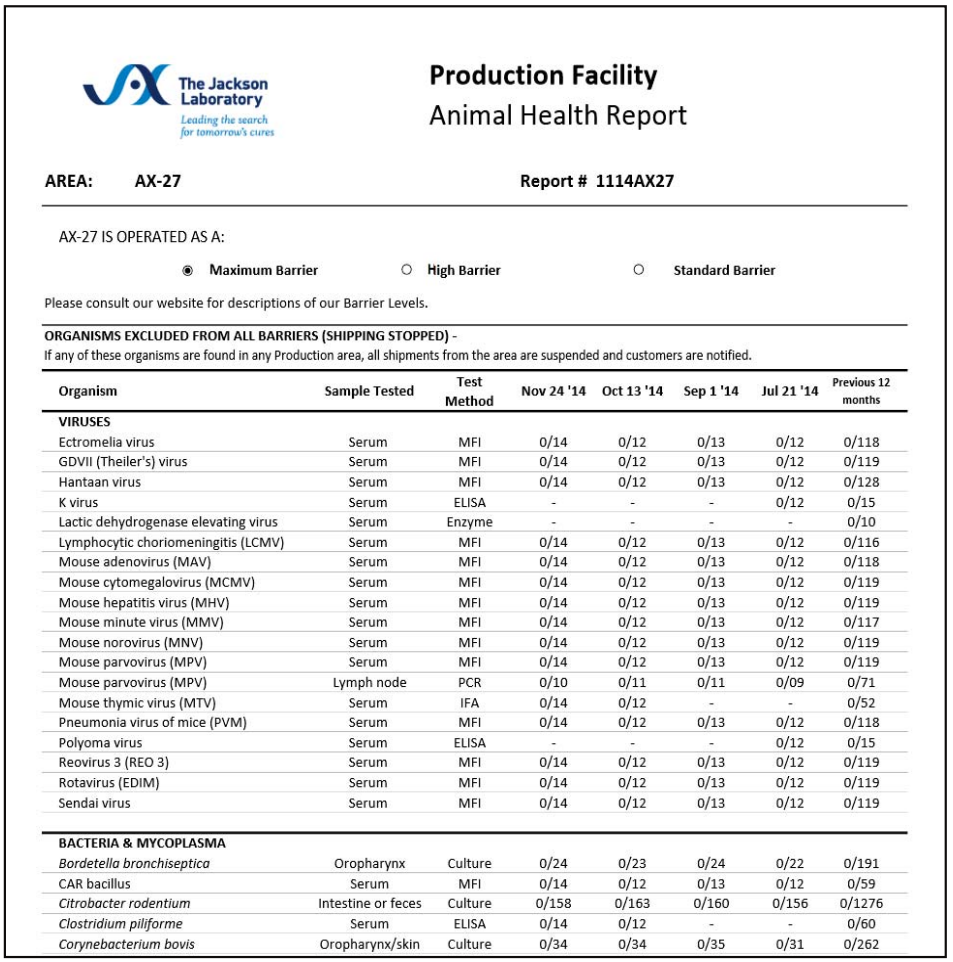
ES = organism is excluded from the barrier; if detected, shipping is stopped and all customers are notified

N = organism is excluded from the barrier; if detected, efforts will be made to eliminate it and the finding will be noted on the health report for the room, but shipping will not be stopped and customers will not be notified

T = organism is tolerated; if detected, the finding will be noted on the health report, but no efforts will be made to eliminate it, shipping will not be stopped, and customers will not be notified

Any concern about agents for which we monitor, but do not stop shipments should be communicated to us in advance or at the time orders are placed (see below).

Organism*	Maine Production, Repository & Breeding Services Barriers			Maine Research Barriers			California Production, Breeding Services & Preclinical Services (*in vivo*) Barriers			
							Production & Breeding Services Barriers	Breeding Services Isolators	Preclinical Services (*in vivo*)	
	Maximum	High	Standard	Elevated	Intermediate	Low	Maximum		level 1	level 2
Viruses										
Ectromelia virus (agent causing mouse pox)	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
GDVII (Theiler's mouse encephalomyelitis) virus	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
Hantaan virus (hantavirus)	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
K virus	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
Lactic dehydrogenase elevating virus	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES
Lymphocytic	ES	ES	ES	ES	ES	ES	ES	ES	ES	ES



The Jackson Laboratory
Leading the search for tomorrow's cures

Production Facility
Animal Health Report

AREA: **AX-27** **Report # 1114AX27**

AX-27 IS OPERATED AS A:

◉ **Maximum Barrier** ○ **High Barrier** ○ **Standard Barrier**

Please consult our website for descriptions of our Barrier Levels.

ORGANISMS EXCLUDED FROM ALL BARRIERS (SHIPPING STOPPED) -
If any of these organisms are found in any Production area, all shipments from the area are suspended and customers are notified.

Organism	Sample Tested	Test Method	Nov 24 '14	Oct 13 '14	Sep 1 '14	Jul 21 '14	Previous 12 months
VIRUSES							
Ectromelia virus	Serum	MFI	0/14	0/12	0/13	0/12	0/118
GDVII (Theiler's) virus	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Hantaan virus	Serum	MFI	0/14	0/12	0/13	0/12	0/128
K virus	Serum	ELISA	-	-	-	0/12	0/15
Lactic dehydrogenase elevating virus	Serum	Enzyme	-	-	-	-	0/10
Lymphocytic choriomeningitis (LCMV)	Serum	MFI	0/14	0/12	0/13	0/12	0/116
Mouse adenovirus (MAV)	Serum	MFI	0/14	0/12	0/13	0/12	0/118
Mouse cytomegalovirus (MCMV)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Mouse hepatitis virus (MHV)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Mouse minute virus (MMV)	Serum	MFI	0/14	0/12	0/13	0/12	0/117
Mouse norovirus (MNV)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Mouse parvovirus (MPV)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Mouse parvovirus (MPV)	Lymph node	PCR	0/10	0/11	0/11	0/09	0/71
Mouse thymic virus (MTV)	Serum	IFA	0/14	0/12	-	-	0/52
Pneumonia virus of mice (PVM)	Serum	MFI	0/14	0/12	0/13	0/12	0/118
Polyoma virus	Serum	ELISA	-	-	-	0/12	0/15
Reovirus 3 (REO 3)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Rotavirus (EDIM)	Serum	MFI	0/14	0/12	0/13	0/12	0/119
Sendai virus	Serum	MFI	0/14	0/12	0/13	0/12	0/119
BACTERIA & MYCOPLASMA							
Bordetella bronchiseptica	Oropharynx	Culture	0/24	0/23	0/24	0/22	0/191
CAR bacillus	Serum	MFI	0/14	0/12	0/13	0/12	0/59
Citrobacter rodentium	Intestine or feces	Culture	0/158	0/163	0/160	0/156	0/1276
Clostridium piliforme	Serum	ELISA	0/14	0/12	-	-	0/60
Corynebacterium bovis	Oropharynx/skin	Culture	0/34	0/34	0/35	0/31	0/262

Health Status Reports: http://jaxmice.jax.org/health/index.html

NSG Mice

Treatment of infection

- Antibiotic treatment of *individual* mice:
  - Baytril (enrofloxacin)
    - 5-20 mg/kg SC as directed by your verterinarian
  - Others (amoxicillin, cephalexin)
  - No evidence of effectiveness as prophylatic treatment
- *Pneumocystis* containment
  - Sulfa-Trimethoprim: 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
- Rederivation to eliminate opportunists from colony

Slate AR., et al., *JAALAS*. 2014.

Marcotte H., et al., *J Infect Dis*. 1996

Humanization

Health Considerations

- Possible human pathogens require ABSL2 housing & BSL2 laboratory
- Possible mouse pathogens from human donor, e.g., LCMV
  - Test tissues (MAP test, and/or culture bio-materials)
- 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

Warning Signs

- Non-specific clinical problems
- Unthriftiness, diarrhea, wasting, sickliness
- Weight loss
- Weakness, lethargy
- Acute and/or premature death
- Breeding problems, including:
  - Embryonic death
  - Small litters
  - Small, weak, and/or sickly pups
  - Pup mortality

Work With Your Veterinarian!

- When breeding performance suddenly declines
- Mice appear unhealthy (ruffled fur, hunched, reduced mobility/activity)
- Spontaneous death
- Positive culture/sentinel mice results

Summary

A little prevention goes a long way!

Handling and Care of the NSG Mouse

Thank you!

In need of mouse breeding and colony management expertise to advance your research?

Contact your regional representative today

www.jax.org/jaxmice/support/regionalcontacts

Contact technical support

www.jax.org/jaxmice/support/techsupport-index

JAX® Mice, Clinical & Research Services

1-800-422-6423 • 1-207-288-5845
jaxservices@jax.org • www.jax.org/jaxmice