

CENTER OF
COMPARATIVE
MEDICINE &
PATHOLOGY

In Focus

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WELCOME HSS!

The CCMP is pleased to welcome the Hospital for Special Surgery (HSS) to the jointly operated MSKCC-Weill Cornell Animal Care and Use Program (ACUP). The inclusion of HSS has enhanced and facilitated collaboration amongst our faculty and expands our research endeavors as we enter into this new partnership.

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Highly Immunodeficient Mice: A Double-Edged Sword

The past two decades have seen an explosion in the use of increasingly more immunodeficient mice in biomedical research, the latest examples being the NSG, NRG and NOG strains¹. Immunodeficient mice have great advantages, such as the ability to accept xenografts from genetically distant species. For example, human immune cells can be administered to recreate the human immune system or to investigate specific cell types². Immunodeficient mice also support the growth of human cancer cells, which provides opportunities to study the biology and potential treatment of human neoplasia. These benefits do not come without risk as the impaired immune status of these strains makes them highly susceptible to a number of environmental and opportunistic bacterial and fungal pathogens. Many of these organisms are ubiquitous and cannot be eliminated from the environment. Therefore, researchers face the challenge of studying mice that are under constant risk of attack from agents that can cause disease and/or affect research data.



The **Athymic Nude** and the **Severe Combined Immuno Deficient (SCID)** mouse mutations started it all.

The nude mouse is a genetic mutation that causes a deteriorated or absent thymus, resulting in an inhibited immune system due to a greatly reduced number of T cells. The genetic basis of the nude mouse mutation is a disruption of the **FOXN1** gene.[1][2]

The **SCID** mouse is characterized by the complete inability of the adaptive immune system to mount, coordinate, and sustain an appropriate immune response, usually due to absent or atypical T and B lymphocytes. ~exc. Wikipedia

Image credit: <https://www.jax.org/strain/007850>

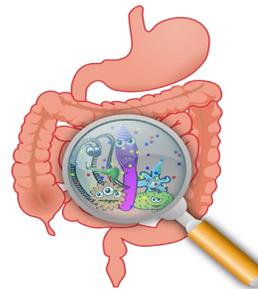
Since the 1960s, the nude mouse (Foxn1nu), the first immunodeficient mouse described, has been a mainstay in studies involving transplanted tissues or cells. Nude mice lack a thymus and functional T lymphocytes and readily engraft many solid tumors. However while still used frequently, their use is limited by the presence of functional innate and adaptive

immune systems as well as high natural killer (NK) cell activity. Some of these shortcomings were addressed in the early 1980s with the discovery of the spontaneously occurring "SCID" mutation, which leads to deficient activity in the enzyme Prkdc, a protein kinase involved in DNA repair. Because V(D)J recombination relies on this DNA repair machinery, recombination is impaired resulting in a lack of mature T or B lymphocytes⁴. This mutation was complemented by the creation of Rag1null and Rag2null knock-out mice using gene targeting in 1992. These models are unable to undergo V(D)J recombination, causing a similar absence of functional T or B cells but without the 'leakiness'* seen in SCIDs. These mice support the growth of a wider variety of cell types and tumors from other species, but are again limited by the presence of functional complement proteins and NK cells, which inhibit the engraftment of hematopoietic stem cells (HSCs) and some foreign tumors. In order to ameliorate these limitations, SCID mice were
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The Effects of Gut Microbiota on Rodent Models

Characterizing the gut microbiome (GM) and determining its role in health and disease has recently become a subject of many research and popular science articles. The GM is the collective genome of all organisms residing within the gastrointestinal tract. As we continue to learn more about the GM of rodents, we are beginning to understand its effects on specific research models. By considering the GM of rodents, we can potentially improve reproducibility of experimental models. This article will review the current understanding of the rodent GM, its known impact on rodent models, and some potential ways to manage or mitigate the GM as a variable in future research.

The mammalian GM contains over 1000 different species, including over 10¹⁴ bacteria and large numbers of smaller populations of Actinobacteria, archaea, eukaryotes, and viruses¹. The



The recently intensified study of gut microbiota gives cause to reconsider—You are what you eat (and drink) and this holds for mice as well.

Image credit: https://microbiomemusing.files.wordpress.com/2015/10/anatomy-160524_1280.png

rodent GM shares similar bacterial species with humans, but vary in predominating phyla. The predominant phyla in rodents are Firmicutes and Bacteroidetes, which are smaller populations of Actinobacteria, Proteobacteria, Verrucomicrobiota, Spiro-

chaeta, Tenericutes, Deferribacteres, and TM7². The GM also varies by gut segment, with the small intestine having the least species diversity and the colon the most. Feces contains GM species from all segments of the gastrointestinal tract². Some specific species differences exist between humans and rodents. The GM of rodents contains *Mucispirillum schaedleri*, a component of the altered Schaedler flora, while no family members have been identified in humans². *Faecalibacterium prausnitzii* is abundant in humans and reduces colitis in several models, but is absent from some mouse colonies³. Segmented filamentous bacteria (SFB) have been evaluated in several inflammatory disease models, but there is still debate as to whether these species are found in humans after one year of age⁴.

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Highly Immunodeficient Mice: A Double-Edged Sword

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The compromised immune system of HIDM render them highly susceptible to myriad opportunistic infections. The skin, urinary system, and digestive tract are most commonly affected. Some examples are *Corynebacterium bovis*, a bacterium that causes hyperkeratosis or scaly skin disease in nude mice (A), the gastrointestinal tract is the ideal setting for overgrowth of *Escherichia coli* (B), and *Pseudomonas aeruginosa* can lead to septicemia (C).

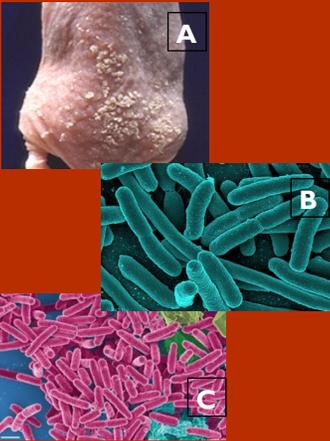


Image credits: A- <http://dora.missouri.edu/mouse/corynebacterium-bovis-hyperkeratitis-associated-coryneform/> B- <http://www.the-cma.org.uk/Articles/Cystitis-is-an-infection-of-the-bladder-where-there-is-inflammation-> C- <http://www.underwateraudio.com/blog/swimmers-ear-101/bill->



Image credits: <http://www.neatorama.com/2013/07/03/Mouse-Armor/>

Body armor being highly impractical, other methods are in place to protect these precious but vulnerable mice including: the use of:

1. Autoclaved (sterile) caging.
2. Antibiotic-containing feed to reduce pathogen exposure or treat infections prophylactically.
3. Biosafety cabinets (BSCs) that protect both the user and animal, delivering HEPA filtered air directional airflow to contain contaminants within the BSC.
4. Performing all procedures using aseptic technique.
5. Appropriately decontaminating and handling instruments/materials that come in contact with HIDM

crossed with non-obese diabetic (NOD) mice, which have defects in innate immunity including decreased NK cell activity and lack of hemolytic complement⁵. The latest generation of immunodeficient mouse strains have additional targeted mutations to the IL2-receptor gamma chain (IL2gr), which is responsible for the signaling of several cytokine receptors. The resulting mice (NSG, NRG, NOG) completely lack functional NK cells in addition to the deficiencies already described. These strains are capable of accepting HSC grafts as well as hematologic cancer cells that could not be grown in earlier developed immunodeficient strains. However, these highly immunodeficient mice (HIDM) are also more susceptible to opportunistic pathogens, requiring the implementation of specialized husbandry and operational procedures.

The most common opportunistic infections of HIDM involve the skin, urinary system, and digestive tract, although infection is not exclusive to these sites⁶. In the skin, bacteria such as *Staphylococcus spp.* or *Streptococcus spp.* have been associated with dermatitis. Another common skin contaminant is *Corynebacterium bovis*, a bacterium that causes hyperkeratosis, or scaly skin disease in nude mice as well as dermatitis in other immunocompromised strains. In the urinary tract, bacteria such as *Enterococcus spp.* and *Klebsiella spp.* can cause ascending infections, which are among the most common causes of unexpected death in NSG mice⁷. The gastrointestinal tract is the ideal setting for overgrowth of bacteria such as *Escherichia coli* or *Clostridium spp.* In addition to affecting specific organ systems, some bacteria can also cause septicemia, a systemic infection that affects multiple organ systems by disseminating through the blood. These bacteria include *Pasteurella pneumotropica*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*, among others. As the use of HIDM increases, new organisms will likely be identified that compromise their health and affect their use as research subjects.

There are myriad options available to house mice, ranging from the use of gnotobiotic isolators to open cages with minimal handling precautions. When housing HIDM, the former is usually impractical and use of the latter poses significant risk to the animals. Therefore, a middle ground is generally utilized. For example, autoclaved (sterile) caging, or antibiotic-containing feed may be used to reduce pathogen exposure or treat infections prophylactically. It is also important to utilize the proper equipment when

handling HIDM. For instance, changing stations located in standard rooms utilize a downward HEPA-filtered air current to reduce the user's exposure to particulates but do not provide adequate protection when working with biohazards. On the other hand, biosafety cabinets (BSCs) are designed to provide both user and animal protection. These cabinets deliver HEPA-filtered air to the work surface and utilize directional airflow to contain contaminants within the BSC. Using a BSC is required when working with HIDM engrafted with human-derived tissues or cells. Instruments/materials coming in contact with HIDM can also be a source of infection, so it is important that these are appropriately sterilized and that all procedures are performed using aseptic technique.

Ultimately each individual study will call for a different approach depending on the risk of infection, potential effects on data, and the added burden of additional precautions. The Research Animal Resource Center has implemented some standard measures when housing HIDM in our facilities. First, certain rooms are dedicated to housing HIDM and all animals entering these rooms must arrive from approved vendors or undergo comprehensive testing to ensure that they arrive free of specified pathogens. Within the rooms, all animals are housed in sterile cages and given food/H₂O that has been gamma-irradiated and/or autoclaved. All rooms are equipped with BSCs in which all animal handling should take place. These rooms are designated to be entered before standard holding rooms, to reduce the risk of tracking opportunistic pathogens from those locations. Additional personal protective equipment (PPE) is also donned just prior to entry to provide added user protection and reduce the tracking of microbes from other parts of the facility. Although this set of baseline precautions will help, they will not eliminate all infections. Users should remain aware of the risk for infections and any unexpected illness or deaths should be reported to Veterinary Services, who will determine a cause and advise on additional precautions that could benefit the study.

~Nick Tataryn, DVM

*The term leakiness is used to describe the phenomenon in which some scid mice develop a small number of functional T and B lymphocytes. Although the number of cells is typically less than 1% of normal, they occasionally exceed that level and contribute to graft rejection.

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Host-microbial interactions can affect research models via microbial-associated molecular patterns (MAMPs), epitopes, and enzymes through food metabolism. MAMPs are molecules such as lipopolysaccharide and peptidoglycan of gut flora that activate the innate immune system, e.g., toll like receptors on immune cells and enterocytes. The resulting cytokine stimulation may influence models of diabetes⁵ and atherosclerosis⁶ via chronic inflammation. Additionally, behavioral models may be affected through activation of the gut-brain axis⁷. Epitopes are molecular patterns found on antigens that stimulate the formation of and bind to antibodies and cells of the adaptive immune system. Epitopes are usually non-self proteins, including those found on bacteria within the GM. Epitope differences that accompany changes in GM have been shown to alter the expression of Th1, Th17, Th2, and regulatory T cells⁸. These have been shown to influence autoimmune, inflammatory, and immunoprotective models⁷. Differences in microbial enzyme production have been shown to affect the gastrointestinal mucosal barrier⁹, which can result in changes in pathogen and metabolic status.

Individual rodent research models impacted by the GM are too extensive to review comprehensively in this article. The review paper by Hansen et al (2015)² offers a comprehensive assessment of the models known to be affected by changes or differences in the GM. The models that seem to be most affected are those related to metabolic syndromes and autoimmune, allergic, neural and behavioral diseases. There are also studies examining the role of GM variations in cancer^{10,11}. These broad categories are likely to be expanded upon as investigators continue to characterize the rodent GM.

Importantly, the rodent GM varies significantly

between vendors, vivaria, and even rooms within a vivaria¹². The GM of rodents seems to be primed early in life and remains relatively stable⁸. Traditionally, genetics were thought to determine much of the GM. However, the impact of genetics is estimated to be only 20%, with greater influence exerted by host immune function and environment¹³. Mice housed together or living in open top caging environments develop similar GM over time⁵. The caging itself has also been shown to influence the GM. Mice housed in open top cages have increased levels of *Enterobacteriaceae*, *Bacteroides*, and *Lactobacillus spp.* and lower levels of *Bifidobacteria spp.* when compared to mice housed in individually ventilated cages¹⁴. The diet should remain constant throughout a study, as changes in sugar, fat, protein, and micronutrients have been shown to change the GM and affect research models used in various studies². Drinking water also has the potential to alter research models. In a study looking at diabetes development in NOD mice, mice with SFB were only prevented from developing the phenotype when given acidified drinking water.¹⁵

In conclusion, it is important to consider the GM of rodents when planning, executing and publishing research. Differences in GM can significantly affect research models, especially when working with models of inflammation, autoimmunity, and allergies. For research models known to be influenced by GM, investigators should consider housing all their animals consistently or consider additional cages to achieve statistical power across GM groups. When preparing research for publication, it is important to include as much detail as possible regarding vendors, housing, and environment to increase research reproducibility.

~Andrew Gorman, DVM

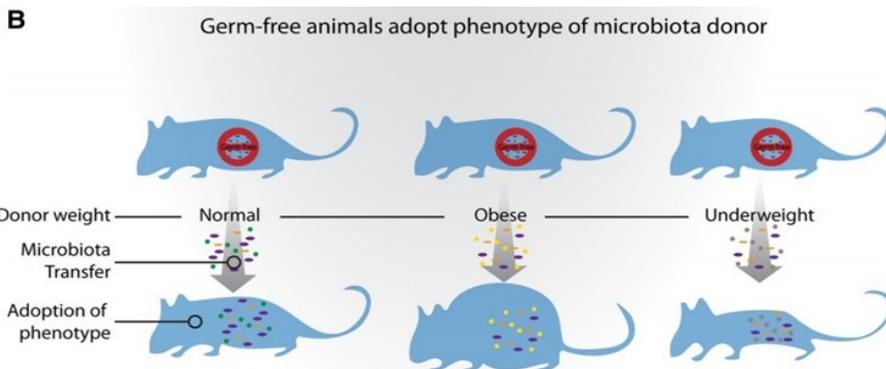


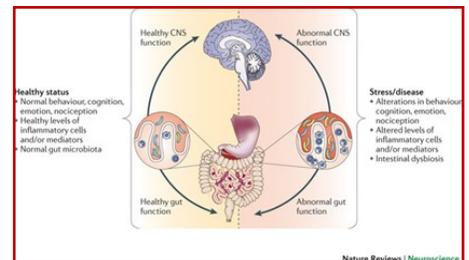
Figure B: GF animals adopt the phenotype of the microbiota donor. Previously GF mice display an obese phenotype on receipt of a microbiota transplant from obese mice or when humanized with a microbiota from an obese individual. Similarly, GF animals lose weight upon receipt of a microbiota transfer from animals who have exhibited rapid weight loss after gastric bypass surgery. Mini review: Gut Microbiota: The Neglected Endocrine Organ Gerard Clarke, et al. Credit: <http://press.endocrine.org/doi/pdf/10.1210/me.2014-1108>

A single research experiment with mice may use dozens, hundreds or even thousands of research subjects. What is the best way to identify individual mice? Historically, ear punching, ear tagging, tattooing or toe clipping have been useful for keeping track of individual animals. More recently, new technology has become available to distinguish individual mice.

An identification method should be easy to apply and read. Additionally, the method should be cost effective (especially in mice) and should not impact the animal's welfare. Some methods, such as ear punching or toe clipping, may have the added advantage in that tissue can be collected and used for genotyping. Disadvantages of traditional identification methods include accuracy (ear punches may close or be traumatized), ear tags may result in wire bar lid entrapment or may fall out, tattooing can be time-consuming and requires special skill for application, and clipped toes may be difficult to read.

Examples of new identification methodologies include JaxTags™, implantable microchips and p-Chips®. JaxTags™ (Figure 1) are 3D bar-coded ear tags that use a hand held scanner to read the codes. The tags are small (so they rarely get caught in the wire bar lid) and they are lightweight. The individual identification numbers are not readily visible to the naked eye and JaxTags™ are not MRI compatible. JaxTags™ cost \$2.75 per mouse implanted at the vendor. The associated bar code scanner costs approximately \$600.

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Numerous rodent research models are focused on the effects of gut microbiota (GM) on obesity but there is an urgent need to take notice of these effects on other areas of study as well.

Image credit: http://www.nature.com/nrn/journal/v13/n10/fig_tab/nrn3346_F3.html

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Technological Advances in Mouse Identification New Pathologist Welcomed to the CCMP

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RFID microchips are 11 – 12 mm and are implanted subcutaneously using a specialized syringe or a lancet. Each chip is encoded with a unique alphanumeric code and the codes are easily distinguished with an electronic reader (Figure 2). Standard microchip implants are too large to be used in neonatal mice, but a smaller size (8 mm) can be implanted in weanlings. Costs vary depending on the vendor and the brand of microchip. Locus Technology sells German-manufactured Trovan Electronic microchip transponders for \$5.50 each. Microchip readers range in price from \$350 to \$745, depending on whether they are hand-held or high performance readers.

Another implantable ID device is the p-Chip[®] mouse tagging system, the smallest electronic tagging device available, developed by PharmaSeq. p-Chips[®] measure 0.5 mm x 0.5 mm x 0.2 mm, and weigh only 85 micrograms. Because of their small size, p-Chips[®] can be injected subcutaneously into a rodent's tail (Figure 3). They have an added benefit that they are compatible with all common imaging techniques (including MRI) and they can withstand radiation exposure up to 40

Gray. The PharmaSeq system is costly, approximately \$2800 for the reader (Figure 4) and tags for 100 mice.³ The reader is the largest expenditure at \$2490.

These newer identification options are not only costly, but each method has its advantages and disadvantages.

For convenience, most mouse vendors offer to identify animals prior to shipment. Taconic Biosciences has teamed up with PharmaSeq and now offers p-Chip[®]-implanted mice at an additional cost of \$12.60 per mouse. Most rodent vendors offer an array of identification services for an additional cost.

For studies involving small numbers of animals (fewer than 100), ear punching remains the most commonly used method of identification as it is fairly reliable and inexpensive. As an alternative to tail tipping, the punch can also be used for genotyping. For studies involving large numbers of mice, newer identification methods may be warranted and advantageous, but they are costly.

~Melissa Nashat, DVM, PhD



Figure 1: JaxTag™¹



Figure 2. Microchipped mice being

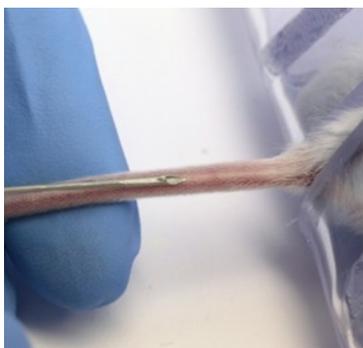


Figure 3. Subcutaneous injection of a p-Chip[®] tag into a mouse tail³

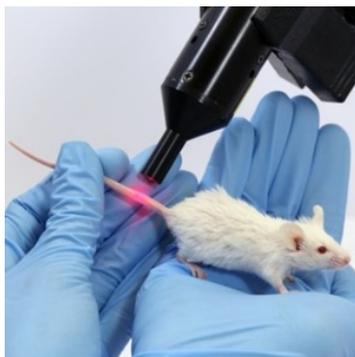


Figure 4. Reading of a p-Chip[®] tag with a handheld reading wand³

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Alessandra Piersigilli, DVM, Ph.D

The Center of Comparative Medicine and Pathology's Laboratory of Comparative Pathology is extremely pleased to welcome Alessandra Piersigilli, DVM, Ph.D. as a Comparative Pathologist. Alessandra received her masters degree in veterinary medicine in '02 from the University of Camerino (Italy). Her experience and focus in experimental pathology and preclinical animal model validation began with her doctoral studies at the Sant'Anna School of Advanced Studies in Pisa (Italy) and at the Italian National Research Council. Her thesis: "Protocols for validation of biomedical devices" led to her Ph.D. in '08. Subsequently she served as a toxicologic pathologist at Abiogen Pharma (Pisa, Italy), Harlan Laboratories (Itingen, Switzerland) and Roche (Basel, Switzerland). Between '09 and '12 Alessandra completed a residency program in veterinary anatomic pathology at the University of Bern, becoming a diplomate of the European College of Veterinary Pathology (ECVP) in '13. Most recently she served at the EPFL as a Comparative Pathologist-Scientist collaborating on projects encompassing immunology, infectious diseases, cancer biology, and metabolic disorders. In '15 she co-founded at the University of Bern the first Swiss platform for comparative pathology (COMPAT) as a joint venture with the Swiss Institute of Pathology and the Institute of Animal Pathology. Her professional interests include cancer biology, the prognostic value of molecular-morphologic signatures and animal model validation.



Welcome Spring!

Image credit: <https://www.pinterest.com/pin/296745062919781905/>