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Brief Reviews

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# Of Mice, Dirty Mice, and Men: Using Mice To Understand Human Immunology

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**Mouse models have enabled breakthroughs in our understanding of the immune system, but it has become increasingly popular to emphasize their shortcomings when translating observations to humans. This review provides a brief summary of mouse natural history, husbandry, and the pros and cons of pursuing basic research in mice versus humans. Opportunities are discussed for extending the predictive translational value of mouse research, with an emphasis on exploitation of a "dirty" mouse model that better mimics the diverse infectious history that is typical of most humans.** ***The Journal of Immunology*****, 2017, 199: 383–388.**

The house mouse, *Mus musculus*, represents the dominant in vivo mammalian model in modern biomedical research. It is said that *Mus* comes from musaka, the Sanskrit word for thief, highlighting the close but somewhat acrimonious relationship between mice and humans. House mice may have the widest geographical distribution of any mammalian species with the exception of humans. *M. musculus* originated in central Asia and has followed humans across the globe by both land and sea. Although house mice have adapted to live in a variety of climates from arid to tropical, they often compete poorly in the wild with other mouse species and are largely dependent on the trappings of civilization for their success. So, although dogs may be man's best friends, mice may be humankind's closest mammalian companion. Mice are more closely related to humans than canines as well, sharing a common ancestor only ~65–75 million years ago (1).

*M. musculus* is comprised of a complex of species. *M. m. domesticus* colonized Western Europe, Africa, the Near East, the Americas, and Australia. *M. m. musculus* colonized Central and Eastern Europe and China. *M. m. castaneus* spread into Southeast Asia and *M. m. molossinus* into Japan (2). The widespread distribution of mice across the globe has been facilitated by their willingness to live in close proximity to humans. Their success as a species can also be attributed to their breeding strategy, resulting in small populations that adapt readily to their surroundings (3). They live in social groups called demes that are composed of a dominant breeding male, a hierarchy of females, subordinate males, and juveniles. This results in a high degree of inbreeding that, combined with their high mutation rates, contributes to their ability to adapt quickly to environmental changes (3, 4). Mice are omnivorous, nocturnal, adapt well to temperature extremes, and with their ability to jump and chew through small openings, they are well poised to take advantage of human food sources in fields, homes, and granaries (5). Although such behaviors prove beneficial for the survival and propagation of the mouse, consumption and contamination of food stores have prompted the view of mice as a pest species. However, hobbyists took an interest in breeding and selling mice with unusual coat colors and behavioral patterns. The breeding of fancy mice in the late nineteenth and early twentieth centuries led to creation of the laboratory mouse, a mixture of all four subspecies that make up the *M. musculus* complex (https://www.jax.org/research-in-action/why-mouse-genetics).

### *Mice in biomedical research: the good, the bad, and the ugly*

The use of mice as laboratory animals began with the study of genetics by scientists in the early twentieth century. Mendelian inheritance was demonstrated in mice by French scientist Lucien Cuenot in 1902 and Clarence Cook Little at Harvard in 1910 (6). Little began creating the first inbred mouse strains (DBA in 1909, C57BL/6 in 1921) to aid in tumor studies and, with Ernest Tyzzer, established basic principles of tissue transplantation (6, 7). Questions surrounding genetics of human cancer spurred the creation of several other inbred strains with different levels of tumor susceptibility, including the C57, C58, and C3H strains. During the mid-twentieth century, the mouse began to play an increasingly important role in immunology research. Mouse models were critical for understanding Ab–Ag interaction, lymphoid differentiation, and the response to infectious agents (6). Use of inbred, congenic, and recombinant congenic mice revealed how polymorphic MHC genes regulate the mammalian immune system (7, 8).

Many key advances in biomedicine and immunology made during the last century may not have been possible without the

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Abbreviations used in this article: SPF, specific pathogen-free.



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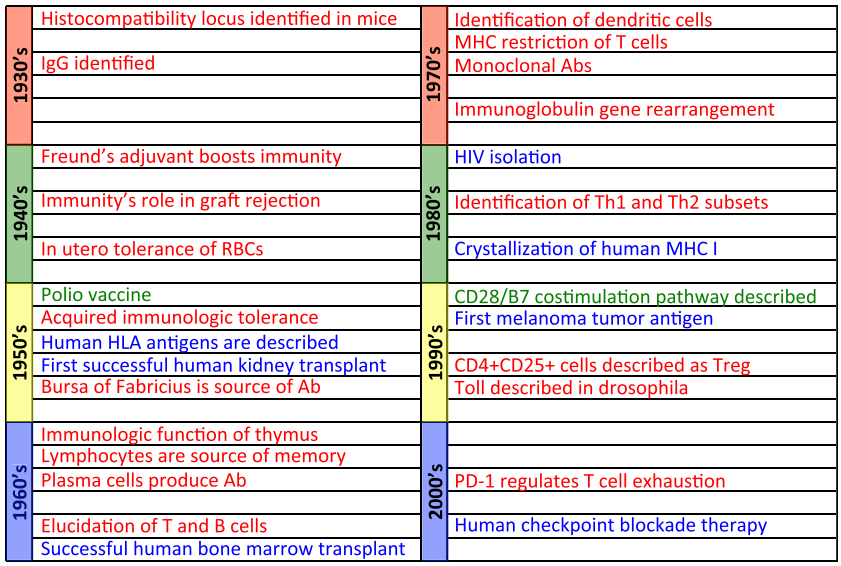


**FIGURE 1.** Landmark discoveries in immunology. Selections from the American Association of Immunologist's timeline. The species or species-derived material with which experiments were principally performed are indicated by color: human (blue), animal (red), or both human and animal (green).

study of animals, in particular mice (Fig. 1). Genetic linkage mapping, genome sequencing, sophisticated strategies for gene manipulation, and the ability to transfer cells from one inbred mouse to another without eliciting immunological rejection all helped accelerate the application of mice for investigation of human diseases and immunology. Mice and humans share >90% of the same genes. Because the genetic content of all mammals is highly homologous, gene discovery in humans can often be predicted in mice and vice versa (9). Not only do mice develop spontaneous mutations, but there now exists the ability to control genetics through breeding and manipulation of the genome via conditional and inducible transgenic and gene knockout strains allowing the study of complex genetic diseases (1, 10, 11). In addition to these important advantages, mice are small, easy to handle and transport, and can be easily maintained in a laboratory setting. They are prolific breeders and have a short generation time, allowing access to large numbers of animals quickly. With short life spans, their entire life cycle and disease processes can be studied during the course of only ~2 y, versus decades in humans.

However, despite considerable homology, there are significant physiological and genetic differences that impede the development of mouse models that capture essential features of human disease (12–15). Although the conclusions have been questioned on methodological grounds, some have proposed that the mouse transcriptional response to inflammatory perturbations poorly mimics that of humans (16–18). Complex human diseases are often tackled in mice with blunt approaches to hasten disease progression or to induce diseases not naturally observed in mice. Such strategies have a mixed track record for inspiring therapies that succeed in the clinic. For instance, atherosclerosis develops poorly in typical mouse strains without employing severe genetic defects, such as knocking out ApoE, which is not a normal feature of afflicted humans (19). Cancer, a very complicated and multifactorial spectrum of diseases, is an example of both the weaknesses and strengths of mouse models: overreliance on a handful of transplantable tumor cell lines has missed critical characteristics of the adaptations employed by slowly evolving human cancers; however, at the same time, studies in mice revealed the roles of the inhibitory receptors that led to checkpoint blockade, a paradigm shift in current immunotherapy (20).

Mouse studies on acute and chronic infectious diseases have proven valuable for understanding the foundations for protective immunity, and mouse models have helped understand ways in which pathogens take the upper hand (such as $CD8^+$ T cell exhaustion in AIDS). Still, species-specific idiosyncrasies of many important pathogens limit the ability of mouse models to recapitulate key aspects of human–pathogen interactions and highly evolved immune defense strategies. Herpes viruses are exquisitely tailored to their host species, precluding direct evaluation of a human pathogen, and even closely related viruses may have developed unique characteristics (such that findings with mouse CMV may not necessarily be predictive for human CMV). Differences in pathophysiology may arise even when mouse models can be established, such as the failure of *Mycobacterium tuberculosis* infection in mice to reproduce key features of human disease, including latency and highly organized granulomas (21).

Consequently, there is the sense that mouse models can misdirect efforts to cure unphysiologic diseases that are intrinsic only to the model, and not the human condition (22). This has led to impressive mouse therapies that have failed to impact the actual disease in humans. This outcome could be blamed on strategic failures in how to design experiments and model systems that minimize limitations while maximizing opportunities for clinical relevance. Nevertheless, the imperfections of current mouse models have led to a growing call to tilt limited immunology research resources away from animals and toward humans themselves (16). Although human studies have and will continue to be essential to the overall immunology discovery program, the perceived advantages of human research must be balanced with its inherent limitations, the chief ones being limited opportunities to derive appropriate samples under suitably controlled conditions. For example, much of human immunology relies on studies of blood, which fails to capture local immune responses and characterize resident lymphocyte and innate immune system components (which likely comprise most of the immune system). Indeed, it is somewhat shocking that careful description of the T cell populations present in diverse normal human tissues

was only recently reported (23, 24). So, although some (by no means all) of the hurdles in human immunology research may be addressed by force of will and ebullient spending, these substantial limitations force the question of whether we should not be too quick to lose faith in mouse immunology research.

Indeed, although the differences between immune responses in mice and humans are reflexively attributed to genetics, lifespan, or the quirks of particular species–pathogen relationships, significant data suggest that environmental differences in laboratory mouse husbandry are also a contributing factor. Whereas mice can forage over a broad temperature range, their nests are typically in a thermoneutral zone of 30–32°C (25), and there is growing concern that laboratory mice are chronically subjected to cold stress (26). This involves an increased basal metabolic rate that is 50–60% higher than mice housed under thermoneutral temperatures, glucocorticoid production, and sustained activation of the sympathetic nervous system (27). This deviation has also been reported to alter responsiveness to LPS, the febrile response, and markedly influences immunological endpoints, including the response to infection and cancer. This has led to the suggestion that simply increasing the temperature in the vivarium to what is most comfortable for mice (rather than their human caregivers) could increase the translation value (for an excellent review, see Ref. 26). Another aspect of the environment in which laboratory mice are raised, and a central focus of this review, is the impact of natural microbial exposure.

### *Specific pathogen-free mouse husbandry*

Early on in the use of laboratory mice, controlling the introduction of pathogens into colonies was difficult. Animals were often housed in wooden cages that were difficult to clean and there were not effective means to identify pathogens (28). Starting in the mid-1900s there has been an increasing focus on identifying strategies to control the spread of pathogens in laboratory animal facilities. It was not until the 1980s that modern filter top microisolation cages became commercially available (29). These systems are still commonly used in animal facilities today.

As immunologists—encouraged by transplant and cancer biologists—increased their use of inbred mice and developed strains with compromised or aberrant immune systems, the pressure grew to develop housing approaches that would allow reliable and reproducible ways to maintain these animals. Pathogens that might have only eliminated some unfortunate individuals in a wild mouse population could decimate a colony of inbred mice. Alternatively, uncharacterized infections could unpredictably alter immune response properties, leading to misinterpretation of phenotype. Without implementation of containment and screening procedures, these genetically well-defined mouse strains might become more of a liability than an asset. The phrase "specific pathogen-free" (SPF) dates to the late 1950s, being used to describe the microbiological status of mouse colonies that are free from a defined list of pathogens that includes exogenous viruses, bacteria, and parasites (30, 31). Use of such barrier housing criteria was in widespread use by the early 1970s (32, 33). Although free from these pathogens, the complete microbiota of SPF colonies may not be known, and SPF mice were clearly recognized as being distinct from germ-free or axenic mice, which were first generated around the same time. The selection of excluded agents, and thus the definition of SPF, can vary considerably from institution to institution, but includes many common pathogens that mice are routinely exposed to in the wild.

Rigorous biocontainment is the most effective tool used to control the introduction of unwanted pathogens into mouse facilities. Mice in SPF colonies are generally housed under barrier conditions. While "barrier" can have many meanings, in many facilities it denotes primary housing in microisolation cages (with filtered tops) or individually vented cages, with both approaches used to prevent the introduction of pathogens into the cage from the environment. Additional steps include use of aseptic technique when handling animals and appropriate disinfection or sterilization of cages, equipment, food, and water that comes in contact with the mice.

To protect existing SPF colony animals, many facilities will not accept direct import of animals from colonies or facilities that have tested positive for an agent on their exclusion list, or they will insist on a lengthy quarantine process or expensive rederivation prior to import. With the routine implementation of these practices, adherence to SPF colony management has become ingrained in the psyche of most cellular immunologists and as a result it is now difficult to appreciate that impact of these policies: specifically, that the laudable goal of protecting vulnerable animals from life-threatening pathogens also means that these animals have a profoundly unphysiological infectious history, making them quite unlike mice, and humans, in the wild.

### *Getting more from the mouse model*

Evidence that microbial experience impacts the immune response of laboratory mice is compelling. At its most extreme, the development of gnotobiotic, or germ-free, mice has revealed the profound impact of commensal organisms on everything from host metabolism to pathogen vulnerability. Gut microbiota correlates with obesity, health status, and metabolic and inflammatory diseases (34–36). Many experimentalists have noted that a key phenotype of a certain mouse strain is no longer reproducible upon changing laboratory locations, or simply by housing their mice in a different room at the same institution. A noted example is the NOD mouse strain, which develops diabetes efficiently in very "clean" animal rooms but shows substantially less disease incidence when colonized by certain commensal microbes (37, 38). Mice purchased from different vendors vary considerably in the presence of Th17 cells within the intestinal mucosa. Littman and colleagues (39) pursued the basis for this discrepancy, elegantly demonstrating that the difference could be attributed to a single member of the gut microbiota.

Intentional pathogen experience in SPF mice resulted in unanticipated yet substantial effects on graft rejection (40), resistance to infection (41, 42), and de novo adaptive immune responses (43–45). Elegant studies comparing healthy monozygotic and dizygotic human twins revealed that variations in cell population frequencies, cytokine responses, and serum proteins were determined not only by genetics, but also substantially a consequence of nonheritable factors, consistent with the interpretation that environmental and microbial exposure drives much of the immune system variation among individuals (46, 47). In summary, our immune systems have

evolved to live in the microbial world we inhabit, and microbial experience profoundly influences steady-state immune function and development of de novo responses.

These observations raise many questions: What are we missing by conducting most mouse experiments under SPF conditions? Are there practicable methods to return laboratory mice to a more physiologic level of microbial experience? If so, would such an endeavor have value?

We recently examined these issues, initially studying feral mice that had completely natural exposure to environmental pathogens, and mice raised in commercial pet stores without barrier housing. In such "dirty" mice, we saw a dramatic increase in the frequency of memory-phenotype $CD8^+$ T cells in both lymphoid and nonlymphoid tissues, with the latter resembling resident memory T cells (48). These characteristics are similar to what is observed in adult humans (e.g., Ref. 49). With the premise that infectious agents drive these changes in immune populations, we also tested whether cohousing pet store animals with laboratory mice led to acquisition of dirty characteristics by the latter group. This was indeed the case: increased numbers of circulating and tissue-resident memory CD8 T cells accompanied colonization of the mice by diverse SPF-designated pathogens (48). These changes were not limited to CD8 T cells: differentiated $CD4^+$ T cell subsets, innate lymphoid cells, and myeloid populations were increased in numbers within various lymphoid and nonlymphoid sites, and serum Igs of diverse isotypes were significantly elevated (48). Again, these characteristics are analogous to immunologically experienced adult humans. Those similarities were quantitatively and qualitatively investigated by comparing gene expression profiles in PBMCs from SPF and dirty mice, compared with neonatal and adult humans. Intriguingly, many gene expression patterns that distinguish adult from neonatal humans were the same as those that distinguished dirty mice from SPF mice (48). Most striking among these was a substantial elevation in expression of genes regulated by type I IFNs in dirty mice and adult humans (compared with SPF mice and neonatal humans, respectively), something that was also seen in SPF mice that were deliberately infected by a series of defined pathogens (48, 50). Taken together, these data suggested that cohoused inbred mice, by having a more physiological exposure to natural mouse pathogens, were a more accurate reflection of the adult human immune system, and hence would be more relevant to translation of mouse immunology studies.

Although use of dirty mice presents opportunities for new dimensions in immunology research, the generation and maintenance of these mice is far from trivial. By our definition, dirty mice harbor numerous pathogens that are excluded from modern SPF animal facilities, and contamination is a serious risk (48). Some pathogens, such as pinworm eggs, remain viable in the environment for long periods of time, and numerous SPF-excluded pathogens are notorious for efficient transmission. Hence, dirty mice must be isolated away from SPF colonies. We achieved this using an animal biosafety level 3 facility, which clearly exceeds the safety level needed for personnel but provides highly effective containment. Other options may be to use a facility in which no other rodent

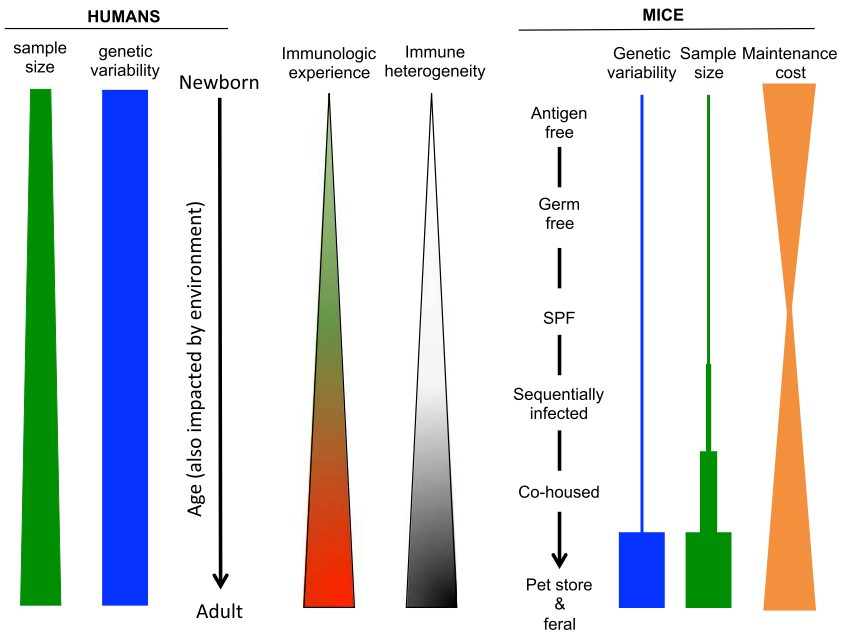


**FIGURE 2.** Practical considerations for research in humans and in mice with varying degrees of immune experience. Humans exhibit significant genetic variability, unlike studies that employ inbred strains of mice. Humans also exhibit significant variability in immunological experience, the effects of which tend to accumulate with age, but also vary by environmental factors. Immune experience in mice can be controlled through diet, sterile derivation, husbandry practices, and sourcing genetically outbred pet store and feral mice from environments outside of biocontainment. These practices impact the cost of mouse studies, sometimes in unexpected ways. For example, whereas feral mice are cheap to obtain, biocontainment in a laboratory setting may be quite expensive (institutional costs will vary and may require animal biosafety level 3 housing to protect SPF vivaria from contamination). In general, increased underlying variability increases the complexity of immunologic assays and the cohort sizes needed to test the specific influence of a chosen variable. Thus, reproducible observations may be discoverable in smaller experimental groups of inbred laboratory mice as compared with outbred organisms with heterogeneous immune experience. This figure does not highlight technical and logistical considerations that often favor mice (e.g., sample availability, longitudinal studies in tissues, sophisticated approaches that require gene manipulation, or a level of invasiveness not permissive in humans), the availability of genetically outbred and outcrossed SPF mice, or the issue that some questions are best addressed in humans (e.g., when the phenomena under investigation is highly species specific or for which appropriate mouse models have not been developed).

colonies are housed, or in a quarantine area located away from regular housing rooms. In all cases, measures need to be in place to minimize the risk of pathogen spread by fomites (such as use of dedicated caging and cage sanitation equipment), directional airflow to reduce airborne spread of pathogens outside of the animal room, and strictly enforced traffic patterns and hygiene practices for personnel who may work with dirty and with SPF mice.

Creating a dirty mouse colony can be done in a number of ways. As discussed, mice obtained from pet stores or captured in the wild are often exposed to multiple bacterial, viral, and parasitic pathogens such as mouse hepatitis virus, mouse parvovirus, mouse encephalitis virus, *Mycoplasma pulmonis*, fur mites, and pinworms. Many of these pathogens establish persistent infections that can be spread to inbred mice by cohousing (48), and some can be transmitted from contaminated bedding (i.e., fomites). This approach introduces immunological experience to laboratory mice with known genetics and is compatible with genetic-dependent experimental approaches such as gene knockout mice. Whether an animal will seroconvert upon exposure depends on dose, agent and age, immune status, genetic composition of the infected animal (51), as well as a substantial element of luck. Accordingly, not all dirty mice will have the same infection profile, and utilizing these methods may result in significant variation between mice. Alternatively, dirty mice can be generated by deliberate infection of SPF mice with a selected series of pathogens. Reese et al. (50) used this approach to inoculate mice with pathogens that model those that commonly infect humans in early childhood (including mouse hepatitis virus 68, murine CMV, and *Heligmosomoides polygyrus*, an intestinal helminth). They found that exposure to these pathogens altered the immune system to partly resemble that of pet store mice. However, selecting the pathogens that best model naturally acquired infections in mice is not simple. Some microbes that might be assumed to be prevalent in wild or pet store mice (such as murine CMV) may be infrequent, and the only microbes that matter for immunological experience may not be those that are on the SPF excluded list. Hence, there are good reasons to advocate for use of both the natural transmission and deliberate infection models of generating dirty mice.

These considerations also raise the question of experimental reproducibility, something that has garnered significant attention within scientific circles as well as the media (12, 14, 52–55). Clearly this may be a concern when dirty mice are generated by natural transmission between animals, as this invites heterogeneity in the timing and nature of infections. This is a multifaceted issue, but two aspects are relevant for this review. The first is that the immune system in SPF mice can be quite dramatically perturbed by changes in the microbiota (such as the effects of segmented filamentous bacteria on frequencies of Th17 in the C57BL/6 mouse gut, and the incidence of diabetes in NOD mice discussed earlier). We would propose that these effects may in fact be magnified because SPF mice have such modest immunological experience, and perhaps more diverse pathogen exposure would, ironically, induce more stability to these phenotypes. Second, taking a further step back perhaps now is the time to question whether standardization is feasible, and, if so, whether it is actually desirable. If the end goal is to robustly model phenomena observed in humans, most of whom have diverse microbial experience, should not our mouse models be similarly diverse? For example, before moving to an expensive clinical trial, it might be reassuring to validate therapeutics in both SPF and dirty mouse models to filter out modalities that are highly sensitive to unique environmental perturbations. In these ways, the dirty mouse model may be seen as a complement rather than any kind of replacement for current studies of the immune system in SPF mice and humans.

## Conclusions

In summary, although expanding studies on human cells and tissues is a laudable objective for immunology research, the considerable benefits offered by the mouse model should secure its place in future work on the basic underpinnings of the immune system (see Fig. 2). That is not to discourage continuance of the longstanding efforts aimed at enhancing the physiological relevance and translatability of mouse models. Normalizing the immunological experience of laboratory mice is one step toward this goal, but it should be viewed as an element in the larger progression to improve the value of mice as a model for the human immune system.

## Disclosures

The authors have no financial conflicts of interest.

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