HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

J Immunol. 2020 July 01; 205(1): 3–11. doi:10.4049/jimmunol.2000171.

New Insights into the Immune System Using Dirty Mice

Sara E. Hamilton[*,†,‡,§], **Vladimir P. Badovinac**[¶,‖,#], **Lalit K. Beura**[**], **Mark Pierson**[*], **Stephen C. Jameson**[*,†,‡,§], **David Masopust**[†,‡,§,††], **Thomas S. Griffith**[†,‡,§,‡‡,§§]

[*]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

[†]Microbiology, Immunology, and Cancer Biology Ph.D. Program, University of Minnesota, Minneapolis, MN

[‡]Center for Immunology, University of Minnesota, Minneapolis, Minnesota

[§]Masonic Cancer Center, University of Minnesota, Minneapolis, MN

[¶]Department of Pathology, University of Iowa, Iowa City, IA

[‖]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA

[#]Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa City, IA

[**]Department of Molecular Microbiology and Immunology, Brown University, Providence, RI

[††]Department of Microbiology and Immunology, University of Minnesota, Minneapolis, MN

[‡‡]Department of Urology, University of Minnesota, Minneapolis, MN

[§§]Minneapolis VA Health Care System, Minneapolis, Minnesota

Abstract

The mouse (*Mus musculus*) is the dominant organism used to investigate the mechanisms behind complex immunological responses, due to their genetic similarity to humans and our ability to manipulate those genetics to understand downstream function. Indeed, our knowledge of immune system development, response to infection, and ways to therapeutically manipulate the immune response to combat disease were in large part delineated in the mouse. Despite the power of mouse-based immunology research, the translational efficacy of many new therapies from mouse to human is far from ideal. Recent data has highlighted how the naïve, neonate-like immune system of specific pathogen-free (SPF) mice differs dramatically in composition and function to mice living under barrier-free conditions (i.e., 'dirty' mice). In this review, we discuss major findings to date and challenges faced when using dirty mice and specific areas of immunology research that may benefit from using animals with robust and varied microbial exposure.

Introduction

Laboratory mouse models represent an undeniable asset in our understanding of immunology and human disease. Mice and humans share >90% genetic homology (1), and

Correspondence: Thomas S. Griffith, University of Minnesota, Department of Urology, MMC Mayo, 420 Delaware St. SE, Minneapolis, MN 55455. Ph: 612-624-8269, Fax: 612-626-0428, tgriffit@umn.edu.

their usage has led to the discovery of a significant number of genes and pathways fundamental for understanding disease processes and designing effective treatment. Additionally, the power to transfer immune cells between hosts, ability to create genetic knockout strains, conditional and inducible deletions, and transgenics are all important advantages to study disease processes within a fairly short timeline. However, many examples now exist whereby use of the laboratory mouse has misdirected clinical approaches, failing to positively impact humans and costing millions of research dollars in the process (2, 3). The question then is how to improve the 'value' of experimental rodent research in order to increase the success rate of translating preclinical findings to the clinic. One option is for experimental immunologists to supplement existing or developing new models with variables known or expected to influence the readout, outcome, and/or conclusions. Unfortunately, it is also fair to note that most of the envisioned improvements will likely increase the complexity, cost, and in some cases limit the number of researchers able to fulfill the new model requirements. Here, we will discuss new insights into immunology research using models based on mice with a history of microbial exposure.

Humans are exposed to pathogenic and commensal microbes on a daily basis from birth, which (together with vaccination) matures the immune system to provide long-lasting protection against future microbial challenge. Immunological memory is minimal in most mice used in biomedical research due to specific pathogen free (SPF) housing conditions, in contrast to the experienced immune systems of wild mice or mice obtained from neighborhood pet stores (hereafter collectively referred to as "dirty" mice (4)). The primary (and most obvious) feature of dirty mice that distinguishes them from traditional SPF laboratory mice is that their exposure to multiple mouse pathogens (which are normally excluded under SPF conditions) was found to mature the murine immune system to more closely resemble that seen in adult humans (4–6). It is now appreciated that the baseline status of the immune system can have significant implications on the cellular composition and intensity of subsequent immune responses. Our group published data outlining the use of dirty mice to evaluate the composition and basic function of the immune system to challenge (compared to SPF mice), but additional work from a number of laboratories has reinforced the concept and value of using dirty mice in preclinical investigation (Table 1 (4–10)). Interestingly, each publication has used slight variations in methodology to generate the dirty mice used for study. The following information will be based on our experience with and data generated using our "pet store mouse cohousing" model, but we will highlight various aspects of the other dirty mouse models used throughout our discussion.

Models of microbially-experienced "dirty" mice

Cohousing model.

To separate genetic and environmental effects on the cellular composition and function of the immune system, we have relied heavily on a model where age-matched cohorts of inbred strains of (initially SPF) laboratory mice are maintained in SPF housing conditions or housed in the same cage with a commercial pet store mouse raised without barrier housing. This system is similar to the one used by Lindner et al., who cohoused SPF mice with wild-caught mice to study how symbiotic host-microbe interactions within the gut modulate

secretory antibody production (11). Regardless of the type of dirty mouse used, the cohousing model permits the direct comparison of the immune systems in the SPF and microbially-experienced genetically-identical, age-matched mice. Variation exists in the variety of microbes the pet store mice carry, and the extent of microbial exposure can in part be measured by serological assays and microbiome sequencing (4, 12). It is important to note that serological assessment primarily detects antibody generated after exposure to a set of well-defined viruses (e.g., parvovirus, MHV, MCMV) and a small number of bacteria, parasites, and fungi. Hence, it is also likely the pet store mice harbor a number of microbes that are not detected by the most commonly used serological tests.

During the first week of cohousing, there is rapid expansion of the T cell compartment along with transition from a predominantly naïve ($CD44^{lo}CD62L^{hi}$) to an activated/memory phenotype ($CD44^{hi}CD62L^{lo}$). The laboratory mice lose ~10% of their body weight during the initial 14 days of cohousing, and we see ~20% mortality rate (mostly within the first 21 days of cohousing; unpublished data and (4)). By 6 weeks, the number of T cells stabilize and the mice do not appear overtly ill. After 60 days of cohousing, 30-80% of the T cells in the blood have an activated/memory phenotype ($CD44^{hi}CD62L^{lo}$), as opposed to 10-15% in SPF mice (4, 12). In addition, the frequency of KLRG1 expressing cells, which exist at only about 1% in SPF mice, increases to 20-40% of the CD8 T cell compartment in most mice after cohousing (4, 12). These features have been fairly reproducible in multiple strains of inbred (i.e., C57BL/6, BALB/c, C3H) and outbred Swiss Webster cohorts of mice (unpublished observations). Additional immunological changes with cohousing include increased numbers of immune cells within nonlymphoid tissues (4), increased IgA repertoire diversity (11), increased circulating inflammatory cytokines and chemokines (12), and increased numbers of neutrophil and macrophage populations in the blood (all compared to SPF mice (12)). Importantly, we have not observed increased expression of markers that indicate exhaustion (e.g., PD-1 or LAG3) on the T cells in cohoused mice, suggesting effector function is likely maintained.

Sequential infection model.

A more regulated and defined way to generate mice with an experienced immune system involves the sequential infection of SPF mice with a panel of known experimental pathogens, such as the model used by Reese et al. where laboratory mice were infected with mouse hepatitis virus, murine cytomegalovirus, influenza, and *H. polygyrus* (9). In contrast to the bolus and likely asynchronous exposure to microbes experienced during cohousing with pet store mice, the controlled sequential infection to a panel of chosen microbes (using experimentally defined doses and routes of infection) greatly improves consistency within and between experiments. Furthermore, the use of known pathogens allows the researcher to draw upon a larger pool of reagents and previously published data specific to each pathogen used to investigate immune system function and interpret results. Sequential infection models using well-established experimental BSL1 and BSL2 pathogens also eliminate the need for special housing facilities (i.e., BSL3 for pet store mice) and procedures to prevent the spread of unknown pathogens. Unfortunately, there is not enough data in hand to know the extent to which infection with a defined, limited set of pathogens (in the absence of the commensal microbe transfer that occurs with cohousing) fully or adequately replicates the

immunological experience of humans. Furthermore, it is difficult to determine what combinations of laboratory-strain pathogens would be appropriate and adequate to achieve the same impact as what occurs with a more diverse and natural microbial exposure history. These caveats being raised, it is still noteworthy that the gene expression profiles of sequentially infected mice do more closely match humans than SPF mice, and many of the phenotypic changes to immune cells noted in the cohousing model were also observed (5).

Natural microbiota transfer model.

Human beings, like other mammals, are colonized by microbes at all epithelial barriers from birth (13). Being the largest body surface exposed to the environmental factors, the gastrointestinal (GI) tract is populated with a myriad of microorganisms (mostly bacteria, fungi, and viruses) that vary along the GI tract because of unique physiologic conditions (14–17). The composition of the gut microbial communities can be altered in a variety of ways, including antibiotic treatment, diet, and co-morbidities associated with disease (18, 19). Work from the last 25 years has unveiled how alterations in the gut microbiome can have profound effects on the function of the systemic immune system (20, 21). Moreover, advances in genomic sequencing technology have given researchers the tools to precisely define the different microbes within the gut. Genetically identical laboratory mice from different vendors have unique gut microbiota (22, 23). Similarly, the bacterial gut microbiome of laboratory mice is significantly different from wild-caught mice (11). Recent work from Rosshart and Rehermann has revealed just how much the wild microbiome can affect host immune fitness (10). Transfer of the natural commensal microbes from wild mice to laboratory mice, either by gavage or naturally from mother to offspring, created a matured host circulating immune system that conferred greater resistance to infection. Interestingly, C57BL/6 mice born from pseudo-pregnant wild female mice (a.k.a. “wildling” mice) replicated human immune responses when used in preclinical models that failed clinically. The need for capturing wild mice and performing embryo transfers into pseudo-pregnant females can make this model somewhat more complex than others, but the fact that the natural microbiota are stable over numerous generations suggests commensal transfer from wild mother to “wildling” offspring is sufficient to obtain a phenotypically and functionally more mature immune system. In a related model, Graham and colleagues simply transferred laboratory mice to an outdoor enclosure that exposed the mice to soil, vegetation, and weather in a more natural habitat (24–26). These “rewilded” mice showed maturation/differentiation of the T cell compartment, increases in circulating granulocytes, and alterations in gut microbiota similar to what has been observed in other dirty mouse models. Interestingly, the increase in granulocytes related to increased gut colonization by several fungal taxa (most notably *Aspergillus* species) (26). It is unclear it the gut microbiota changes that occur during “rewilding” will persist between generations, as seen in the “wildling” model. Moreover, construction of the outdoor facility may be beyond the means for some. Regardless, the compelling data generated using the “wildling” or “rewilding” models further demonstrate the increased similarity between microbially-experienced mice with humans and the power of using them in “transitional-translational” studies.

Challenges in using dirty mice

Housing.

Despite the physiological benefits of using dirty mice to study the immune system, there are several challenges to using a mouse model with varied microbial experience. The first is logistical, in that most facilities that house mice for research work diligently to exclude select pathogens (e.g., mouse hepatitis virus, *Mycoplasma pulmonis*, *Clostridium piliforme*, and *Enchephalotozoon cuniculi*) normally present in the wild. The desire to restrict/eliminate the microbial contaminants commonly found in many institutional animal facilities was initiated in the 1960's to increase the reproducibility of the data generated (e.g., within groups in a single experiment, experiment-to-experiment, and institution-to-institution) and limit the number of unknown experimental variables, and the call for improved cleanliness has only intensified over time (27). Ways in which to overcome this logistical hurdle include housing dirty mice in barrier-free facilities at distant locations, creating new outdoor spaces (24), or increasing the containment of dirty mice by working at BSL3 level. The main reason for having such elaborate housing for the dirty mice, as well as restrict the traffic pattern of the investigator (i.e., enter SPF housing before interacting with the dirty colony on the same day) is to prevent the spread of any microbes to the normal barrier facility. These housing restrictions frequently create new financial hurdles for researchers – necessitating additional internal or external support to subsidize the work. In addition, some institutions may not have or be less willing to work with the researcher to create suitable space to establish a colony of this kind. Alternatively, outdoor housing (24) eliminates the needs for a BSL3 facility, but sufficient space and enclosure specifications must be available to ensure animal health and safety.

Analysis of male mice.

Sex as a biological variable is becoming increasingly appreciated as a critical factor in both basic immunology research and preclinical studies. Certain areas of research can easily justify using a single sex of mouse for experiments, such as the exclusive use of male or female mice for prostate or ovarian cancer research, respectively. Most other cancers or infectious diseases strike both males and females, driving the push to perform experiments in mice of both sexes to better capture the impact of sex differences on physiology. While the incorporation of mice of both sexes can be easily done using the sequential infection or microbiota transfer ('wildling') models of dirty mice, one difficulty of using the cohousing model is the incompatibility (i.e., fighting) between laboratory and pet store male mice. To overcome this obstacle, we have found that exposing laboratory male mice to the dirty bedding ("fomites") from pet store mice achieves the main goal of maturing the immune systems in male laboratory mice through natural microbial exposure. There are several advantages associated with using fomites to make dirty male mice. From our experience, SPF mice exposed to fomites rarely lose weight, die spontaneously, or develop conditions that require euthanasia (as we have seen with cohousing; unpublished data). Bedding from a single cage of 4-5 pet store mice can provide enough fomites to convert 10-12 small or 6-8 large cages of mice. Fomites also allow for more consistent and uniform exposure (even over several different cages) with a reliable transfer of pathogens. Despite these strengths, we have found the efficiency and effectiveness of fomites is not as robust as with cohousing.

Fomite-generating pet store mice lose their ability to effectively passage microbes over time, which necessitates additional pet store mice and can risk a higher percentage of poorly generated dirty mice. The intensity of conversion (i.e., frequency of effector/memory T cells in the blood) is more erratic than with cohousing (unpublished data), and the variety of pathogens transferred is slightly more limited (Table 2). An alternative approach to fomite exposure would use cohousing with breeding inbred mice to get dirty male and female offspring, which get exposed *in utero* and beyond to various commensal and pathogenic microbes; however, we do not yet have a full account of whether this approach would achieve the same phenotypic and functional changes within the immune system as seen in adult cohoused mice or the multi-generational effects seen in the natural microbiota transfer (‘wildling’) model (10).

Lack of control.

Perhaps the biggest challenge to using dirty mice is becoming comfortable with the uncontrolled nature of “natural” microbial exposure. In addition to the variation in pathogenic and commensal microbes present in wild and pet store mice, not all microbes are efficiently transmitted to cage partners whereas others are more frequently transferred. This variability creates a mixture of exposure for each individual mouse, even within the same cage. Moreover, we are unable to identify every microbe the laboratory mouse has encountered during cohousing with pet store mice using the most robust assays currently available and seroconversion is dependent on a number of factors (e.g., dose, pathogen, and age and genetic composition of the infected animal). Some may view these unknowns as an affront to our scientific instinct to control variables and attempt to discredit the validity of the model, but it can simultaneously be argued that this variability is a more realistic representation of the differential exposure humans encounter by living in the natural environment. Despite this variability, we do not find excessively large numbers of mice per experimental group (5-10 cohoused vs. 3-5 SPF mice) are needed to achieve statistical significance.

Research areas that benefit from the use of dirty mice

Data generated to date suggest the immune system of dirty mice is a more accurate model of the adult human immune system (4, 6, 9–12, 24). Consequently, dirty mice are now recognized as an important tool for improving the expediency of translational research. It is important to emphasize, however, that much work still needs to be done to rigorously show the translational applicability of the dirty mouse model, as well as its breadth within immunology. One immediate extension of future dirty mouse models will include settings in which work with inbred strains of mice will be supplemented with outbred cohorts (or Collaborative Cross (CC)) mice to more accurately reflect the variations in immune outcomes in the genetically diverse human population. However, the cohousing approach may not feasible given that the CC mice consist of a large number of genetically different mice. Rather, SPF CC lines should be initially screened to define a particular phenotype/function/gene locus of interest (28) before moving a specific CC mouse line into a dirty environment. The following sections highlight areas of research which seem particularly pertinent to test how the features of dirty mice echo observations in humans. We will also

consider how this model could best be leveraged to improve vaccines, medications, and treatments for diseases and conditions that have not been adequately revealed through research in SPF mice.

Vaccine Efficacy.

Infectious history clearly impacts subsequent immune responses even to discordant infections. Twin studies show immune system variability is dictated in large part by acquired, and not only genetic, factors (29). Furthermore, chronic viral infections are prevalent in humans which stimulate ongoing immune responses (30). Aside from antigen-specific immunity, increased numbers of antigen-experienced immune cells can improve antigen-independent 'alarm responses', which are a potent mechanism of early pathogen detection and amplification of immune responses to control infection (31, 32). However, extensive immune experience does not always predict improved immune reactivity. Vaccines are frequently less efficacious in individuals from developing countries where the number of previous microbial encounters is increased compared to people in developed areas of the world (33–35). The reasons behind the depressed vaccine-induced response are not fully understood and (as expected) difficult to tease apart in humans.

Despite the accepted influence of previous and ongoing microbial exposures on the immune system, the SPF mouse continues to be a common initial model used in vaccine development. Some investigators have attempted to deal with the issue of an ongoing infection by challenging SPF mice with a single pathogen (typically establishing a chronic viral or parasite infection) followed by the initiation of a new immune response (30, 36–39). These studies have revealed important changes in both innate and adaptive responses dealt by ongoing infection, but are limited by the singularity of the exposure. The use of dirty inbred (and potentially outbred) mice may reveal additional, hitherto unappreciated, differences in the response to vaccines, perhaps highlighting pathways to improved adjuvants or methods of vaccination that are more likely to be successful. Since dirty mice harbor vastly increased numbers of resident memory T cells (similar to humans) (4), they may represent a more relevant animal model for the testing of vaccines aimed at establishing memory cells in barrier tissues, which is likely to be critical for diseases like HIV, tuberculosis, and influenza.

In the realm of vaccine development/testing, the use of mice offers researchers numerous advantages. For basic investigation, vaccine testing in mice eliminates the expense and time constraints needed for the GMP manufacture, FDA approval, and monitoring that would be needed for human application. Interrogation of the murine immune system during vaccine testing can be done at great depth, where it is possible to perform high-throughput analysis of multiple time points and tissues that may contain the critical immune cell populations involved in the protective response. Moreover, studies that rely on gene manipulation (e.g., use of knock-out or reporter mice) give the researcher the opportunity to mechanistically dissect the vaccine response in mice. Challenge studies designed to test vaccine effectiveness are much more feasible in the mouse, though it is possible to conduct such investigation in humans. Despite these advantages, vaccine testing in humans must still be done and the advanced technologies available in the current "-omics" era are making human vaccine

testing more feasible. There are human vs. mouse species differences that have nothing to do with microbial experience, such as species-specific tropisms, that can affect vaccine potency and help to justify human vaccine testing. The addition of dirty mice to the vaccine toolbox will hopefully advance the testing possibilities prior to human evaluation.

Trained immunity.

Host immunity has been classically divided into two arms – the quick-acting, antigen-independent innate response and the slower, but antigen-specific, adaptive response. Moreover, it was long thought that only adaptive immunity was able to establish memory. Data from a variety of organisms – ranging from plants to invertebrates to mice and humans – now clearly demonstrate, however, that prior microbial exposure can also 'train' innate immune cells (e.g., neutrophils, monocytes/Mϕ, and NK cells) to exhibit memory and mediate resistance to secondary challenges (40, 41). Dirty mice exhibit increases in neutrophils, monocytes/Mϕ, and NK cells, as well as increased expression on Toll-like receptors on these populations (10, 12). It remains to be determined whether these increases are driven by pathogenic infection or commensal colonization (or both), but they suggest these populations have the capacity to respond more vigorously to subsequent challenges. To complement the current models using BCG or β-glucan (42), it would be intriguing to use dirty mice to investigate some of the under-investigated areas in trained immunity research. For example, dirty mice could be used to better define the role of the adaptive immune system in the generation and maintenance of memory within innate immune cells, because of the significant increase in mature, antigen-experienced T cells in dirty mice. Furthermore, dirty mice can be used to help delineate the temporal, cellular, metabolic, and epigenetic dynamics of innate memory responses.

Sepsis.

Sepsis is a significant public health burden, striking over 1 million Americans annually (43–45). Sepsis was recently redefined as a 'life-threatening organ dysfunction caused by a dysregulated host response to infection' (46). Early stages of sepsis are marked by hyperinflammation driven by proinflammatory cytokines (i.e., IL-1β, IL-6, IFNγ, and TNF) (47–49). Mouse models of sepsis have been used to define and understand the pathophysiological complications that develop during a septic event (50, 51), as well as developing therapeutic modalities to counteract the profound immunological changes that contribute to sepsis-induced mortality (47, 52, 53). We have been interested in studying a variety of immunological parameters altered in the septic host (54–63), and (like most sepsis researchers) have relied on SPF mice for these experiments. Unfortunately, very few of the pharmacological therapies demonstrating efficacy in preclinical sepsis models have shown similar success in humans, resulting in significant scrutiny of mouse models of sepsis. While SPF housing of laboratory mice has been instrumental in increasing experimental reproducibility in the investigation of sepsis-driven immunoparalysis, it has simultaneously further distanced the mouse as a model from humans largely because SPF mice live their lives with limited exposure to mouse pathogens (64). There have been a handful of reports using humanized NOD SCID γC (NSG) mice to evaluate the acute changes in leukocytes and circulating cytokines/chemokines after sepsis induction (65–70), but species incompatability between some cytokines and their receptors, absence of HLA molecules on

host antigen-presenting cells, and potential for low levels of hematopoietic cell engraftment, human immune cell development and function have limited the ability of humanized mice to faithfully replicate keys aspects of the human immune system (71–73).

Data obtained from cohoused mice after systemic infection with virulent *Listeria monocytogenes* or malaria suggest microbially-experienced hosts can more efficiently control subsequent infections compared to SPF mice (4). With this increased resistance to systemic experimental infection, we used the cecal ligation and puncture (CLP) model of sepsis (74) to study the acute immune response to a systemic polymicrobial infection by gut commensals, expecting cohoused mice to be more resistant than SPF mice. Even though the SPF and cohoused mice had equivalent bacteremia after CLP, the cohoused mice exhibited more severe weight loss and increased acute mortality after sepsis induction. The increased morbidity/mortality in cohoused mice after CLP correlated with exaggerated acute hyperinflammation, as seen by increased serum IL-1β, IL-6, IFNγ, and TNF post-CLP. IL-6 levels in septic humans correlate with disease severity and outcomes (75, 76), while TNF and IL-1β are primary mediators of inflammation-induced activation of coagulation (77) during sepsis. Administration of Ceftriaxone (50 mg/kg) and Metronidazole (30 mg/kg) (78) at 12 and 24 h after CLP did not improve survival of cohoused mice, suggesting that once the hyperinflammatory response is triggered the presence of the inducing microbes becomes less important. Similar outcomes were observed when we used a cecal slurry or LPS endotoxemia model of sepsis. As part of the data published recently by Rosshart et al., the therapeutic benefit of anti-TNF treatment during septic shock was tested in SPF laboratory and dirty wildling mice (10). Prophylactic administration of anti-TNF mAb was able to protect SPF laboratory mice from lethal LPS endotoxemia, but this therapy failed to rescue the wildlings. These data were consistent with clinical results demonstrating the inability of anti-TNF therapy to improve survival in septic shock patients (79). However, it is important to note the anti-TNF mAb was administered to the mice prior to LPS challenge whereas patients were given anti-TNF therapy after sepsis onset. Thus, while the data with the LPS-treated wildling mice suggest they phenocopy sepsis patients in regard to their lack of response to TNF neutralization, these data highlight the potential power of using microbially-experienced mice as a transitional step between traditional SPF mice and humans in the evaluation of immunomodulatory therapeutics designed to lessen the cytokine storm and immune suppression induced by sepsis, with the intention of improving patient outcomes.

Future research directions

It is likely that additional areas of immunology research will benefit from using dirty mice in the future. While the list of future research directions with dirty mice is unlimited, the following areas are just a few of the many where preliminary pieces of data are in hand that demonstrate how the microbial experience history of the host can potentially impact the way the immune system responds.

Tumorogenesis and immunotherapy.

In the last 10-15 years, tumor immunology has experienced an unprecedented explosion in research and the development of improved treatments. Immune checkpoint therapy has revolutionized the way cancer can be treated, and it is clear these current (and future) means of treating cancer have strong foundations in preclinical mouse modeling. Despite these successes, many current mouse models are still heavily scrutinized because they can be poor at modeling tumor control, the impact of immunotherapy, and the adverse events related to therapy that can result in humans. The use of humanized immunodeficient mice bearing human tumors (either patient-derived xenografts or human tumor cell lines) has given cancer researchers another model for examining the therapeutic benefit of various drugs under development, but these models have their limitations, including the lack of function of some mouse cytokines on human immune cell development and function, the possibility of xenogeneic graft-vs-host disease, and presence of host innate immunity. Recent data has also highlighted the potential of harnessing the immunological power in pathogen-specific tissue resident memory T cells to recognize and eliminate tumors (80–86). Inclusion of dirty mice in the preclinical repertoire of reagents used to study tumor formation and/or de novo or therapy-driven immune responses to tumors may give researchers new insight into the dynamic tumor/immune system relationship seen in adult humans with experienced immune systems (87, 88). The addition of dirty mice into tumor immunology may be especially useful with agents designed to stimulate adaptive immune responses, as T cell differentiation or the composition and/or magnitude of the cytokine response may be quite different from that seen in traditional SPF laboratory mice.

The hygiene hypothesis and responses to allergens.

Considerable attention has been given to the concept that the increased incidence of allergen sensitivity (including allergic asthma) in industrialized nations reflects more limited exposure to pathogens, as a consequence of improved public health standards and efficient vaccination programs. Some epidemiological and experimental evidence supports this “hygiene hypothesis” (89, 90), but it is still unclear to what extent the low incidence of allergic diseases in some populations reflects the impact of diverse microbial experience versus exposure to a specific set of microbial factors. Most preclinical studies in mice investigate how individual microbes or microbial products affect the response to allergens in SPF mice, making it hard to extrapolate whether natural exposure to a diverse population of pathogens (as experienced by nearly all humans and modelled in dirty mice) does or does not affect the incidence or severity of allergic/asthmatic responses. In addition, it remains to be determined whether responses to allergens in these populations are simply inhibited or instead are diverted into distinct forms of immune reactivity that do not prompt typical allergic/asthmatic diseases (91, 92). It will be interesting to see to what extent and how the broad microbial exposure experienced by dirty mice alters the immunological detection of and response to allergens.

Aging.

Normal aging results in both intrinsic changes to the immune system (e.g., thymic involution and loss of naive T cell production) as well as accumulating exposures to vaccines and

microbes. These amassed changes frequently result in immune disorders related to either immunosenescence (an insufficient immune response) or inflammaging (an exacerbated immune response) (93). Certain populations, such as HIV-infected people, show early onset of age-related chronic disease and geriatric symptoms that appear unrelated to a specific microbial exposure, suggesting the effects of aging could be accelerated or compounded with some chronic infections (94, 95). Several commonalities have been noted between dirty mice and aged SPF mice, such as persistent, systemic inflammation, elevated numbers of memory T and B cells, exacerbated septic responses, and upregulation of senescence markers like KLRG1 (96). The increased number of senescent cells found in aged mouse models can experimentally lead to a number of age-related pathologies (97). The recent development of senolytic drugs to eliminate senescent cells is an area of active research (98), and it would be useful to test these clinical compounds in dirty mouse models as a test of efficacy. Whether there are conserved pathways between the ageing immune system and the experienced one, or these are two completely independent processes, is an important line of investigation to be considered moving forward.

Neurodegenerative diseases.

Over the last decade, it has become increasingly appreciated that most neurodegenerative diseases including Alzheimer's disease, Amyotrophic Lateral Sclerosis, and Parkinson's disease stems from inflammatory processes in the central nervous system (CNS) and the host immune system is intimately involved in the disease pathogenesis (99). Various CNS-resident and -recruited immune cells directly participate in these neuroinflammatory events. While the cause for these highly complex pathologic processes are likely multifactorial, there are several reports of evidence of viral and bacterial involvement (100–102). However, most preclinical animal model studies of neurodegenerative diseases continue to use transgenic mice reared in the ultra-hygienic SPF conditions. Although these studies have provided important information regarding basic aspects of disease process and changes in the immune system, therapies designed to mitigate immunopathogenesis have yet to translate to humans even though they were efficacious in SPF mouse-based preclinical studies. Inclusion of more microbially enriched dirty environments for rearing various transgenic mouse models has the potential to better recapitulate the disease process as well as successful therapy design.

Conclusions

The use of dirty mice has advanced our understanding of the immune system and moved us closer to replicating human immune experience. Many more studies will be needed, in multiple models of infection, cancer, and other immune perturbations to comprehensively learn the unique features of these mice. Incorporating outbred or Collaborative Cross dirty mice in the future will also reflect variation of immune outcomes in diverse populations and potentially uncover underlying genetic causes. Critically, more studies are needed directly comparing data from humans given vaccines or therapies alongside dirty and traditionally used SPF mice to determine if the dirty mouse model will consistently lead us to more accurate conclusions about human immune responses.

Acknowledgments

Supported by National Institutes of Health Grants AI116678 (S.E.H.), GM134880 (V.P.B.), AI147064 (V.P.B.), AI084913 (D.M.), GM115462 (T.S.G.), and a Veterans Administration Merit Review Award (I01BX001324; T.S.G.). This work was also supported in part by NIH P30 CA77598 utilizing the Masonic Cancer Center, University of Minnesota Flow Cytometry shared resource.

REFERENCES

1. Mural RJ, Adams MD, Myers EW, Smith HO, Miklos GL, Wides R, Halpern A, Li PW, Sutton GG, Nadeau J, Salzberg SL, Holt RA, Kodira CD, Lu F, Chen L, Deng Z, Evangelista CC, Gan W, Heiman TJ, Li J, Li Z, Merkulov GV, Milshina NV, Naik AK, Qi R, Shue BC, Wang A, Wang J, Wang X, Yan X, Ye J, Yooseph S, Zhao Q, Zheng L, Zhu SC, Biddick K, Bolanos R, Delcher AL, Dew IM, Fasulo D, Flanigan MJ, Huson DH, Kravitz SA, Miller JR, Mobarry CM, Reinert K, Remington KA, Zhang Q, Zheng XH, Nusskern DR, Lai Z, Lei Y, Zhong W, Yao A, Guan P, Ji RR, Gu Z, Wang ZY, Zhong F, Xiao C, Chiang CC, Yandell M, Wortman JR, Amanatides PG, Hladun SL, Pratts EC, Johnson JE, Dodson KL, Woodford KJ, Evans CA, Gropman B, Rusch DB, Venter E, Wang M, Smith TJ, Houck JT, Tompkins DE, Haynes C, Jacob D, Chin SH, Allen DR, Dahlke CE, Sanders R, Li K, Liu X, Levitsky AA, Majoros WH, Chen Q, Xia AC, Lopez JR, Donnelly MT, Newman MH, Glodek A, Kraft CL, Nodell M, Ali F, An HJ, Baldwin-Pitts D, Beeson KY, Cai S, Carnes M, Carver A, Caulk PM, Center A, Chen YH, Cheng ML, Coyne MD, Crowder M, Danaher S, Davenport LB, Desilets R, Dietz SM, Doup L, Dullaghan P, Ferriera S, Fosler CR, Gire HC, Gluecksmann A, Gocayne JD, Gray J, Hart B, Haynes J, Hoover J, Howland T, Ibegwam C, Jalali M, Johns D, Kline L, Ma DS, MacCawley S, Magoon A, Mann F, May D, McIntosh TC, Mehta S, Moy L, Moy MC, Murphy BJ, Murphy SD, Nelson KA, Nuri Z, Parker KA, Prudhomme AC, Puri VN, Qureshi H, Raley JC, Reardon MS, Regier MA, Rogers YH, Romblad DL, Schutz J, Scott JL, Scott R, Sitter CD, Smallwood M, Sprague AC, Stewart E, Strong RV, Suh E, Sylvester K, Thomas R, Tint NN, Tsonis C, Wang G, Wang G, Williams MS, Williams SM, Windsor SM, Wolfe K, Wu MM, Zaveri J, Chaturvedi K, Gabrielian AE, Ke Z, Sun J, Subramanian G, Venter JC, Pfannkoch CM, Barnstead M, and Stephenson LD. 2002 A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. Science 296: 1661–1671. [PubMed: 12040188]

2. Mak IW, Evaniew N, and Ghert M. 2014 Lost in translation: animal models and clinical trials in cancer treatment. Am. J. Transl. Res 6: 114–118. [PubMed: 24489990]

3. Wong CH, Siah KW, and Lo AW. 2019 Estimation of clinical trial success rates and related parameters. Biostatistics 20: 273–286. [PubMed: 29394327]

4. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, Thompson EA, Fraser KA, Rosato PC, Filali-Mouhim A, Sekaly RP, Jenkins MK, Vezys V, Haining WN, Jameson SC, and Masopust D. 2016 Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature 532: 512–516. [PubMed: 27096360]

5. Japp AS, Hoffmann K, Schlickeiser S, Glauben R, Nikolaou C, Maecker HT, Braun J, Matzmohr N, Sawitzki B, Siegmund B, Radbruch A, Volk HD, Frentsch M, Kunkel D, and Thiel A. 2017 Wild immunology assessed by multidimensional mass cytometry. Cytometry A 91: 85–95. [PubMed: 27403624]

6. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, Hickman HD, McCulloch JA, Badger JH, Ajami NJ, Trinchieri G, Pardo-Manuel de Villena F, Yewdell JW, and Rehermann B. 2017 Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell 171: 1015–1028 e1013. [PubMed: 29056339]

7. Huggins MA, Jameson SC, and Hamilton SE. 2019 Embracing microbial exposure in mouse research. J. Leukoc. Biol 105: 73–79. [PubMed: 30260516]

8. Masopust D, Sivula CP, and Jameson SC. 2017 Of Mice, Dirty Mice, and Men: Using Mice To Understand Human Immunology. J. Immunol 199: 383–388. [PubMed: 28696328]

9. Reese TA, Bi K, Kambal A, Filali-Mouhim A, Beura LK, Burger MC, Pulendran B, Sekaly RP, Jameson SC, Masopust D, Haining WN, and Virgin HW. 2016 Sequential Infection with Common Pathogens Promotes Human-like Immune Gene Expression and Altered Vaccine Response. Cell host & microbe 19: 713–719. [PubMed: 27107939]

10. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, McCulloch JA, Anastasakis DG, Sarshad AA, Leonardi I, Collins N, Blatter JA, Han SJ, Tamoutounour S, Potapova S, Foster St Claire MB, Yuan W, Sen SK, Dreier MS, Hild B, Hafner M, Wang D, Iliev ID, Belkaid Y, Trinchieri G, and Rehermann B. 2019 Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science 365: eaaw4361. [PubMed: 31371577]

11. Lindner C, Thomsen I, Wahl B, Ugur M, Sethi MK, Friedrichsen M, Smoczek A, Ott S, Baumann U, Suerbaum S, Schreiber S, Bleich A, Gaboriau-Routhiau V, Cerf-Bensussan N, Hazanov H, Mehr R, Boysen P, Rosenstiel P, and Pabst O. 2015 Diversification of memory B cells drives the continuous adaptation of secretory antibodies to gut microbiota. Nat. Immunol 16: 880–888. [PubMed: 26147688]

12. Huggins MA, Sjaastad FV, Pierson M, Kucaba TA, Swanson W, Staley C, Weingarden AR, Jensen IJ, Danahy DB, Badovinac VP, Jameson SC, Vezys V, Masopust D, Khoruts A, Griffith TS, and Hamilton SE. 2019 Microbial Exposure Enhances Immunity to Pathogens Recognized by TLR2 but Increases Susceptibility to Cytokine Storm through TLR4 Sensitization. Cell Rep. 28: 1729–1743 e1725. [PubMed: 31412243]

13. Levy O 2007 Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat. Rev. Immunol 7: 379–390. [PubMed: 17457344]

14. O'Hara AM, and Shanahan F. 2006 The gut flora as a forgotten organ. EMBO Rep. 7: 688–693. [PubMed: 16819463]

15. Berg RD 1996 The indigenous gastrointestinal microflora. Trends Microbiol. 4: 430–435. [PubMed: 8950812]

16. Ruan W, Engevik MA, Spinler JK, and Versalovic J. 2020. Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration. Dig. Dis. Sci 65: 695–705. [PubMed: 32067143]

17. Sender R, Fuchs S, and Milo R. 2016 Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 14: e1002533. [PubMed: 27541692]

18. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, and Knight R. 2009 Bacterial community variation in human body habitats across space and time. Science 326: 1694–1697. [PubMed: 19892944]

19. De Filippo C, and Lionetti P. 2013 Impact of diet on gut microbiota in the globalized world. Functional Food Reviews 5: 13–22.

20. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, and Littman DR. 2009 Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139: 485–498. [PubMed: 19836068]

21. Villarino NF, LeCleir GR, Denny JE, Dearth SP, Harding CL, Sloan SS, Gribble JL, Campagna SR, Wilhelm SW, and Schmidt NW. 2016 Composition of the gut microbiota modulates the severity of malaria. Proc. Natl. Acad. Sci. U. S. A 113: 2235–2240. [PubMed: 26858424]

22. Rasmussen TS, de Vries L, Kot W, Hansen LH, Castro-Mejia JL, Vogensen FK, Hansen AK, and Nielsen DS. 2019 Mouse Vendor Influence on the Bacterial and Viral Gut Composition Exceeds the Effect of Diet. Viruses 11: 435.

23. Robertson SJ, Lemire P, Maughan H, Goethel A, Turpin W, Bedrani L, Guttman DS, Croitoru K, Girardin SE, and Philpott DJ. 2019 Comparison of Co-housing and Littermate Methods for Microbiota Standardization in Mouse Models. Cell Rep. 27: 1910–1919 e1912. [PubMed: 31067473]

24. Leung JM, Budischak SA, Chung The H, Hansen C, Bowcutt R, Neill R, Shellman M, Loke P, and Graham AL. 2018 Rapid environmental effects on gut nematode susceptibility in rewilded mice. PLoS Biol. 16: e2004108. [PubMed: 29518091]

25. Lin JD, Devlin JC, Yeung F, McCauley C, Leung JM, Chen YH, Cronkite A, Hansen C, Drake-Dunn C, Ruggles KV, Cadwell K, Graham AL, and Loke P. 2020 Rewilding Nod2 and Atg16l1 Mutant Mice Uncovers Genetic and Environmental Contributions to Microbial Responses and Immune Cell Composition. Cell Host Microbe.

26. Yeung F, Chen YH, Lin JD, Leung JM, McCauley C, Devlin JC, Hansen C, Cronkite A, Stephens Z, Drake-Dunn C, Fulmer Y, Shopsin B, Ruggles KV, Round JL, Loke P, Graham AL, and

Cadwell K. 2020 Altered Immunity of Laboratory Mice in the Natural Environment Is Associated with Fungal Colonization. Cell Host Microbe.

27. Lane-Petter W 1962 Provision of pathogen-free animals. Proc. R. Soc. Med 55: 253–256. [PubMed: 14462027]
28. Martin MD, Sompallae R, Winborn CS, Harty JT, and Badovinac VP. 2020. Diverse CD8 T cell responses to viral infection revealed by the Collaborative Cross. Cell Reports In press.
29. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, Shen-Orr S, Dekker CL, Swan GE, Butte AJ, Maecker HT, and Davis MM. 2015 Variation in the human immune system is largely driven by non-heritable influences. Cell 160: 37–47. [PubMed: 25594173]
30. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, and Virgin H. W. t. 2007 Herpesvirus latency confers symbiotic protection from bacterial infection. Nature 447: 326–329. [PubMed: 17507983]
31. Chu T, Tyznik AJ, Roepke S, Berkley AM, Woodward-Davis A, Pattacini L, Bevan MJ, Zehn D, and Prlic M. 2013 Bystander-activated memory CD8 T cells control early pathogen load in an innate-like, NKG2D-dependent manner. Cell Rep. 3: 701–708. [PubMed: 23523350]
32. Schenkel JM, Fraser KA, Vezys V, and Masopust D. 2013 Sensing and alarm function of resident memory CD8(+) T cells. Nat. Immunol 14: 509–513. [PubMed: 23542740]
33. French N, Nakiyingi J, Carpenter LM, Lugada E, Watera C, Moi K, Moore M, Antvelink D, Mulder D, Janoff EN, Whitworth J, and Gilks CF. 2000 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. Lancet 355: 2106–2111. [PubMed: 10902624]
34. Gruber JF, Hille DA, Liu GF, Kaplan SS, Nelson M, Goveia MG, and Mast TC. 2017 Heterogeneity of Rotavirus Vaccine Efficacy Among Infants in Developing Countries. Pediatr. Infect. Dis. J 36: 72–78. [PubMed: 27755463]
35. Stelekati E, and Wherry EJ. 2012 Chronic bystander infections and immunity to unrelated antigens. Cell Host Microbe 12: 458–469. [PubMed: 23084915]
36. Crosby EJ, Goldschmidt MH, Wherry EJ, and Scott P. 2014 Engagement of NKG2D on bystander memory CD8 T cells promotes increased immunopathology following Leishmania major infection. PLoS Pathog. 10: e1003970. [PubMed: 24586170]
37. Lin JS, Mohrs K, Szaba FM, Kummer LW, Leadbetter EA, and Mohrs M. 2019 Virtual memory CD8 T cells expanded by helminth infection confer broad protection against bacterial infection. Mucosal Immunol. 12: 258–264. [PubMed: 30361537]
38. Rolot M, Dougall AM, Chetty A, Javaux J, Chen T, Xiao X, Machiels B, Selkirk ME, Maizels RM, Hokke C, Denis O, Brombacher F, Vanderplasschen A, Gillet L, Horsnell WGC, and Dewals BG. 2018 Helminth-induced IL-4 expands bystander memory CD8(+) T cells for early control of viral infection. Nat. Commun 9: 4516. [PubMed: 30375396]
39. Stelekati E, Shin H, Doering TA, Dolfi DV, Ziegler CG, Beiting DP, Dawson L, Liboon J, Wolski D, Ali MA, Katsikis PD, Shen H, Roos DS, Haining WN, Lauer GM, and Wherry EJ. 2014 Bystander chronic infection negatively impacts development of CD8(+) T cell memory. Immunity 40: 801–813. [PubMed: 24837104]
40. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, and Xavier RJ. 2016 Trained immunity: A program of innate immune memory in health and disease. Science 352: aaf1098. [PubMed: 27102489]
41. Netea MG, Quintin J, and van der Meer JW. 2011 Trained immunity: a memory for innate host defense. Cell Host Microbe 9: 355–361. [PubMed: 21575907]
42. Song WM, and Colonna M. 2018 Immune Training Unlocks Innate Potential. Cell 172: 3–5. [PubMed: 29328917]
43. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M, and Sepsis Definitions Task F. 2016 Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama 315: 775–787. [PubMed: 26903336]
44. Vincent JL, Marshall JC, Namendys-Silva SA, Francois B, Martin-Loeches I, Lipman J, Reinhart K, Antonelli M, Pickkers P, Njimi H, Jimenez E, Sakr Y, and investigators I. 2014 Assessment of

the worldwide burden of critical illness: the intensive care over nations (ICON) audit. Lancet Respir. Med 2: 380–386. [PubMed: 24740011]

45. Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, Jaton K, Giulieri S, Delaloye J, Opal S, Tracey K, van der Poll T, and Pelfrene E. 2015 Sepsis: a roadmap for future research. Lancet Infect. Dis 15: 581–614. [PubMed: 25932591]
46. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, and Angus DC. 2016 The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama 315: 801–810. [PubMed: 26903338]
47. Hotchkiss RS, Monneret G, and Payen D. 2013 Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat. Rev. Immunol 13: 862–874. [PubMed: 24232462]
48. Hotchkiss RS, and Nicholson DW. 2006 Apoptosis and caspases regulate death and inflammation in sepsis. Nat. Rev. Immunol 6: 813–822. [PubMed: 17039247]
49. Rittirsch D, Flierl MA, and Ward PA. 2008 Harmful molecular mechanisms in sepsis. Nat. Rev. Immunol 8: 776–787. [PubMed: 18802444]
50. Stortz JA, Raymond SL, Mira JC, Moldawer LL, Mohr AM, and Efron PA. 2017 Murine Models of Sepsis and Trauma: Can We Bridge the Gap? ILAR J. 58: 90–105. [PubMed: 28444204]
51. Weber GF, and Swirski FK. 2014 Immunopathogenesis of abdominal sepsis. Langenbecks Arch. Surg 399: 1–9. [PubMed: 24121723]
52. Patil NK, Guo Y, Luan L, and Sherwood ER. 2017 Targeting Immune Cell Checkpoints during Sepsis. Int. J. Mol. Sci 18.
53. Hotchkiss RS, Monneret G, and Payen D. 2013 Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect. Dis 13: 260–268. [PubMed: 23427891]
54. Cabrera-Perez J, Babcock JC, Dileepan T, Murphy KA, Kucaba TA, Badovinac VP, and Griffith TS. 2016 Gut Microbial Membership Modulates CD4 T Cell Reconstitution and Function after Sepsis. J. Immunol 197: 1692–1698. [PubMed: 27448587]
55. Cabrera-Perez J, Condotta SA, James BR, Kashem SW, Brincks EL, Rai D, Kucaba TA, Badovinac VP, and Griffith TS. 2015 Alterations in antigen-specific naive CD4 T cell precursors after sepsis impairs their responsiveness to pathogen challenge. J. Immunol 194: 1609–1620. [PubMed: 25595784]
56. Condotta SA, Khan SH, Rai D, Griffith TS, and Badovinac VP. 2015 Polymicrobial sepsis increases susceptibility to chronic viral infection and exacerbates CD8+ T cell exhaustion. J. Immunol 195: 116–125. [PubMed: 25980007]
57. Danahy DB, Anthony SM, Jensen IJ, Hartwig SM, Shan Q, Xue HH, Harty JT, Griffith TS, and Badovinac VP. 2017 Polymicrobial sepsis impairs bystander recruitment of effector cells to infected skin despite optimal sensing and alarming function of skin resident memory CD8 T cells. PLoS Pathog. 13: e1006569. [PubMed: 28910403]
58. Danahy DB, Jensen IJ, Griffith TS, and Badovinac VP. 2019 Cutting Edge: Polymicrobial Sepsis Has the Capacity to Reinvigorate Tumor-Infiltrating CD8 T Cells and Prolong Host Survival. J. Immunol 202: 2843–2848. [PubMed: 30971442]
59. Duong S, Condotta SA, Rai D, Martin MD, Griffith TS, and Badovinac VP. 2014 Polymicrobial sepsis alters antigen-dependent and -independent memory CD8 T cell functions. J. Immunol 192: 3618–3625. [PubMed: 24646738]
60. Gurung P, Rai D, Condotta SA, Babcock JC, Badovinac VP, and Griffith TS. 2011 Immune unresponsiveness to secondary heterologous bacterial infection after sepsis induction is TRAIL dependent. J. Immunol 187: 2148–2154. [PubMed: 21788440]
61. Jensen IJ, Winborn CS, Fosdick MG, Shao P, Tremblay MM, Shan Q, Tripathy SK, Snyder CM, Xue HH, Griffith TS, Houtman JC, and Badovinac VP. 2018 Polymicrobial sepsis influences NK-cell-mediated immunity by diminishing NK-cell-intrinsic receptor-mediated effector responses to viral ligands or infections. PLoS Pathog. 14: e1007405. [PubMed: 30379932]
62. Sjaastad FV, Condotta SA, Kotov JA, Pape KA, Dail C, Danahy DB, Kucaba TA, Tygrett LT, Murphy KA, Cabrera-Perez J, Waldschmidt TJ, Badovinac VP, and Griffith TS. 2018

Polymicrobial Sepsis Chronic Immunoparalysis Is Defined by Diminished Ag-Specific T Cell-Dependent B Cell Responses. Front. Immunol 9: 2532. [PubMed: 30429857]

63. Strother RK, Danahy DB, Kotov DI, Kucaba TA, Zacharias ZR, Griffith TS, Legge KL, and Badovinac VP. 2016 Polymicrobial Sepsis Diminishes Dendritic Cell Numbers and Function Directly Contributing to Impaired Primary CD8 T Cell Responses In Vivo. J. Immunol 197: 4301–4311. [PubMed: 27798171]
64. Foster HL 1959 Housing of disease-free vertebrates. Ann. N. Y. Acad. Sci 78: 80–88. [PubMed: 13824112]
65. Lapko N, Zawadka M, Polosak J, Worthen GS, Danet-Desnoyers G, Puzianowska-Kuznicka M, and Laudanski K. 2017 Long-term Monocyte Dysfunction after Sepsis in Humanized Mice Is Related to Persisted Activation of Macrophage-Colony Stimulation Factor (M-CSF) and Demethylation of PU.1, and It Can Be Reversed by Blocking M-CSF In Vitro or by Transplanting Naive Autologous Stem Cells In Vivo. Front. Immunol 8: 401. [PubMed: 28507543]
66. Laudanski K, Lapko N, Zawadka M, Zhou BX, Danet-Desnoyers G, and Worthen GS. 2017 The clinical and immunological performance of 28 days survival model of cecal ligation and puncture in humanized mice. PLoS One 12: e0180377. [PubMed: 28715505]
67. Skirecki T, Drechsler S, Hoser G, Jafarmadar M, Siennicka K, Pojda Z, Kawiak J, and Osuchowski MF. 2019 The Fluctuations of Leukocytes and Circulating Cytokines in Septic Humanized Mice Vary With Outcome. Front. Immunol 10: 1427. [PubMed: 31297113]
68. Skirecki T, Kawiak J, Machaj E, Pojda Z, Wasilewska D, Czubak J, and Hoser G. 2015 Early severe impairment of hematopoietic stem and progenitor cells from the bone marrow caused by CLP sepsis and endotoxemia in a humanized mice model. Stem Cell Res. Ther 6: 142. [PubMed: 26272069]
69. Unsinger J, McDonough JS, Shultz LD, Ferguson TA, and Hotchkiss RS. 2009 Sepsis-induced human lymphocyte apoptosis and cytokine production in “humanized” mice. J. Leukoc. Biol 86: 219–227. [PubMed: 19369639]
70. Ye C, Choi JG, Abraham S, Wu H, Diaz D, Terreros D, Shankar P, and Manjunath N. 2012 Human macrophage and dendritic cell-specific silencing of high-mobility group protein B1 ameliorates sepsis in a humanized mouse model. Proc. Natl. Acad. Sci. U. S. A 109: 21052–21057. [PubMed: 23213216]
71. Brehm MA, Shultz LD, Luban J, and Greiner DL. 2013 Overcoming current limitations in humanized mouse research. J. Infect. Dis 208 Suppl 2: S125–130. [PubMed: 24151318]
72. Laudanski K, Stentz M, DiMeglio M, Furey W, Steinberg T, and Patel A. 2018 Potential Pitfalls of the Humanized Mice in Modeling Sepsis. Int. J. Inflam 2018: 6563454. [PubMed: 30245803]
73. Shultz LD, Brehm MA, Garcia-Martinez JV, and Greiner DL. 2012 Humanized mice for immune system investigation: progress, promise and challenges. Nat. Rev. Immunol 12: 786–798. [PubMed: 23059428]
74. Rittirsch D, Huber-Lang MS, Flierl MA, and Ward PA. 2009 Immunodesign of experimental sepsis by cecal ligation and puncture. Nat. Protoc 4: 31–36. [PubMed: 19131954]
75. Chousterman BG, Swirski FK, and Weber GF. 2017 Cytokine storm and sepsis disease pathogenesis. Semin. Immunopathol 39: 517–528. [PubMed: 28555385]
76. Spittler A, Razenberger M, Kupper H, Kaul M, Hackl W, Boltz-Nitulescu G, Fugger R, and Roth E. 2000 Relationship between interleukin-6 plasma concentration in patients with sepsis, monocyte phenotype, monocyte phagocytic properties, and cytokine production. Clin. Infect. Dis 31: 1338–1342. [PubMed: 11095999]
77. Schulte W, Bernhagen J, and Bucala R. 2013 Cytokines in sepsis: potent immunoregulators and potential therapeutic targets--an updated view. Mediators Inflamm. 2013: 165974. [PubMed: 23853427]
78. Meng M, Klingensmith NJ, Liang Z, Lyons JD, Fay KT, Chen CW, Ford ML, and Coopersmith CM. 2019 Regulators of Intestinal Epithelial Migration in Sepsis. Shock 51: 88–96. [PubMed: 29424793]
79. Fisher CJ Jr., Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, Abraham E, Schein RM, and Benjamin E. 1996 Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion

protein. The Soluble TNF Receptor Sepsis Study Group. N. Engl. J. Med 334: 1697–1702. [PubMed: 8637514]

80. Amsen D, van Gisbergen K, Hombrink P, and van Lier RAW. 2018 Tissue-resident memory T cells at the center of immunity to solid tumors. Nat. Immunol 19: 538–546. [PubMed: 29777219]
81. Rosato PC, Wijeyesinghe S, Stolley JM, Nelson CE, Davis RL, Manlove LS, Pennell CA, Blazar BR, Chen CC, Geller MA, Vezys V, and Masopust D. 2019 Virus-specific memory T cells populate tumors and can be repurposed for tumor immunotherapy. Nat. Commun 10: 567. [PubMed: 30718505]
82. Duhen T, Duhen R, Montler R, Moses J, Moudgil T, de Miranda NF, Goodall CP, Blair TC, Fox BA, McDermott JE, Chang SC, Grunkemeier G, Leidner R, Bell RB, and Weinberg AD. 2018 Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors. Nat. Commun 9: 2724. [PubMed: 30006565]
83. Erkes DA, Smith CJ, Wilski NA, Caldeira-Dantas S, Mohgbeli T, and Snyder CM. 2017 Virus-Specific CD8(+) T Cells Infiltrate Melanoma Lesions and Retain Function Independently of PD-1 Expression. J. Immunol 198: 2979–2988. [PubMed: 28202614]
84. Scheper W, Kelderman S, Fanchi LF, Linnemann C, Bendle G, de Rooij MAJ, Hirt C, Mezzadra R, Slagter M, Dijkstra K, Kluin RJC, Snaebjornsson P, Milne K, Nelson BH, Zijlmans H, Kenter G, Voest EE, Haanen J, and Schumacher TN. 2019 Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers. Nat. Med 25: 89–94. [PubMed: 30510250]
85. Simoni Y, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, Yeong JPS, Nahar R, Zhang T, Kared H, Duan K, Ang N, Poidinger M, Lee YY, Larbi A, Khng AJ, Tan E, Fu C, Mathew R, Teo M, Lim WT, Toh CK, Ong BH, Koh T, Hillmer AM, Takano A, Lim TKH, Tan EH, Zhai W, Tan DSW, Tan IB, and Newell EW. 2018 Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature 557: 575–579. [PubMed: 29769722]
86. Danahy DB, Berton R, and Badovinac VP. 2020. Cutting Edge: Antitumor immunity by pathogen-specific CD8 T cells in the absence of cognate antigen recognition. J. Immunol.: In press.
87. Park SL, Gebhardt T, and Mackay LK. 2019 Tissue-Resident Memory T Cells in Cancer Immunosurveillance. Trends Immunol. 40: 735–747. [PubMed: 31255505]
88. Park SL, Mackay LK, Waithman J, and Gebhardt T. 2019 Tissue-resident memory T cells orchestrate tumour-immune equilibrium. Cell Stress 3: 162–164. [PubMed: 31225511]
89. Garn H, and Renz H. 2007 Epidemiological and immunological evidence for the hygiene hypothesis. Immunobiology 212: 441–452. [PubMed: 17544829]
90. Okada H, Kuhn C, Feillet H, and Bach JF. 2010 The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol 160: 1–9.
91. Bloomfield SF, Rook GA, Scott EA, Shanahan F, Stanwell-Smith R, and Turner P. 2016 Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. Perspect. Public Health 136: 213–224. [PubMed: 27354505]
92. Haspeslagh E, Heyndrickx I, Hammad H, and Lambrecht BN. 2018 The hygiene hypothesis: immunological mechanisms of airway tolerance. Curr. Opin. Immunol 54: 102–108. [PubMed: 29986301]
93. Thomas R, Wang W, and Su DM. 2020 Contributions of Age-Related Thymic Involution to Immunosenescence and Inflammaging. Immun. Ageing 17: 2. [PubMed: 31988649]
94. Hearps AC, Angelovich TA, Jaworowski A, Mills J, Landay AL, and Crowe SM. 2011 HIV infection and aging of the innate immune system. Sex. Health 8: 453–464. [PubMed: 22127030]
95. Zapata HJ, and Shaw AC. 2014 Aging of the human innate immune system in HIV infection. Curr. Opin. Immunol 29: 127–136. [PubMed: 24997358]
96. Nikolich-Zugich J 2018 The twilight of immunity: emerging concepts in aging of the immune system. Nat. Immunol 19: 10–19. [PubMed: 29242543]
97. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, and Verdin E. 2019 From discoveries in ageing research to therapeutics for healthy ageing. Nature 571: 183–192. [PubMed: 31292558]
98. Paez-Ribes M, Gonzalez-Gualda E, Doherty GJ, and Munoz-Espin D. 2019 Targeting senescent cells in translational medicine. EMBO Mol. Med 11: e10234. [PubMed: 31746100]

99. Chitnis T, and Weiner HL. 2017 CNS inflammation and neurodegeneration. J. Clin. Invest 127: 3577–3587. [PubMed: 28872464]

100. De Chiara G, Marcocci ME, Sgarbanti R, Civitelli L, Ripoli C, Piacentini R, Garaci E, Grassi C, and Palamara AT. 2012 Infectious agents and neurodegeneration. Mol. Neurobiol 46: 614–638. [PubMed: 22899188]

101. Readhead B, Haure-Mirande JV, Funk CC, Richards MA, Shannon P, Haroutunian V, Sano M, Liang WS, Beckmann ND, Price ND, Reiman EM, Schadt EE, Ehrlich ME, Gandy S, and Dudley JT. 2018 Multiscale Analysis of Independent Alzheimer's Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus. Neuron 99: 64–82 e67. [PubMed: 29937276]

102. Xue YC, Feuer R, Cashman N, and Luo H. 2018 Enteroviral Infection: The Forgotten Link to Amyotrophic Lateral Sclerosis? Front. Mol. Neurosci 11: 63. [PubMed: 29593492]

TABLE 1.

Mouse models of microbial exposure. The models highlighted in the text are compared to traditional SPF mice and "dirty" humans.

Model	Strengths	Weaknesses
Human	• Genetic diversity (outbred) • Experienced immune system • Males and females can be used to address sex as a biological variable	• Significant regulations limit *in vivo* "hypothesis-testing" studies
SPF mouse	• Can be inbred or outbred • Infection history is controllable • Numerous strains readily available to identify and interrogate immune cells • Male and female mice can be used to address sex as a biological variable	• Naïve immune system • Microbiome is more uniform • Can be highly susceptible to pathogens
Wild mouse	• Natural pathogen exposure since birth • Diverse microbiome • Experienced immune system leads to enhanced protection to new infections	• Unknown infection history • Unknown genetics and age • Increased cost for dedicated housing • Mice must be caught and may carry pathogens dangerous to humans (e.g., Hantavirus)
Cohoused with pet store mice	• Can be inbred or outbred • Can be age-matched with SPF mice • Natural pathogen exposure • Conversion of the naïve mouse immune system to a mature/effector phenotype is efficient (<10% of cohoused mice experience a poor conversion) and robust	• Increased cost for dedicated housing (e.g., BSL-3) • Microbial exposure can vary with each cohort • Cohousing can result in death depending on the pet store mouse used • Unknown variation in microbiome composition • Restricted to female mice
Sequential infection	• Infection history is known • Using known pathogens draws on a larger pool of knowledge to interpret results • Can be done with BSL1- and BSL2-level pathogens • Male and female mice can be used	• Requires prior selection of a 'correct' set of pathogens • Uses laboratory defined routes of exposure instead of natural routes • Milder conversion than observed with other methods
Exposure to dirty bedding ("fomites")	• Mice rarely die or develop conditions that require euthanasia (as seen in cohoused model) • Reduced variability – fomites allow for a more consistent and uniform exposure over different cages with a reliable transfer of pathogens • Male and female mice can be used	• Fomite generating petstore mice lose their ability to convert SPF mice over time which requires additional petstore mice and can create a higher percentage of low converted SPF mice • The intensity of conversion as measured is more erratic and the variety of pathogens transferred is different than with cohousing
Natural microbiota transfer/rewilding	• Mice live in a more natural outdoor environment • Phenotype is transferred from one generation to the next aiding in standardization • Natural pathogen exposure since birth • Male and female offspring mice can be used	• In "rewilding" model, outdoor enclosures must be constructed that prevent escape and deter natural predators • In "wildling" model, wild mice must be captured and embryo transfers are technically challenging • Unknown variation in microbiome composition • Increased cost for dedicated housing

Table 2.

Microbial experience determined by serology testing. % of positive mice tested is indicated

	Laboratory (n=9)	Cohoused (n=828)	Fomite (n=105)	Pet store (n=365)
Viruses				
Pathogen-free	100	0.5	0.0	0.5
Rotavirus (EDIM)	0.0	0.0	1.0	41.6
Mouse Hepatitis Virus	0.0	46.7	64.8	86.8
Murine Norovirus	0.0	80.1	84.0	43.3
Mouse Parvovirus NS1	0.0	16.5	25.7	66.0
Mouse Parvo Virus Type 1	0.0	19.2	21.0	72.9
Mouse Parvo Virus Type 2	0.0	16.5	23.8	74.5
Minute Virus of Mice	0.0	17.1	37.1	71.0
Theiler's Murine Encephalomyelitis Virus	0.0	84.5	92.4	61.6
Sendai Virus	0.0	8.0	0.0	61.4
Ectromelia Virus	0.0	0.0	0.0	0.0
Lymphocytic Choriomeningitis	0.0	3.9	0.0	6.3
Mouse Adenovirus 1 and 2	0.0	4.7	5.7	7.1
Mouse Cytomegalovirus	0.0	0.0	0.0	0.8
Polyoma Virus	0.0	0.2	0.0	0.5
Pneumonia Virus of Mouse	0.0	1.6	0.0	5.8
Reovirus	0.0	0.5	0.0	1.4
Bacteria				
Cillia-Associated Respiratory Bacillus	0.0	0.0	0.0	0.0
Mycoplasma Pulmonis	0.0	57.1	0.0	53.7
Clostridium Piliforme	0.0	0.1	12.4	9.9
Parasites/Protozoa/Fungi				
Enchephalitozoon Cuniculi	0.0	5.4	5.4	20.3
Pinworm	No	Yes	Yes	Yes
Mites	No	Yes	No	Yes