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Review Article

Mouse hygiene status—A tale of two environments for mast cells and allergy

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ABSTRACT

Animal models, including those employing the use of house mice (*Mus musculus*), are crucial in elucidating mechanisms in human pathophysiology. However, it is evident that the impreciseness of using laboratory mice maintained in super-hygienic barrier facilities to mirror relevant aspects of human physiology and pathology exists, which is a major limitation in translating mouse findings to inferring human medicine. Interestingly, free-living wild mice are found to be substantially different from laboratory-bred, specific pathogen-free mice with respect to various immune system compartments. Wild mice have an immune system that better reflects human immunity. In this review article, we discuss recent experimental findings that address the so-called "wild immunology", which reveals the contrasting immune features between laboratory-raised mice and their wild companions as well as laboratory mice that have been exposed to a natural rodent habitat. A particular focus will be given to the development of pulmonary mast cells and its possible impact on the use of "naturalized" or "rewilded" laboratory mice as experimental asthma models.

Advantages and limitations of mouse models in research

Animal models have long been used to better understand human physiology and the pathological mechanisms of comparable diseases in humans. The development of the modern pharmaceutical industry also heavily depends on the use of experimental animals, which serve to identify and validate the putative drug candidates, as well as to test treatment modalities of the diseases that the animals can model. A number of advantages have set house mice (*Mus musculus*) aside from other animals as the model species of choice to imitate human physiology and pathology in pre-clinical models.[1] Because of their small size, mice can be maintained with cost-efficient husbandry for research purposes, achieving easy synchronization of the majority of the environmental factors, including day–night cycles, temperature, and supply of food and water. Genetic manipulation is relatively less costly and straightforward for mice that have a short lifespan with a high reproductive rate. Interestingly, humans and mice have comparable anatomical and physiological features despite their huge size differences. In fact, much of our current knowledge of immunology is based on research using mouse models.

However, there is a growing concern that conventional laboratory mice maintained in ultra-hygienic barrier conditions do not precisely reproduce human pathophysiology, including immune-related features.[2] Thus, limitations in extrapolating data derived from murine models to humans are evident as there are certain areas where humans and mice differ substantially. Despite the overwhelmingly promising results arising from animal modelling of human diseases in preclinical studies, the overall success rate of drug candidates that can pass vigorous clinical trials can be as low as a single-digit number,[3–8] which questions the translational values of modelling human diseases using animals. The overall cost of successfully developing a novel drug for marketing, which usually takes more than a decade, is estimated to be higher than 1 billion US dollars.[9] Therefore, the appropriate selection of an adequately validated animal model that can predict human pathophysiology is essential to bridge the translational gap to the clinic, thus avoiding the waste of resources.[10]

Although one possible explanation is the flawed or misleading animal models that have been adopted for mimicking human pathophysiology, the lack of precision in data extrapolation from mice to humans may be due to discrepancies between mice and humans concerning their respective precise physiology and histology, as a result of their fundamental divergence at the genetic level over a long evolutionary history. In evolutionary terms, mice and humans diverged almost 80–100 million years ago.[11,12] Accumulated differences owing to such an early divergence may lead to

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variations in the protein expression and functionality between the two species. Alternatively, the discrepancies may also reflect an artifact, which is a more recent event, arising from the generation and breeding of laboratory strains of mice.[1,13] This is a reasonable assumption as laboratory-based studies of clean mice with limited exposure to antigens are unlikely able to precisely predict the immune features of humans who are exposed to a much more diverse range of environmental antigens.

Wild mice as a better model to reflect human immunity

Accumulating experimental evidence suggests that certain discrepancies between humans and laboratory mice, in terms of immunological features or responses, stem from variations in the gut microbiome, a complex collection of microorganisms, including bacteria, viruses, protozoa, and fungi located in the gut of humans and animals. Limited microbiome diversity is described for laboratory mice which live in specific pathogen-free (SPF) barrier facilities.[14] It is well-accepted that gut microbiota can effectively shape the immune signatures of individual organisms including both humans and mice.[15] Furthermore, lung microbiota also contributes to the regulation of mouse airway immune responses.[16,17] A diverse spectrum of microbial communities at relevant body sites is required to facilitate adequate immune system education. Therefore, humans and ultra-clean laboratory mice could have evolved distinct functional phenotypes and mechanisms of their respective immune systems as a result of the profoundly divergent microbiomes they possess.

The SPF mice that are bred and maintained in an ultra-hygienic barrier display reduced diversity and complexity of microbiota compared with free-living house mice whose habitats are drastically different from an SPF barrier facility.[18] Hugely contrasting immunological features have been well documented between laboratory-raised mice and wild-raised mice.[14,19–23] It was predicted and has recently been experimentally demonstrated that reduced richness and complexity of microbiota is a major factor that accounts for the deviation of immunity of SPF laboratory mice away from that of the wild mice and possibly humans.[18]

Thus, the term “wild immunology” has been coined to refer to research that focuses on the study of the immune response of rodents in their natural habitat.[24] A thorough understanding of “wild immunology” is assumed to be the key link between conventional animal models using SPF-class laboratory mice and real human pathophysiology. Indeed, growing evidence has demonstrated that the natural environment, in comparison to the artificially created ultra-clean laboratory setting, increases the chances of exposure to various types of microbes. Nature-derived biomaterials enriched with microbes and allergens can remarkably change not only the specificity but also the activation threshold of the immune system. These environmental factors can profoundly generate a divergent immunophenotype in wild mice in contrast to SPF mice.

It is believed that both the innate and adaptive immune systems are subject to the stimulation and training of the microbiome that is extraordinarily different between conventional laboratory-raised mice and wild-raised mice.[18] Consequently, emerging studies have already revealed intriguing findings contrasting the immunological discrepancies between these two populations of rodents with genetic identity but living in strikingly different environments by analysing various immune parameters including molecular, cellular, and functional aspects. Free-living wild mice demonstrate immune features that are closer to those of adult humans.[14] Boysen and colleagues found that natural killer (NK) cells from wild mice tend to express higher levels of a number of activation markers, including CD69, KLRG1, granzyme B, and NKp46. Wild mice also have increased frequencies of NK cells in peripheral lymph nodes compared with SPF laboratory mice.[21] These findings suggest the pre-activation of NK cells in wild mice, probably indicating a strong influence by the environment on these cells. In addition to NK cells, other immune cells are also found to be associated with a greater overall level of activation in wild mice, including effector CD4 T cells and CD8 T cells, B cells, dendritic cells, and macrophages.[14,20,22] In response to stimulation through CD3 and CD28, splenic T cells from wild mice release increased levels of IFN-γ, IL-1β, and IL-4, cytokines critical for regulating adaptive immunity.[22] However, cytokine production by splenocytes is largely suppressed in response to pathogen-derived stimulatory ligands in wild mice.[22] This might represent immune exhaustion as a result of the intensive challenge of microbial antigens to the immune system in wild mice, which is an evolutionary protective mechanism. Humoral immune responses are also observed to be modulated by the living environment where mice reside. Wild mice have higher concentrations of total IgG and IgE compared with hygienic laboratory-bred mice. In response to immunization, wild mice not only have augmented levels of antigen-specific antibodies but also increase the avidity of these antibodies.[20,22]

Strategies for generating naturalized laboratory mice

Although wild populations of house mice are predicted to be a more relevant model to reflect human immune responses,[25] it is practically difficult to use wild-caught mice for routine experimental use because of a number of issues. First, the availability of wild mice is unpredictable and it is not easy to catch wild mice to accumulate enough numbers within a short reasonable period. Second, wild mice are diverse in gender, age, body size, and genetic background,[26] which renders it difficult to use gender- and age-matched cohorts in research, resulting in increased individual variation. Third, genotyping is necessary to confirm the *M. musculus* identity because quite many other rodent species can masquerade as house mice in appearance. Therefore, “naturalizing” laboratory mice to let them possess immunological phenotypes that reflect those of their wild companions has been suggested.[23,27] Various methods are adopted to generate “naturalized” or “rewilded” laboratory mice to expand and diversify the gut microbiota of these mice.[23,28] These manipulations will make laboratory mice “dirty” while still maintaining their genetic uniformity. These naturalized models are expected to improve the predictive ability of mouse-based translational research.

Gut microbiota is predicted to be a defining determinant in shaping wild mouse immune phenotypes in contrast to laboratory mice maintained in SPF conditions.[29] Furthermore, it is reported that geographic distance, which is correlated to different microbial cues, contributes much more than the genetic distance in the formation of gut microbiota diversity in mice.[30,31] Therefore, manipulating the gut microbiota by direct faecal transfer has been attempted. Oral gavage of pooled faecal material from wild-caught mice to laboratory-raised, germ-free mice shifts the microbiome diversity towards wild mice, resulting in higher quality host fitness including resistance against viral infection and tumorigenesis, compared with germ-free mice engrafted with SPF laboratory mouse faeces.[29]

Co-housing of laboratory-bred, super-clean mice with “dirty”, wild-caught mice leads to remarkably changed gut microbiota in the former after 2–3 months.[32,33] Co-housing of laboratory mice with pet store mice, which are also maintained in a less hygienic condition, can convert the immune phenotypes and signatures of these laboratory mice from originally resembling human neonates to adult humans.[14] Co-housing with wild mice or pet shop mice increases the frequency of effector memory T cells and enhances their protection against systemic *Listeria monocytogenes*

infection.[34] Elevated levels of memory T cells and late-stage NK cells, which represent antigenic experience and trained immunity, are recorded in the laboratory mice co-housed with wild mice.[33] Deliberately infecting laboratory mice with a range of microorganisms that mice normally encounter in their natural habitat makes their pre- and post-vaccination circulatory immune signatures closer to those of pet store-raised mice and adult humans.[35] Co-housing with pet store mice dampened both humoral and cellular immune responses in laboratory mice,[36,37] which is predicted to be a better model to reflect human responses. Co-housing with pet store mice can also modulate Toll-like receptor signalling.[38] Viral transmission between co-housed mice has been investigated,[39] which may also impact the immune dynamics of the recipient mice.

Instead of co-housing laboratory mice with “dirty” mice, either wild or those from the pet store, housing clean laboratory mice in a predator-free outdoor enclosure allows the self-acquisition of microbes with notable increases in intestinal fungi, which leads to enhanced differentiation of T cell populations and circulating granulocytes.[40] Conventional laboratory strains of mice maintained in an outdoor enclosure with a more natural environment become more susceptible to deliberate intestinal worm infection.[41] A more comprehensive phenotypic analysis of immunological parameters in inflammatory bowel disease-prone strains of genetically manipulated mice after rewilding in such an outdoor enclosure has been carried out.[42] Environmental differences can certainly drive variation in population frequencies of immune cells in the blood and lymph nodes, while cytokine responses to microbial stimulation are affected more by genetic factors.[42]

Yet another approach has also been attempted to establish a naturalization model, by exposing conventional laboratory mice to bedding materials from a domestic animal farmhouse, which include sawdust, soil, compost, twigs, and hay, to allow the recapitulation of a wild mouse habitat in an indoor farmyard-type setting. Mice born and raised in this purpose-built indoor housing facility show distinct changes in immune parameters and gut microbiota.[43] Following this naturalization process, laboratory C57BL/6 mice have an increased relative abundance of Proteobacteria and a reduced abundance of Bacteroidetes,[43] consistent with the microbiota pattern found in wild mice.[29,44]

A seminal study demonstrates that breeding laboratory mouse progeny using a wild mouse as a surrogate mother generates the so-called “wildling” mice which have genetic identity with their paternal laboratory mouse while possessing the microbiota and many of the immune phenotypes of their surrogate mother which is a true wild mouse.[44]

There are certain research challenges when employing mouse naturalization or rewilding models. A major issue is the consistency of the study design which can affect reproducibility. The globally different geographic locations are correlated to gut microbiota variations.[30,31] In addition, seasonal microbiota restructuring has been observed.[45] Therefore, spatiotemporal discrepancies in the microbiota patterns may confound the study results. Futhermore, it is reported that infection with Campylobacter can extensively shape the pattern of the microbiota of the Eurasian harvest mice (*Micromys minutus*),[46] a cousin of the house mice (*M. musculus*) from which the conventional laboratory mouse strains are derived. Thus, we can predict that accidental infection with certain microbial species during their exposure to naturally derived materials or contact with wild mice may modify the microbiota composition of laboratory mice, hence the immune phenotype disruption. These might well be the reasons accounting for the discrepancies in the literature concerning the comparison between clean SPF mice and “dirty mice”. For example, wild mice are reported to have increased humoral immune responses in certain studies.[20,22] However, laboratory mice are found to have dampened humoral and cellular immune responses after co-housing with pet store mice.[36,37]

Wild mice and naturalized laboratory mice for allergy and asthma research

Allergy is one of the most prevalent human diseases affecting the quality of life of more than one billion people across the globe. Allergy incidence and prevalence have been steadily increasing over recent decades. This increasing trend is particularly notable for allergic asthma and rhinitis, which has led to an enormous socioeconomic burden worldwide.[47] Currently, more than 300 million people suffer from asthma globally, and this number may likely increase by another one-third by 2025.[48] Innovative asthma and allergy treatment approaches to tackle this chronic disease are yet to be designed.[49]

Mounting experimental studies addressing wild immunology have provided implications for allergy and asthma.[50] Wild-raised mice have augmented serum levels of not only IgG but also IgE, in comparison to their SPF counterparts.[19,22] Exposure of mice early in life to a farming environment protects them from allergen-induced skin contact hypersensitivity.[51] Infection by *Schistosoma mansoni* suppresses allergic airway inflammation in a Treg-dependent manner.[52] Chronic *Trichinella spiralis* infection prevents the development of allergic airway remodelling in asthmatic mice.[53] Farm dust and endotoxin protect against allergy through the induction of ubiquitin-modifying enzyme A20 in lung epithelium cells.[54] Immune imprinting effects of microbial or helminth infection on offspring for suppressing allergic airway inflammation have also been reported.[55,56] Co-housing pet store mice with laboratory mice results in a transient reduction of lung eosinophilia to intranasally delivered allergens probably through inhibiting mouse lung group 2 innate lymphoid cells (ILC2).[57]

Exposure to various types of individual microbial components has also been implicated in suppressing allergic inflammation. Administration of unmethylated CpG DNA, mimicking the action of microbes, reduces the risk of developing airway allergic inflammation in mice by expanding regulatory lung interstitial macrophages.[58] Simultaneous instillation of flagellin and the experimental allergen ovalbumin (OVA) can suppress experimental asthma by generating regulatory dendritic cells and Tregs.[59] The importance of microbial-derived carbohydrates on immune suppression has been observed. Intranasal administration of *Toxoplasma gondii*-derived molecules reduces allergic lung inflammation possibly as a result of the immunomodulation by carbohydrates.[60] Oral exposure to capsular polysaccharides derived from a commensal bacterium effectively protects mice from the development of experimental asthma through mobilizing the production of immune-suppressive cytokine IL-10.[61]

Besides fundamentally shaping the microbiota, microbial exposure may also alleviate asthmatic responses through desensitization based on the presence of shared epitopes in the microbial component (e.g., *Streptococcus pneumonia*) and allergen (e.g., house dust mite).[62] Interestingly, mice treated with inactivated soil-derived materials have decreased levels of pro-inflammatory cytokines, possibly reflecting the importance of biodiversity in shaping the murine immune system even in a sterile environment.[63]

Despite the overwhelming experimental evidence supporting the “hygiene hypothesis” first introduced by David Strachan more than three decades ago,[64] studies addressing the effect of microbial exposure on the development of allergic responses that cannot reconcile with the hygiene hypothesis have also been reported. Using a similar approach for generating the wildling mice as reported previously,[44] Ma and colleagues showed that these “dirty”

mice, having been in contact with wild microbial flora, develop stronger asthmatic inflammation compared with SPF mice (bioRxiv preprint doi: https://doi.org/10.1101/2021.03.28.437143).

Mast cells in wild mice and naturalized laboratory mice

Mast cells are immune cells of the myeloid lineage, which have been classically described as one of the major effector cells involved in the development of allergic pathology by virtue of their potential to secrete a variety of allergic mediators.[65] Mast cells express FcεRI, the Fc receptor that binds IgE with high affinity. Because of its high affinity, FcεRI is constitutively occupied by IgE. Following cross-linking of IgE bound to adjacent FcεRI molecules expressed on mast cells by plurivalent allergens, FcεRI transduces cellular activation signals, resulting in mast cell degranulation and the release of proinflammatory mediator molecules, a process that typically initiates or exacerbates allergic inflammation.

Mast cells are derived from hematopoietic progenitors in the bone marrow. Guided by various chemotactic factors, these progenitors migrate to the vascularized tissues where they further differentiate into mature mast cells with phenotypic heterogeneity.[66] Mast cells are abundantly expressed in the skin, serosal tissues, and mucosal membranes, such as the airways and gastrointestinal tracts. Tissue-specific distribution of mast cells is dependent on various mediators including local expression of mast cell differentiation and growth factors. It is reported that upon allergen-mediated sensitization in the airways, locally produced CCL2 recruits mast cell progenitors which express CCR2.[67] Stem cell factor (SCF) is one of the major growth and differentiation factors for mast cells which express cKit, the receptor for SCF, throughout their lifespan.[68] In addition to SCF, mast cell growth and differentiation are also driven by several other cytokines including IL-3.[69] Tissue mast cells can be further differentiated phenotypically according to the functional need for them through spatiotemporal stimulation under defined microenvironmental conditions. For example, tryptase/chymase-producing mast cells are predominantly found in inflamed human lungs whereas tryptase-producing mast cells are dominant in lung parenchymal tissues under homeostasis.[70–72] Not only is mast cell density increased at sites of allergic inflammation, but also can a positive correlation be established between mast cell tissue density and the severity of allergic symptoms.[73]

A particular point that attracted our attention is the observation that commonly used SPF laboratory mice, including both the C57BL/6 and BALB/c strains, do not have lung parenchymal mast cells, which stands in clear contrast to the abundant distribution of mast cells in human lungs.[74] Intrigued by this observation, as well as the accumulating experimental evidence suggesting discrepant immune phenotypes between SPF and wild mice, we have recently explored the lung tissue mast cell distribution in wild, free-living house mice trapped in both Norway and India,[75] as well as pet store mice from Taiwan (our unpublished observation). We discovered that wild mice and pet store mice which live in a natural, unhygienic environment did have lung parenchymal mast cells. The absence of lung parenchymal mast cells in conventional laboratory mice may happen as a regulatory result of both genetic and environmental factors. Interestingly, we further demonstrated that lung parenchymal mast cells have appeared in naturalized “dirty” laboratory mice which have spent their entire life in a semi-natural environment established in Oslo, Norway.[75] This observation argues for a strong environmental impact on mast cell distribution in the lung tissue. However, our observation cannot exclude any genetic influences on pulmonary mast cell distribution. We observed that wild mice trapped in India had substantially more lung parenchymal mast cells compared with their wild companions in Norway, which may be distantly related to Indian wild mice.[75] Of course, one may also argue that the differential lung mast cell density in Indian wild mice and Norwegian wild mice can arise from fundamental differences in the environmental factors they have experienced. We have recently tried to reproduce the naturalization model in Hong Kong. Our data demonstrate that only neonatal exposure promoted pulmonary accumulation of mast cells, which stands in contrast to the failure of lung mast cell recruitment after adult exposure (our unpublished observation). Table 1 summarizes lung parenchymal mast cell densities in mice from various locations and after different treatment conditions. Increased lung mast cell accumulation after neonatal exposure was accompanied by augmented levels of cytokines critical for mast cell differentiation and growth, including SCF, IL-33, and TGF-β (our unpublished observation). Our observation on lung mast cell number regulation by comparing neonatal and adult exposure to a natural environment is consistent with literature reports showing that certain immunological deficits can only be reversed if mice are colonized with microorganisms within a pre-weaning time window, an early-life interval critical for microbe-mediated immune education.[76,77]

The currently established mouse allergy and asthma models mainly depend on the use of laboratory-raised, inbred strains of mice (e.g., C57BL/6 and BALB/c). In order to overcome the poor response to asthmatic provocation by these laboratory mice probably as a result of the absence of lung parenchymal mast cells, various approaches have been designed to induce mast cell recruitment into the lung tissues of laboratory mice. Intraperitoneal sensitization with OVA in the absence of adjuvant, followed by chronic intranasal OVA instillation, leads to the pulmonary recruitment of mast cells.[78] Alum-adjuvanted intraperitoneal sensitization with OVA, followed by daily challenges using OVA-containing aerosol for one week,[79] intranasal OVA instillation twice weekly for at least one month,[80] or three consecutive daily intratracheal OVA administration,[81] induces mast cell distribution in the lung tissues. In contrast to these artificial models of mast cell lung recruitment, we could possibly assume that the naturalization of laboratory mice for the enrichment of lung parenchymal tissue mast cells may provide a better tool for modelling human

Table 1
Comparison of the lung parenchymal tissue mast cell density in mice from various sources and treatment.

Location	Source	Treatment	Age at examination	Mast cell density (cells/mm^2)
Taiwan	Pet store	–	Unknown	0.59 ± 0.12
India	Wild	–	Unknown	1.26 ± 0.24
Norway	Wild	–	Unknown	0.72 ± 0.26
	C57BL/6	SPF	8 weeks	0.03 ± 0.02
	C57BL/6	Neonatal Exposure	8 weeks	0.27 ± 0.08
Hong Kong	C57BL/6	SPF	8 weeks	0.02 ± 0.01
	C57BL/6	Neonatal Exposure	8 weeks	0.42 ± 0.18
	C57BL/6	SPF	16 weeks	0.09 ± 0.05
	C57BL/6	Adult Exposure	16 weeks	0.17 ± 0.08
	C57BL/6	Neonatal Exposure	16 weeks	0.40 ± 0.18

SPF, specific pathogen-free; neonatal exposure refers to the study design whereby the mice were bred in a purpose-built indoor facility with farm-derived bedding materials, and the offspring were collected for examination when they reached adulthood; adult exposure refers to the study design in which 8-week-old SPF mice were transferred to the facility with farm-derived materials, where they were maintained for another 8 weeks before examination; mast cell density is calculated based on the number of mast cells in the parenchymal lung tissues, excluding areas with small airways and pulmonary vessels, and the values represent mean ± SEM.

pulmonary physiology, although further experimental studies are warranted to assess the suitability of the use of these "dirty" models in pre-clinical asthma research.

Human asthma is typically manifested with both an acute-phase response and a late-phase response. The late-phase response is believed to be caused by mast cell activation and release of pro-inflammatory cytokines a couple of hours later, which may attract other inflammatory effector cells to the lung tissue, exacerbating the disease symptoms at a late time point.[82] It is well known that the conventional mouse asthma models based on the use of SPF laboratory mice cannot model the late-phase response, probably due to their profound lack of lung parenchymal mast cells.[65,74] Therefore, the lack of constitutive lung parenchymal mast cells has challenged the relevance of mouse asthma models for understanding human asthma, which is assumed to be responsible for the failure of preclinical studies.[74] There is a pressing need to generate more faithful mouse asthma models that reflect the human immune landscape, including the distribution of lung mast cells. The naturalization of laboratory mice may provide a practical solution to the restoration of lung parenchymal mast cells in mice that can be genetically defined and properly controlled. These naturalized laboratory mice may be exploited for the development of mouse asthma models that may truthfully manifest features mediated by mast cell activation, thus more closely recapitulating human asthma. Interestingly, the wildling mice are reported to develop airway inflammation to a heightened level compared with laboratory-raised mice (bioRxiv preprint doi: https://doi.org/10.1101/2021.03.28.437143). However, mast cell pulmonary residency is not assessed in these wildling mice, and it would be of interest to investigate the mast cell recruitment in the lungs of these wildling mice and its correlation with stronger asthmatic responses.

In addition to the lung tissue, mast cells located in other tissue sites may also be impacted by environmental factors. It is reported that germ-free mice have lower numbers of skin mast cells and these mast cells demonstrate an immature phenotype, probably

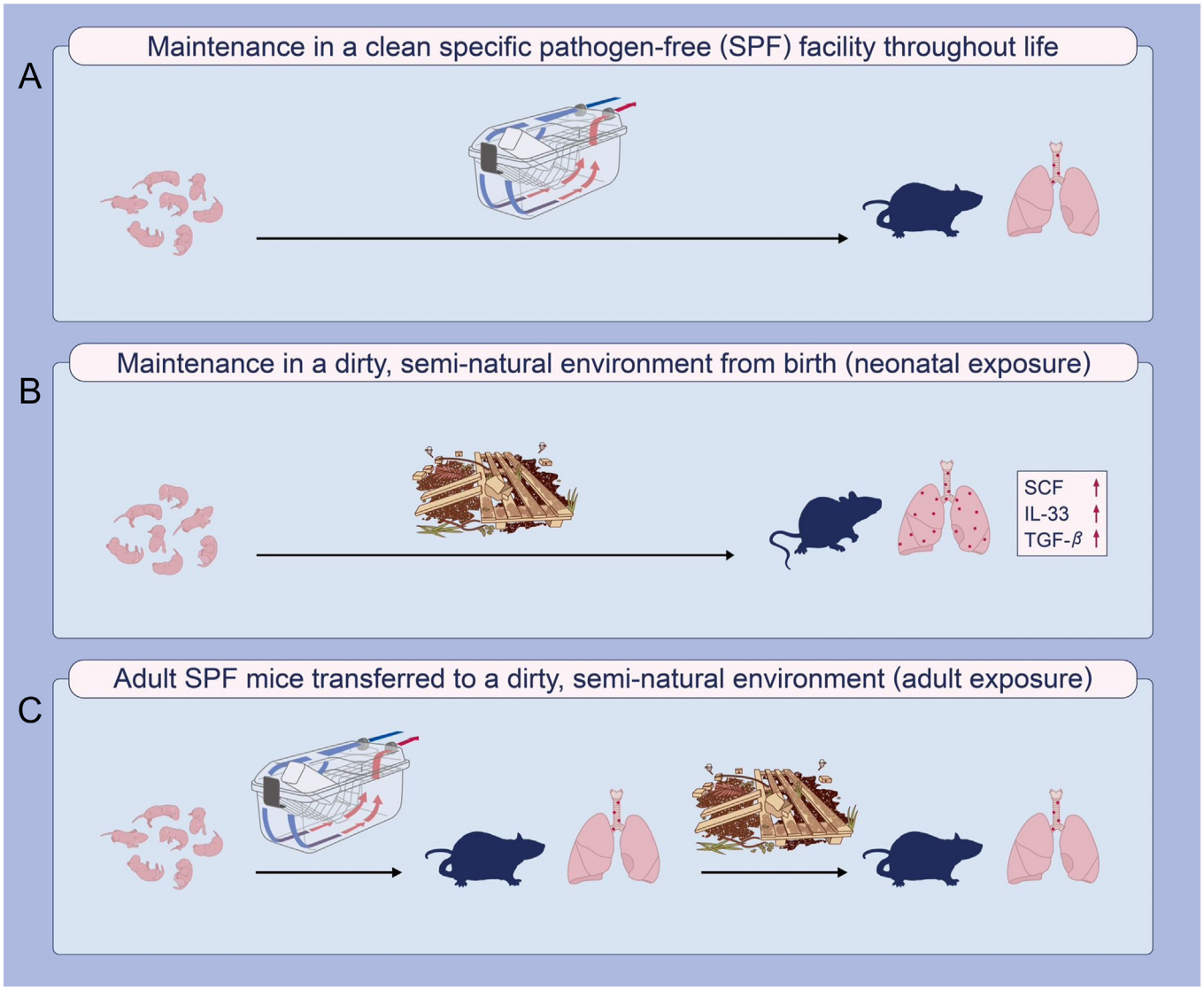

Fig. 1. A graphical representation explains a possible environmental impact on the development of lung parenchymal mast cells in mice. Laboratory (lab) mice raised in ultra-hygienic, specific pathogen-free (SPF) barrier conditions profoundly lack lung parenchymal mast cells **(A),** in contrast to the enriched distribution of mast cells in human lungs (not depicted). Interestingly, mast cells are found in lung parenchymal tissues of free-living house mice (not depicted). Support for an environmental impact on the development of lung mast cells in these mice comes from the fact that mast cells do appear in the lung tissues of lab mice bred in a semi-natural environment with farm-derived bedding materials, simulating neonatal exposure **(B)**. However, clean adult mice, even after exposure to the nature-derived bedding materials, fail to develop lung parenchymal mast cells **(C)**.

owing to their reduced local production of SCF. Co-housing of the germ-free mice with SPF mice can restore the normal mast cell density in the skin.[83] This report has investigated the impact of microbiota on the development of skin mast cells by looking at the lower end, i.e., from the lack of microbes, an extremely low microbiome status, to a normal SPF level of microbiome. The effect of a richer microbiome, such as that of the wild mouse or the naturalized laboratory mouse, on the development of skin mast cells awaits further investigation, although our preliminary observation did not observe any modulation of mouse ear mast cell densities between wild mice and SPF laboratory mice, or between SPF laboratory mice and naturalized laboratory mice (our unpublished observation).

Another factor related to the functionality of mast cells that sets wild mice and naturalized mice aside from SPF mice is the circulating levels of IgE. It has long been revealed that wild mice have increased levels of IgE.[19,20,22] Furthermore, naturalized mice also showed elevated levels of IgE compared with clean laboratory mice, although the levels have not reached as high as those in the wild mice derived from the same region.[43] It is generally assumed that increased levels of IgE, together with other immunoglobulin species, in wild rodents reflect a pressure from the heavy parasite load in these creatures that live in extremely unhygienic conditions.[84] IgE-mediated mast cell activation, with massive degranulation leading to release of abundant pre-formed mediators, pro-inflammatory lipids, and a multitude of cytokines and chemokines, is a hallmark of acute allergic reactions. The precise roles of modulated levels of IgE in wild mice and naturalized mice in mast cell activity warrant further clarification.

Concluding remarks

Animal models are indispensable not only for elucidating immunological mechanisms, including those regulating allergy and asthma, but also for developing therapeutic strategies. Mice will continue to serve as a valuable research tool for the study of allergy and immunology. Although research based on the use of mouse models has contributed substantially to our knowledge in understanding the roles of mast cells in asthma and allergy, the existing mouse models can only reflect certain, but not all, aspects of human asthmatic responses. Preclinical research based on the use of animal models that reflect biological mechanisms and features deviating from human immunology may explain the inconsistencies between predictions of animal models of immunology and human clinical trial outcomes. Refining existing mouse asthma models or establishing new models with a higher degree of clinical relevance may make preclinical research extrapolatable, thus avoiding the waste of research resources. Indeed, the majority of asthma drugs that pass the most vigorous preclinical testing still fail clinical trials. Among the reasons for the high failure rate of drug development, the lack of adequate animal models suitable for drug screening constitutes a major one. Optimizing animal models, including mouse models, used for predicting human diseases and treatments may greatly reduce the cost of drug discovery. Our recent work has established a mouse naturalization model, which can restore lung parenchymal mast cells (Fig. 1). Naturalized laboratory mice with a “wild gut” and redistributed mast cells in tissues may be further exploited to confirm their applicability and advantages in allergy and asthma research.

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Conflict of interest

The authors have no conflict of interest to declare.

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