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Modelling human immune responses using microbial exposures in rodents

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The extent and diversity of exposures to microbial stimuli have a crucial role in regulating the capacity of a host to mount an immune response to a challenge, such as vaccination, making exposure history an important factor to optimize in rodent models.

Free-living animals are exposed to trillions of commensal and pathogenic microorganisms, which begins before birth and ultimately results in colonization by a diverse microbiota. The 'microbial exposome' is the summation of one's lifetime exposures to microorganisms. This includes the endogenous microbiota and infectious organisms, such as multicellular parasites and ectoparasitic arthropods, which may be chronically or transiently present. The microbial exposome is a dominant driver in the development and regulation of immune responses. Individual and cumulative microbial and infectious encounters train and shape the host immune system. This in turn may target or tolerate specific microorganisms to sculpt endogenous communities and determine host responses to immune challenges, such as vaccination. However, our understanding of the consequences of specific microbial exposures to human health and immunity remains elusive. In this Comment, we provide a general overview of how the microbial exposome may influence the immune response to vaccination, and we discuss ongoing efforts to leverage microbial exposures in mouse models to better mimic human immune responses.

Microbial exposure and immune responses to vaccination

Perhaps one of the most readily studied immune responses in humans is to vaccines, which are standardized and administered widely at known intervals, facilitating comparative studies. The continuing COVID-19 pandemic and the recent emergence or re-emergence of pathogens such as monkeypox and poliovirus have highlighted the ongoing need to optimize prophylactic vaccine regimens to elicit strong and long-lasting immunity. Antigen-specific antibody- and immune cell-mediated responses have critical roles in the protection induced by vaccination, but these immunological responses are highly variable among individuals. Compared with individuals living in low- and middle-income countries, individuals in high-income countries tend to exhibit more robust humoral and/or cellular immune responses to vaccination, with differential responses reported for influenza, yellow fever and Ebola candidate vaccines[1]. These distinct immune responses correlate with substantial differences in cumulative microbial exposures. Individuals from low- and middle-income countries tend to have much greater microbiota diversity, as well as exposure to viral and helminthic infections, compared with individuals from high-income countries[1]. There are numerous host factors (for example, age, genetics) and other extrinsic factors (for example, diet, environment) that can influence vaccine immunogenicity and/or efficacy. However, emerging evidence from clinical studies and animal models supports the idea that the microbial exposome is an additional crucial factor modulating human vaccine responsiveness[1,2]. Antibiotic-induced perturbation of the gut microbiota can alter immune responses to vaccination in both mice and humans[3,4], and viral co-infections have been linked to diminished vaccine responsiveness[5]. The mechanisms by which microbial exposures shape immune responses to orally and parenterally administered vaccines are being studied extensively. Microbiota-targeted interventions, such as synbiotics, prebiotics and probiotics, are also in evaluation for their capacity to optimize immune responses to vaccination[6] (Clinicaltrials.gov; for example, NCT01616693, NCT05157425, NCT04798677 and NCT04884776). The integration of clinical multi-omics data (metagenomics, metabolomics, metaproteomics) with host variables (immunological parameters, genetic markers, demographics) continues to improve our understanding of the associations between key microbial taxa and products and immunological phenotypes[7]. However, moving beyond correlative studies to investigate the specific effects of microorganisms and their metabolites in enhancing or suppressing vaccine responses will require effective in vivo models capable of appropriately representing the breadth of human immune responses.

Rodent models for immune responses to microorganisms

House mice (*Mus musculus*) serve as one of the most fundamental in vivo platforms for modern biomedical research. These animals are highly tractable for genetic manipulation, immune cell transfer between hosts, and kinetic tissue analysis, thus providing key insights into immunology and pathophysiology. However, to increase research reproducibility, almost all current studies are conducted using specific pathogen-free (SPF) mice that live in barrier housing and are protected from natural exposure to many pathogenic and commensal microorganisms. A series of powerful recent analyses have shown that, compared with free-living mammals, SPF-housed laboratory mice have profoundly underdeveloped immune systems that lack differentiated effector memory T cells[8,9]. This deficiency in immune development may help explain why the results of some preclinical studies in SPF mice are discordant with clinical outcomes in humans[10]. Furthermore, subtle microbiota variation among SPF mice (for example, mice obtained from different vendors or breeding cohorts) can be associated with dramatic alterations in immune or colitis phenotypes[8,11]. This raises the possibility that in the absence of robust microbial exposures, minor differences in endogenous bacterial communities may confer outsized effects. Importantly, it has also recently emerged that intentional exposure to the microbiota and pathogens common in mice raised outside barrier facilities can restore the unnaturally naïve immune systems of SPF mice to a more mature state. These microbially

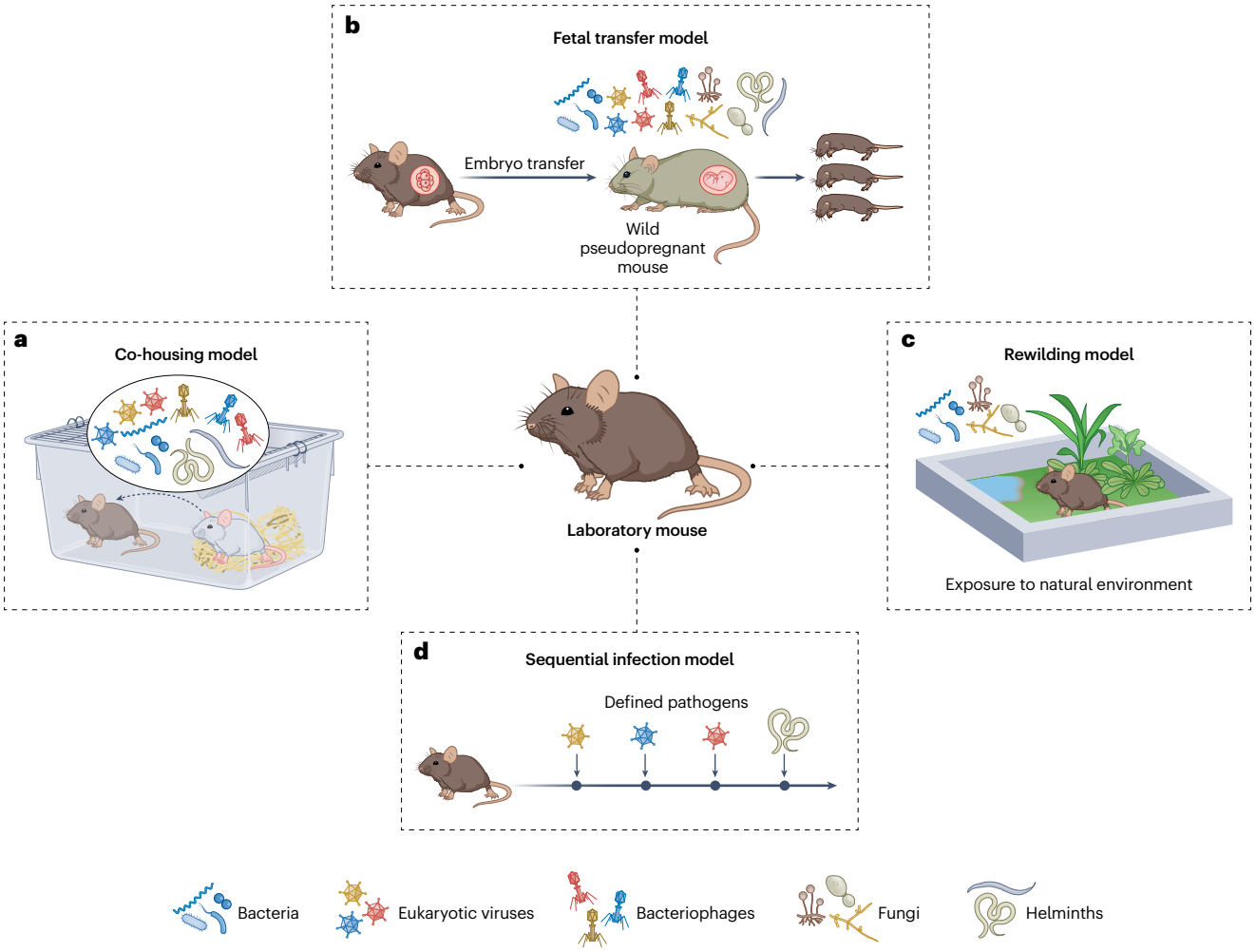

Fig. 1 | Rodent models using microbial exposure to enhance relevance to human immune responses. a–**c**, Laboratory mice are maintained in SPF housing conditions that limit their microbial exposomes. Co-housing laboratory mice with pet-shop mice (**a**), performing fetal transfer of laboratory mouse embryos to pseudopregnant wild mice (**b**), or rewilding by exposing mice to a natural environment (**c**) can reproduce natural microorganism exposures and variably transmit agents that can include bacteria, eukaryotic viruses, bacteriophages, fungi, helminths and/or mites to SPF laboratory mice. **d**, Intentional sequential infection of laboratory mice with a panel of known pathogens provides an alternate method to enhance microbial exposures.

exposed, or 'dirty' mice, may better mimic adult humans[9], which may render them substantially more useful as preclinical models. The restoration of natural rodent microbial exposures is thus a developing research direction.

Inbred SPF laboratory mice exhibit immune systems more similar to neonatal humans than adult humans. By contrast, wild mice captured from free-living barn populations or commercially available mice from pet shops exhibit massively altered steady-state levels of innate and adaptive immune cell numbers, serum cytokines and antibodies[12]. While wild or pet-shop mice can be directly used for experimentation, given the outbred nature of these 'dirty' animals, studying them foregoes the benefits of genetically defined laboratory mice. These assets include phenotypic consistency and reagents such as tetramers that have been developed specifically for them. There are also many other variables for wild or pet-shop mice that often cannot be controlled, including species, age, diet, condition and non-microbial exposures, which can be affected by geography and climate.

Generating 'dirty' mouse models

Numerous distinct strategies have been devised to generate microbially experienced 'dirty' mice in well-defined genetic backgrounds, such as the commonly used and highly tractable C57BL/6 or 'black 6' lines. Co-housing laboratory mice with pet-shop mice, transfer of laboratory mouse fetuses to pseudopregnant wild mice, and 'rewilding' models all incorporate enhanced microbial exposures (Fig. 1). Each approach comes with inherent advantages and disadvantages. Co-housing, where SPF mice are kept in the same cage as pet-shop mice (Fig. 1a), transfers microbiota and natural mouse pathogens, including viruses, fungi, helminths and mites, to laboratory mice at physiological doses through natural routes, such as faecal–oral transmission. This model successfully reproduces wild mouse immune phenotypes, including the frequency and activation status of innate and adaptive immune cells in the blood and secondary lymphoid organs of co-housed mice[9,13]. In addition to better recapitulating human transcriptional signatures associated with vaccination, co-housing is also associated with reductions in

humoral and protective T cell responses to vaccination. This indicates that SPF mice exhibit exaggerated adaptive immune responses to these challenges compared with co-housed mice[13]. 'Wildlings', or laboratory mice born to wild mice after fetal transfer (Fig. 1b), reproduce natural microorganism exposure including various microbial and multicellular pathogens from the beginning of life. These mice exhibit a microbiota that is stable over time and is resilient to environmental challenges[8]. Compared with SPF mice, wildlings have been shown to phenotypically replicate human immune responses in preclinical studies of two immune-targeted therapies[8]. In the rewilding model (Fig. 1c), laboratory mice are released into an outdoor enclosure facility with vegetation and barn-like structures but no other mammals, thereby limiting the introduction of natural rodent pathogens. This aims to restore exposure to natural ecological microorganisms. As a result, these rewilded mice exhibit immune system maturation and microbiota alterations associated with acquisition of environmental fungi[14]. While these diverse methods have all yielded potent effects on the immune system, a consistent challenge is the limited control investigators have over which pathogens and microorganisms are passed to the laboratory mice or the timing of infections. This is particularly important because it remains unclear whether broad changes to the endogenous microbiota or exposure to specific pathogens serves as the key driver for immunomodulation. Robust changes to bacterial communities have been consistently observed across all 'dirty' models profiled thus far, and seem to be accompanied by alterations to viral and fungal constituents as well. Further, these approaches often require extended exposure times or an additional generation before experimental mice are available. A separate 'dirty' mouse or outdoor facility is also required, which will increase costs and make broad implementation challenging.

A more reductionist way to generate mice with an experienced immune system is through a series of intentional sequential infections with a panel of known pathogens (Fig. 1d). Sequential infection of C57BL/6 SPF laboratory mice with murine γ-herpesvirus, murine cytomegalovirus, influenza virus and the helminth *Heligmosomoides polygyrus* is sufficient to mature the immune system[15]. Specifically, after sequential infection, gene expression profiles from these exposed mice better resemble those of pet-shop mice than those of SPF mice, as well as adult humans as opposed to neonatal humans. Further, sequentially infected mice exhibit reduced antibody responses to vaccination, similar to other 'dirty' models[13,15]. This approach specifically implicates pathogens as critical exposures for immune system maturation. Like the other transfer models described above, the sequential infection model can leverage established mouse genetics and associated tools. Moreover, unique to this method is that it can be performed in a traditional Biosafety Level 2 animal facility without the need for high containment or nonstandard housing. It is highly feasible to determine the specific effect of single or multiple pathogens on identified phenotypes because the microorganisms used are known. The sequential infection model does have its own limitations. This model requires expertise in preparation of pathogen stocks and can be labour intensive with repeated inoculations. It also requires mice to be maintained for an extended interval while infections are completed. In sum, however, due to its repeatable, flexible and controlled nature, we believe that the sequential infection model is the easiest to implement widely in order to better mimic human immune responses in rodents.

Future outlook

As these rodent models of enhanced microbial exposure exhibit dampened humoral responses to vaccination[13,15], they provide convincing evidence that diverse microbial experience may underlie the suboptimal vaccine responses observed in low- and middle-income countries. However, many important open questions remain. First, what is the optimal degree of variation in immune responses among mice to best mimic humans? Is it more desirable to have a strictly controlled series of exposures as is achieved in the sequential infection model for maximum reproducibility? Or is the level of microbial variability derived from co-housing or rewilding likely to provide a better representation of the varied microbial exposome of humans? How does variability in microbial exposures interact with variability in rodent genetic background, and can tools such as the Collaborative Cross be leveraged in conjunction with microbial exposures to capture human diversity? Which method(s) will yield the clearest experimental results and/or best predict human responses?

Further, as discussed above, the human microbial exposome varies dramatically with age and geography. Can rodent models be designed to effectively capture differing degrees of human exposure? Could combining 'humanized' mice, which have been engrafted with human cells, with the humanizing effects of microbial exposures yield fully optimized models for immune studies?

Additional questions include which specific microorganisms drive immune maturation, how do they mediate these effects, and what is the degree of overlap between taxa that are sufficient to confer maturation? Or alternatively, is there simply a threshold for diverse antigen exposure that must be overcome? Do these microbial exposures mediate other physiological changes in rodents that modulate the immune system indirectly, such as changes in metabolic pathways? Finally, while pathogen exposure has been implicated as sufficient for immune maturation, the role of the endogenous microbiota, which may itself be modulated by infections, in this process has not yet been defined. Does the microbiota mediate direct or indirect contributions to immune maturation? And can robust immune activation render subtle vendor- or facility-associated differences in the microbiota negligible? There is increasing recognition that a reconfiguration of how we approach mouse studies is needed. Further mechanistic insights from these 'dirty' mouse models will be critical to develop the optimized and readily implemented model or models that best represent specific human populations relevant to a given study. And indeed, consideration of how microbial exposures influence other animal models routinely used in vaccination trials, such as non-human primates, will also be of critical importance moving forward.

Our appreciation of the importance of the microbial exposome in modulating immune responses, particularly to vaccines, is increasing. Thus, continued investigations in this area should be vigorously promoted. We propose that the field must prioritize delineation of specific immunological criteria for rodent models that are considered 'human representative'. This consensus should involve a consortium of investigators active in this area and include a shared definition of appropriate ranges for a subset of immune assays. Achievement of this would facilitate further refinement of current models and/or development of approaches to microbially enhance the immune system to better model humans.

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