Mice, Models, Microbiota: How Can We More Accurately Reflect Human Disease?

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ABSTRACT

With growing evidence that human disease is affected by the microbiota, many researchers have sought to modulate the microbiomes of mice to improve translational research. Altering their microbiomes, which are usually germ-free or specific pathogen-free, might allow mice to more accurately model human disease and hence produce more applicable findings. However, this has been difficult to apply to individual projects due to the disparity of explained methods and results. In this review, we first describe the immunological functions of the gut microbiota and the methods of altering mice microbiota, from transplantation route to age of transplantation to microbiota source. We then present an approach for how the gut microbiota might be considered when modelling human disease in mice. By organizing findings by type of disease - neurological, immunological, chronic inflammatory, and cancer - we propose that mouse models can be improved by considering the source of the microbiota, the presence or absence of certain microbial phyla, and by timing the transplantation during a physiologically relevant stage of development, such as the first five weeks of life.

Keywords: Microbiota, Translational Research, Mouse Models, Disease Models, Microbiota Transplantation

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WHY USE MOUSE MODELS? HISTORY OF TRANSLATIONAL RESEARCH

Due to its small size, rapid breeding, and genetic tractability, the laboratory mouse (a genetic hybrid between *Mus musculus domesticus* and *M. musculus musculus*) is the animal model of choice for research on human disease. Their utility is conferred by their genetic similarity to humans, since they share 97.5% of their coding genome with us, and hence scientists assume that their metabolic and disease phenotypes likely resemble ours. Therefore, studies on disease phenotype and drug effectiveness in mouse models are predicted to 'translate' to humans.

Translational Research in Mice

When discrepancies between mouse models and their human counterparts were manifest in early studies, scientists were quick to blame genetics¹. Comparing the different species' protein sequences to their functions, the mere 2.5% difference in the coding sequence were explained to alter the activities of proteins and their affinities to binding targets. Scientists therefore proposed that 'humanizing' key mouse cells could increase translational research value. 'Humanization' involves replacing mouse immune cells with human immune progenitor cells. An example is the PBMC humanized mouse, which expresses human peripheral mononuclear blood cells (PBMCs) instead of mouse ones, and therefore can be used as an *in vivo* model to study the human immune system².

This was hoped to solve the problem of low research translatability for mice in diseases affecting non-conserved pathways. However, 'humanization' of the mouse immune system was not a panacea to the problems of translational research. For example, mouse strains bred to model human disease as accurately as possible, such as the non-obese diabetic (NOD) mouse strain to model autoimmune diabetes, still have many differences between the mouse and human phenotypes. Since 'humanization' of mouse cells has failed to solve this problem, scientific consensus began to point towards other players in phenotype, the most prominent of which was the microbiome.

Typically, laboratory mice are housed under specific-pathogen free (SPF) or germ-free (GF) conditions to prevent unpredictable disease outbreaks and to control for the effects of a potential pathogen on disease outcomes. Due to a complete absence of microbes, GF mice develop abnormalities of their immune systems^{3,4}. While this problem exists to a lesser degree for SPF mice, they too show immune defects relative to their counterparts with wild-derived microbiotas as they often lack certain health-benefitting microbial species, such as segmented filamentous bacteria (SFBs)⁵.

Fortunately, the practice of ignoring the microbiota is now reversing; scientists are now focusing on adding instead of removing the microbiota to increase translational research value.

TRANSPLANTATION METHODOLOGIES

There are many procedures to perform microbiota transplantation, with varying effectiveness. The main procedure is oral-fecal transplantation, which involves orally gavaging (force-feeding) mice with fecal pellets. Though GF mice are usually used, mice with pre-existing microbiotas can be fed fecal pellets after repeated antibiotic treatments to displace the original microbiota of the mice. In general, oral antibiotics eliminate 90% of the pre-existing microbiome allowing the newly transplanted microbiota to engraft after a single oral transplant, though multiple fecal-oral transplants allow for greater stability in the new microbiota^{6,7}.

Besides the method of transplantation, there are other variables that must be considered when transplanting microbiomes. They are summarized below:

The overall microbiome diversity. Microbiome alpha-diversity, which indicates the number of different taxa within the community, dictates the diversity of antigens that can be provided to the immune system and can help mature the immune system into an adult-like form⁵. Ideally all mouse model research should be performed using microbiomes that are more diverse than GF or SPF mice.

The presence of certain key species. Each microbial species synthesizes its own metabolites, which can contribute to the training of the immune system by acting as antigens or by participating in modulating the immune response. There are a few key species that we know so far that impact immune development listed in Table 1.

The life stage of the mouse when transplantation occurs. Since irrecoverable development of the adaptive immune system occurs early in the fetal and postnatal period, transplanting a microbiota in the postnatal period (0-5 weeks) is critical for giving mice a healthy adaptive immune system. Nevertheless, transplantations during adulthood still achieve some changes to immune phenotype, especially regarding the innate immune system⁸.

SOURCES OF MICROBIOTA USED IN TRANSPLANTATION

The main sources for microbiota transplants are humans and 'dirty' mice, both of which have higher microbial diversity than typical lab mice. The term 'dirty' refers to more diverse microbiomes derived from wild

Bacterial Species or Taxa	Immunomodulatory Effects in Mice	Mechanism	References
<i>Segmented filamentous bacteria (SFB)</i>	increase Th17 effector pool	produces SAA (serum amyloid antigen) drives Th17 differentiation by IL-1B induction	[14] [15] [16]
<i>Bacteroides fragilis</i>	increase IL-10+ T-cells, increase Th1 effector cells, reduce Tregs	Polysaccharide antigen (PSA)	[17] [18]
<i>Lactobacillus</i> (<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. salivarius</i> , and <i>L. lactis</i>)	anti-inflammatory (many adaptive and innate immune cells involved)	SCFAs and cell-surface carbohydrates which signal via TLRs	[19] [20]
<i>Bacteroides</i> (<i>B. bifidum</i> and <i>B. lactis</i>)	Immunomodulation and metabolic function	SCFAs	[21]
<i>Clostridium</i> (clusters IV, XIVa and XVIII)	Increases Tregs	SCFAs	[22]

Table 1 Bacterial species and taxa which exert immunomodulatory effects. The list includes common bacteria which play important immunomodulation roles.

mice or pet-store mice, since these organisms have the potential to drive greater immunological experience than cleaner laboratory GF or SPF mice. To confirm the differences between dirty and clean lab mice, one study assessed the number of CD8+ effector memory T-cells and found that immunological exposure of the lab mice to the microbes from pet-stores during cohousing increased the population of these cells⁹. This indicates that the microbiota of the pet-store mice was outcompeting the lab microbiotas, but not the reverse, since the laboratory mouse microbiome failed to transplant into the wild mouse¹⁰.

Another factor to consider is the species-specific relationship of the microbiota being transplanted. Dirty microbiotas that are adapted specifically to *Mus musculus* mice can transplant successfully into typical lab mice, whereas human microbiomes cannot transplant as successfully due to inter-species differences in microbial composition and function¹¹. Instead, the composition of the human microbiome when transplanted into mice changes into a new stable state, where phyla related to pre-existing mouse phyla enrich and microbes with metabolites or functions unique to humans are reduced or eliminated. For example, transplantation of asthmatic human microbiomes with genera *Bacteroidaceae*, *Peptostreptococcaeae*, and *Clostridiaceae* into mice that lacked those genera resulted in successful colonization of the first two, but no colonization of the third genera since no mouse-specific *Clostridiaceae* spp. niche developed¹². While this alteration in the microbiota to a mouse-like state

may be in part due to the dietary differences from humans, it will nonetheless have consequences on host phenotype if important immunomodulatory phyla are lost or enriched¹³.

AUTOIMMUNE CHRONIC DISEASE MODELS

Transplanting the human microbiota into mice is another solution to increase research translatability. Due to the bidirectional relationship between disease and the gut microbiota, dysbiosis in the gut microbiome impacts the immune system. Therefore, transplanting dysbiotic microbiotas from humans into mice may replicate this immune phenotype in mice. This section will attempt to summarize how this can be executed successfully, and with what limitations.

Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a neurodegenerative and inflammatory disease where immune cells, both innate and adaptive, destroy the myelin sheath of neurons in the central nervous system. One commonly used mouse model for multiple sclerosis, experimental autoimmune encephalitis (EAE), mimics the human disease process by activating CD4+ T helper cells with specificity towards myelin proteins. The EAE model mice are usually raised in specific pathogen-free (SPF) conditions since germ-free (GF) mice fail to develop EAE¹⁴. Thus, even before conducting experiments with microbiota transplantation, it is evident that the microbiota affects EAE mice as its presence seems to

be a prerequisite for disease.

Improving the EAE model by using human-transplanted microbiota relies on the presence of specific microbial taxa. MS microbiotas are enriched in *A. muciphila* and *Acinetobacter* genera and are reduced in *Parabacteroides* (i.e. *P. distasonis*) relative to MS-free counterparts. During microbiota transplants, *A. muciphila* did not colonize mice, but *Acinetobacter calcoaceticus* did. Their colonization reduced Treg cells and increased IFN γ + Th1 cells, hence indicating a shift from anti- to pro-inflammatory state. Likewise, a separate procedure involving *P. distasonis* transplantation led to increased IL-10 levels, as observed in humans, confirming the IL-10 dependent mechanisms of gut microbes in causing MS pathology. These results are instructive for microbiota-enhanced disease modelling at large since they show that the effects of an individual species in disease pathophysiology are key to mouse modelling. Importantly, in showing that not all human-transplanted bacteria can colonize mice, they also reiterate that mouse models can only model - and not replicate - human disease¹⁵.

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease results in destruction of the colon's absorptive abilities. Though genetic risk factors can influence whether someone develops the disease¹⁶, research has shown that microbial dysbiosis can also promote the development of IBD¹⁷. One study transplanted human microbiomes from IBD patients into model mice and observed an increase in Th17/Th2 cells and a decrease in Tregs, matching patterns observed in humans¹⁸. Similar experiments have identified other relationships between the microbiota and disease progression^{19,20,21}, prompting researchers to investigate the role of the microbiome as a therapeutic platform. The current models for IBD research are usually *in vitro* cell cultures or laboratory mice, both of which lack the research translatability of humanized microbiota mice²⁰.

Although some difficulties have arisen where human microbiota transplants into mice led to variable effects, such as microbiotas from IBD remission patients failing to confer remission in IBD in mice²¹, the general trend has been that transplanting human IBD microbiotas into mice exacerbates IBD-like symptoms^{23,24}. Humanized microbiota mice are already being used to discover drugs, such as PACAP (Pituitary adenylate cyclase-activating polypeptide), which has succeeded in preclinical mouse trials and is set to be used in human clinical trials²⁵.

CANCERS

Although cancer is a genetic disease, its prognosis

and treatment are impacted by the gut microbiota. This is because the microbiota impacts inflammation and immunity systemically and not only at the level of the intestine, and thus may play a role in carcinogenic inflammatory processes. An introductory review on the relationships of the microbiome to cancer development and therapy is given by Nishimura et al., 2019²⁶. This review will focus on the relationship of the microbiota to 3 of the most common cancers (colorectal, lung, and breast).

Colorectal Cancer

Although microbiome-host interactions occur for cancers around the body, they are most easily studied with respect to colorectal cancer (CRC) since the cancer is directly in contact with the intestinal microbiota. Certain microbiome profiles have been linked to the adenoma-adenocarcinoma sequence, which involves genomic instability triggered by the inactivation of tumor-suppressor genes, including APC (adenomatous polyposis coli), and further studies have identified the specific microbial species responsible for driving this mutational sequence²⁷.

The relationship between CRC development in humans and the microbiota involves a few key bacterial species, namely *Fusobacterium nucleatum*, *Bacteroides fragilis*, and pathogenic colibactin-expressing *E. coli* among others^{28,29}. While many of these species produce metabolites that alter the gene expression profiles of colorectal epithelial cells to help trigger cancer, the key driver of CRC mutation is likely *F. nucleatum*, which has an adhesin called FadA that binds to E-cadherin and increases β -catenin signaling to trigger epithelial cell growth³⁰. The effects of *F. nucleatum* are localized to CRC cells since they have a receptor, Fap2, for CRC's upregulated Gal-GalNAc glycoprotein^{31,32}, thereby enriching *F. nucleatum* at sites of colorectal tumors.

Lung Cancer

Lung cancer has been linked to microbial dysbiosis. This includes the gut microbiome (gut-lung axis) as well as the local lung microbiota. Researchers determined that the lung microbiota plays a role in the acceleration of cancer development by inducing a pro-inflammatory environment. Jacks et al. (2018) gave antibiotics to mice with oncogenic mutations and found that antibiotic treatment reduced tumor growth³³. The researchers also identified an increase in *Herbaspirillum* and *Sphingomonadaceae* bacteria in cancerous mouse lungs, which differs from the observed increase in *Helicobacter pylori* and *F. nucleatum* in human lung tumors. To increase correspondence between human and mouse lung microbiotas, a feasibility test was performed by transplanting human *H. pylori* into healthy mouse lungs. This induced a cancer-associated

Figure 1 Mice with more diverse microbiomes (for example, mice colonized with a microbiota derived from a wild mouse) develop an adult-like immune system with more effector and memory T and B lymphocytes, and increased monocytes, granulocytes, and ILCs in peripheral lymphoid and non-lymphoid tissues. There are also fewer pro-inflammatory cytokines and more anti-inflammatory cytokines as the Treg population is increased. A more diverse microbiota leads to more autonomic nervous signaling between the brain and gut. Although this is not labelled in the diagram, a more diverse microbiota also impacts the endocrine system by producing more endocrine hormone analogues.

cytokine profile³⁴, meaning that humanized lung microbiomes could potentially be used as a humanized microbiota lung cancer mouse model.

Interestingly, the above experiments focused only on the lung microbiome and concluded that microbiome effects in lung cancer are local, since the fecal bacterial load remained unaltered. This conclusion fails to recognize the significance of the gut microbiota in impacting the prognosis of lung cancer, since the intestinal microbiome has systemic effects on immunity and inflammation. Hence, intestinal probiotics should be tested for their ability to modulate cancer development. One example is the use of cisplatin, which does not significantly reduce tumors in mice unless administered with the human-derived bacteria *L. acidophilus*³⁵, elucidating an improvement to an existing chemotherapy.

Breast Cancer

Breast cancer is the most common cancer in adult women and one major variable involved in breast

cancer progression is the microbiota. For example, the bacterial species, *Helicobacter hepaticus*, increases neutrophil recruitment to the breast tissues leading to cancer development in certain mouse models³⁶. Another mechanism involves production of estrogen-like compounds by a few human bacterial species, such as *Escherichia coli*, *Klebsiella sp-1-1-55*, *Prevotella amnii*, *Enterococcus gallinarum*, *Actinomyces sp. HPA0247*, *Shewanella putrefaciens*, and *Erwinia amylovora*³⁷. These bacteria provide estrogen-like compounds by metabolizing dietary lignans into estrogen-like compounds such as enterodiol or enterolactone that alter the growth of breast tumors^{38,39}.

DRUG-RESPONSE MODELLING IN MICE

Due to the importance of the microbiota in modulating the immune system and metabolizing drugs, preclinical trials of chemotherapeutics should be performed in humanized microbiota animals. Preclinical studies in conventional lab mice often fail to take the microbiota into account, which may contribute to the low rate of translation of drugs from mouse models to humans.

It is plausible that some human-specific bacteria are metabolizing the drug in an unforeseen way.

In the case of synthetic monoclonal antibodies against CTLA-4 (a checkpoint inhibitor used as an anti-cancer chemotherapeutic agent), genetically similar mice from different facilities had microbiotas differing in relative abundance of *Bifidobacterium* spp. and increases in *Bifidobacterium* frequency were shown to cause increased efficacy of the drug. This finding matched a finding in humans, where the presence of the species *B. thetaiotaomicon* and *B. fragilis* conferred drug responsiveness⁴⁰. Similarly, with checkpoint inhibitors of the PD-1/PD-L1 axis, drug efficacy was shown to be related to the presence or absence of the *A. muciniphila*, since it increased IL-12 and CD4+ T-cells, which are needed to achieve the full drug response. These findings stress that translatability of results depends on the resemblance of the microbiotas of mice to their human equivalents. If warranted, personalized medicine can also be performed using humanized microbiota mice for each patient, where microbiome profiles can be investigated to see how the drug will be metabolized^{41,42}. A summary of microbial taxa associated with responsiveness or non-responsiveness to immune checkpoint inhibitors is given by Wu Kongming et al. (2018)⁴³.

LIMITATIONS AND FUTURE DIRECTIONS

While studies with human-microbiota associated (HMA) mice may increase research translatability, immunological and physiological deficiencies in HMA mice remain that may confound experimental findings⁴⁴. These differences arise from the specificity of the host-microbiota relationship—only 15% of bacterial strains and 4% of microbiome genes are shared between both mice and humans⁴¹. Even when a given bacterial species is shared between two hosts, such as segmented filamentous bacteria (SFB) present in both mice and rats, interchanging the species-specific strains abrogates their functions; indeed, SFB from rat intestines fails to induce Th17 development in mice, highlighting the existence of strain differences among bacterial species⁵. Given that human microbiotas are even more different from mouse ones, non-specific host-microbe interactions in HMA mice are an obvious confounding variable.

To overcome these limitations with HMA mice, the following approaches should be considered. The inspiration for the following was taken from a review by Brett Finlay et al., (2016)⁴⁷. Firstly, when a healthy state is being researched using mice, such as ecological studies about infections, diet, or genetics, it is recommended to use a wild-mouse microbiota. Because wild mice share the similar immunomodulatory bacteria as lab mice, yet have a higher diversity,

transplanting their microbiome will lead to better immune training. Secondly, if a human disease is being modeled in mice, using a disease-specific microbiota transplant from humans, and transplanting during the neonatal window of opportunity will provide the most translatable mouse model. Next, to ensure functional similarity between the transplanted microbiota in the recipient and in the donor, the metabolic profiles of the pre-transplantation and post-transplantation microbiotas should be compared. Although the usual protocol is to assess the relative abundance of different phyla by OTUs (operational transcriptional units, from 16S rDNA sequencing), this comparison is not as good as comparing the metabolic profiles since the microbial metabolites are what exert their mechanisms of action on the host and the metabolic profiles of structurally different microbiotas may be similar. Even if the OTU abundances differ between the donor and recipient, the microbiotas are functionally similar if their metabolic profiles are similar. Finally, humanized microbiota animals are not a replacement for existing animal models; instead, they should be viewed as an addition to the repertoire of diverse models, each with their own specific uses. As discussed with the MS-microbiota transplant mice, there are functional differences between the donor and recipient microbiotas and using multiple mouse models in studies may increase translational research value since different mouse models make up for one another's limitations.

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COMPETING INTERESTS

No competing interests declared.

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