nature biotechnology

Laying better plans for mice

Genetically defined germ-free animal models colonized with defined microbiota are crucial for progress in microbiome research.

This issue presents several articles detailing advances in human microbiome research and how that knowledge may be translated into new treatment options. As large-scale efforts to sequence genomic DNA from microbiota continue apace, evidence is gathering that pathological imbalances in the human microbiota (dysbiosis) are associated with disorders such as colitis, diabetes and obesity and may modulate the body's metabolism of many common pharmaceuticals. Thus far, most of these studies have remained exploratory, enabling cataloging of the human microbiota and delineation of some of the changes in membership—and therefore microbial functions—that correlate with disease. But proving causation and understanding the mechanisms through which dysbiosis might contribute to disease will require the wider adoption of animal models in which factors such as host genotype, microbiome genotype, diet and exposure to drugs can be placed under stringent control.

Since the microbiome field's inception some 15 years ago, a variety of animal models have been adopted to study links between changes in the microbiota and disease pathogenesis. These range from invertebrate models, such as zebrafish, through to mice and large-animal models, such as pigs. As in many other areas of investigation, however, it is the mouse that reigns supreme. Indeed, today nearly 3,500 strains of isogenic mouse models are available for use in research.

In the early days, reciprocal transplantations of native zebrafish and murine microbiota to germ-free recipients revealed the dependence of microbial communities on host selection pressures; for example, germ-free mice colonized with zebrafish microbiota ended up with a more 'mouse-like' than 'zebrafish-like' gut community, and vice versa (*Cell* **127**, 423–433, 2006). More recent studies examined the effects of host genotypes on microbiome membership to further decipher the complex interactions between hosts and their microbes. For example, deficiency of the intracellular receptor NLRP6 in mice results in reduced expression of interleukin-18 and altered fecal microbiota (*Cell* **145,** 745–757, 2011).

And yet the field is still wrestling with how to enhance reproducibility and increase comparability between different studies in germ-free animals.

The first step in addressing variability among experiments is to ensure that germ-free mice are really germ-free. Generating such mice is not trivial and involves axenic embryo transfer and delivery of mice in an aseptic environment. Good husbandry is also critical for maintenance of germ-free conditions. Experienced researchers and technicians appreciate that numerous factors, such as differences among autoclaves, changes in chow and even the length of time that chow is sterilized, can also affect microbiota outgrowth.

There is thus a need to standardize protocols and share the know-how gained in experienced laboratories on how to generate and maintain germ-free animals. Such expertise remains in short supply in this still-young field.

A second step is to further hone our ability to precisely control the microbiota. Just as we would not use outbred mice for experiments aiming to pinpoint functions of genetic loci, some researchers think that we should not use outbred microbiota in animal models. Using a defined standard set of microorganisms (e.g., altered Schaedler flora, which is a mixture of eight defined bacteria) to standardize mouse models, or introducing microorganisms one bacterial genus at a time to monitor effects on basic aspects of physiology and immunology are two of the options that are currently being explored.

Even once a germ-free model has been established, great care should be taken over the ensuing experimental design. One option to reduce intercage variation is to put knockout and wild-type mouse models in cages together so that they share their reconstituted microbiota. Another option is to use sufficient numbers of mice to reduce confounding that might occur owing to any subsequent differences in outgrowth of acquired microbiota. And then there are issues that go beyond establishing the model with defined host and/or microbiome genotypes and robust study design. Does one track genetic exchange among members of the microbiota or exchange between the microbiota and the host? All of these issues have the potential to affect outcomes.

One way good practice could be encouraged and expertise shared with the wider community is through the establishment of central repositories of germ-free mice and germ-free protocols. Although laboratories currently can and do share germ-free mice, clearly this is not feasible on a large scale. Thus, the US National Institute of Allergy and Infectious Diseases is working to establish a consortium of germ-free mice facilities across the United States, which will enable sharing of strains and breeding pairs. It has already established a gnotobiotic mouse facility with three strains of mice and has plans to generate more. And in Europe, the ECGnoto consortium aims to tackle the problem by sharing information and harmonizing the generation and husbandry of germ-free mice. The consortium also has the goal of defining a set of standard microbiota for use in control model mice.

Nature Biotechnology fully supports these initiatives. Until reliable models and standardized animal protocols become more readily available, the ability to adequately probe cause and effect in 'supra-organisms' is likely to be restricted to a handful of laboratories. For microbiome research to move forward, more germ-free animals must be put in the hands of more researchers. And more know-how has to be shared concerning their use. This will enhance the reproducibility of studies of the microbiota. It will enable the research community to reach a consensus on best practice and the minimal information for describing experiments. And ultimately it will enable the field to move from correlation to rigorous proof of disease causation.