Laboratory mice housed under germ-free conditions. Photograph courtesy of Taren M. Thron, California Institute of Technology.

⇒ MILESTONE 3

Gut microbiota transfer experiments in germ-free animals

Germ-free animals are raised under sterile conditions to prevent colonization with bacteria and other microorganisms. These animals (mostly mice and rats, but also guinea pigs and chicks) have a life span similar to that of conventional, normally colonized animals, although they seem to have a slower or impaired growth as well as some anatomical and physiological differences (such as an enlarged cecum). By the 1960s, germ-free animals were a well-established tool in nutritional studies aiming to understand the contribution of the intestinal microbiota to host dietary requirements (for example, with regard to the synthesis of vitamins).

In 1965, Schaedler and colleagues introduced a new use for GF animals: the transfer of bacterial cultures to germ-free mice. Such transfer experiments have been essential in studying the effects of the gut microbiota on the host ever since. In their pivotal study, Schaedler and colleagues reported the results of feeding bacterial cultures isolated from the gut of Nelson–Collins–Swiss (NCS) mice (a colony of albino mice that are free of ordinary mouse pathogens as well as intestinal *Escherichia coli* and *Proteus* spp.) to germ-free mice. The germ-free mice were fed food inoculated with individual bacterial

cultures of several anaerobic isolates. After one week, the numbers and localization of these bacterial strains in the gastrointestinal tract were comparable to those observed in the NCS mice and remained stable for several months, confirming the feasibility of microbiota transfer experiments and their usefulness for studying bacterial gut colonization. Importantly, transfer of a *Bacteroides* strain partially reduced the cecum enlargement typical of germ-free mice, and the offspring of germ-free mice that had been colonized with a mixture of strains inherited those strains and subsequently showed normal cecum size and structure. These results directly showed the important and profound effect of the gut microbiota on host development and physiology.

This landmark study paved the way for further research on the effects of the gut microbiota on the host and on the interactions

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between different species of the gut microbiota. For example, one strain of Lactobacillaceae that could 7α-dehydroxylate bile acids in vitro did not have the same catabolic activity when transferred to germ-free rats until additional bacterial strains were introduced. Another study in germ-free rats also showed the metabolic capacity of the gut microbiota, specifically the reduction of bilirubin to urobilins, which had been assumed by some to be produced by the liver. These initial studies laid the ground for detailed work that explored the links between microbial and host metabolism (MILESTONE 12).

In addition to exploring the metabolic capacity of the gut microbiota, GF animals were also essential for elucidating the close links between bacteria and the host that determine tissue homeostasis and immune system development. One striking example is determination of the role of segmented filamentous bacteria (SFB), which had previously been shown to closely interact with the intestinal epithelium. Experiments that led to monoassociation of germ-free mice with SFB showed that these bacteria are key determinants of intestinal lymphocyte numbers and phenotype in mice. Subsequent studies have identified many links between specific microbial taxa and/or molecules and host immune function (MILESTONE 9).

Germ-free animals have been, and still are, indispensable tools for studying functional relationships between the microbiota and the host — although, as always with animal studies, the comparability and applicability of the results to humans need to be verified. Nevertheless, the early studies using these models have inspired several avenues of microbiota research and highlighted the important effects the gut microbiota have on their host.

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