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Innovations
FROM IDEA TO IND

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Michael Seiler, Taconic Biosciences

TOOLS & TECHNIQUES

BETTER DIRTY

By Karen Tkach, Staff Writer

While squeaky-clean mouse husbandry has established standardized, reductionist models of mammalian physiology, the practice might be limiting the effective translation of mouse research, especially in the age of immunotherapy, according to two academic teams who have characterized how getting mice "dirty" changes the animals' immunological baselines and responses to infections or vaccines.

In studies published in *Nature* and *Cell Host & Microbe* last month, the teams used different methods but converged on the same idea: dirty mice represent adults better, and "clean" mouse models are for babies. "In a lot of studies, the lab mice are starting from a place that's perhaps very similar to a newborn human, but not to most of us," said Stephen Jameson, principal investigator on the *Nature* study and a professor of laboratory medicine and pathology at University of Minnesota.

The studies showed that peripheral blood mononuclear cells (PBMCs) from dirty mice, which were exposed to microbes representing common infections, resembled PBMCs from healthy adults, whereas PBMCs from clean mice were closer to those in cord blood samples from infants. These results offered preliminary evidence that dirty mice could be better than clean ones for testing vaccines or therapeutics targeting the immune system.

Jameson's group studied the effects of pathogens on immune function by co-housing laboratory mice with mice bought at pet stores, creating a model system with orderly genetics and a messy microbial exposure.

In the second study, a group led by Tiffany Reese mimicked human microbial exposure by sequentially infecting mice with pathogens representing common human infections — two herpesviruses, an influenza virus and a helminth (see "Lousy Mouse").

Reese is an assistant professor at University of Texas Southwestern Medical Center. She conducted the study as a postdoctoral fellow in the lab of Herbert "Skip" Virgin at Washington University in St. Louis.

Michael Seiler, portfolio director for commercial genetically engineered models at Taconic Biosciences Inc., told BioCentury the studies created a lot of discussion at the American Association of Immunologists' annual meeting last week. "The manuscripts have been quite provocative for the field of immunology, and have had an impact on the mouse models life science tools industry," he said.

He said the studies feed into the recent groundswell of activity addressing how microbes influence physiology — including the White House's announcement of a National Microbiome Initiative earlier this month — and Taconic is taking note.

Seiler believes that although pharmas aren't leading the way, they are gradually becoming more interested in new animal models that will translate better to humans.

"They're usually the slower market segment to adopt significant changes. But it has become a major subject of conversation with our pharma clients," particularly with the rise of immunology and immuno-oncology therapeutics, he said.

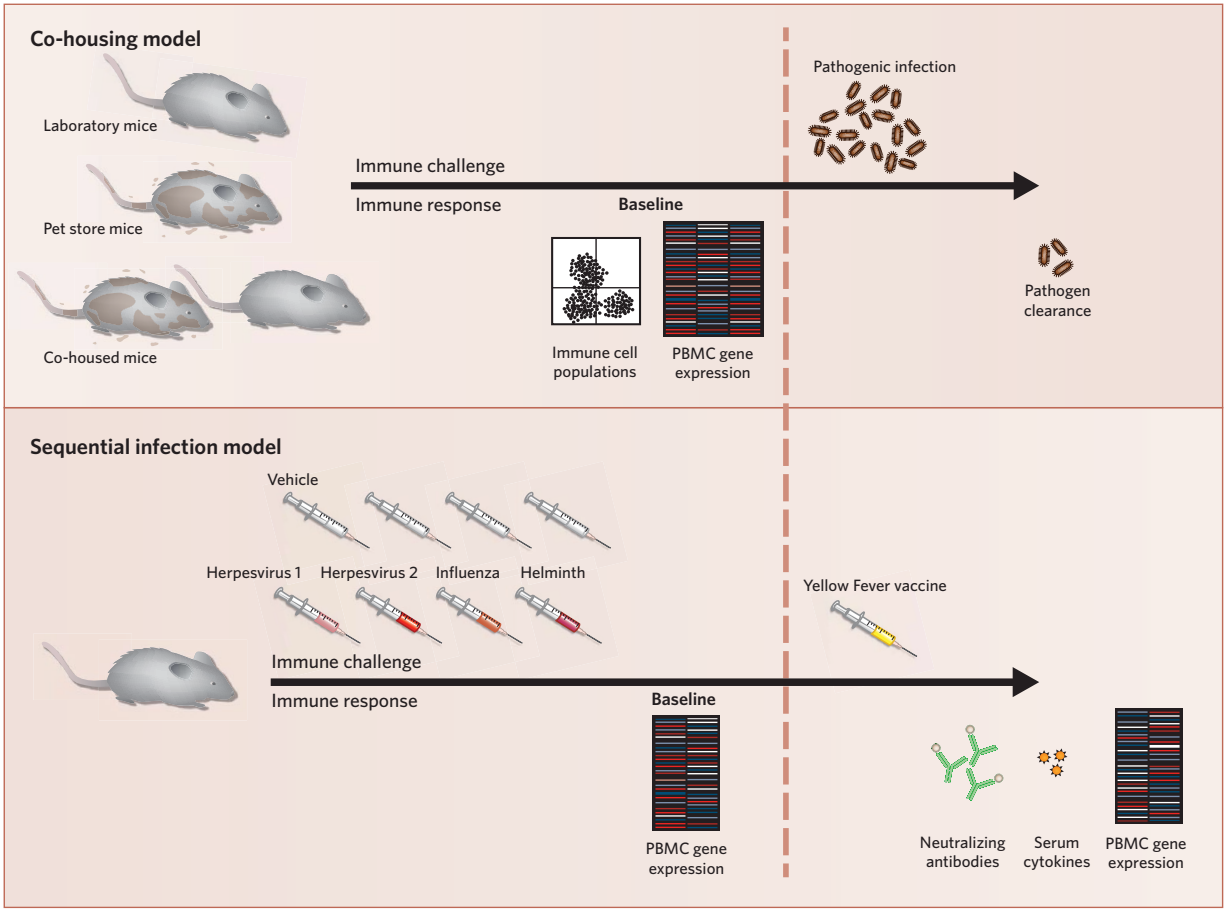
That's supported by comments from Anish Suri, a senior director in the immunosciences team at Johnson & Johnson's Janssen Research & Development LLC unit, who told BioCentury that while the limitations of clean laboratory mice have long been acknowledged among drug developers, there has not been an industry push to develop dirtier models. "I don't think that's a

LOUSY MOUSE

A pair of studies published in *Nature* and *Cell Host & Microbe* introduce two different "dirty" mouse models to characterize how pathogen exposure shapes immune system baselines and responses to perturbations **(red dashed line)** like infections or vaccines.

Co-housing model. In the *Nature* study, the authors compared the immune systems of laboratory mice to those of mice bought in pet stores, and to laboratory mice co-housed with pet store animals. After two months, the authors examined each group's baseline **immune cell populations** in blood, lymphoid and non-lymphoid tissues. They also analyzed peripheral blood mononuclear cell (**PBMC**) **gene expression**. The mice were then exposed to **pathogenic infections** such as *Listeria monocytogenes*, and tested for **pathogen clearance**.

Sequential infection model. In the *Cell Host & Microbe* study, laboratory mice were sequentially infected with two different herpesviruses (**herpesvirus 1**, **herpesvirus 2**), **influenza**, and a **helminth**, and then compared to naïve mice injected with **vehicle**. After at least five weeks, the authors assayed baseline gene expression in PBMCs from both groups. The mice were then immunized with a **yellow fever vaccine**, and the team monitored production of yellow fever-targeting **neutralizing antibodies**, production of **serum cytokines** and the kinetics of **PBMC gene expression**.



really prominent thing that has emerged. But is the information valuable? Absolutely."

Suri said Janssen's approach has been to limit use of animal models to mechanistic studies. "Very soon after that we try to go to translational data sets from human subjects whenever possible. We've been bitten a few times where the mouse was not the best test tube for a clinical indication."

BUGGING OUT

The two teams used RNA microarray analyses to get broad snapshots of how microbial exposure influences gene expression in PBMCs.

Although the two labs began their studies independently, they collaborated during the data analysis stage to compare their respective microarray results using the same tools. In addition to making comparisons between dirty and clean groups in their own experiments, the groups looked for similarities across each other's results, and for similarities to human data from the literature.

The teams identified sets of related genes whose expression is strongly boosted or down-regulated in the different PBMC samples, with a system that accounted for co-regulation between the genes.

Both teams showed microbial experience strongly up-regulated type I interferon immune response genes. "It's the same sets of genes coming up again and again, and they're shared between humans and mice, so we think we're homing in on the ones that have functional relevance," said Jameson.

He noted that similarities in the transcriptional responses across the two studies suggested the pet store-based model, in which the pathogens were not fully known, was capturing relevant immune activity. "The nice thing about similarity with their work is that there, they do know what the animals were exposed to, and we're getting very similar overall results."

He added that despite concerns of "massive variability," the pet store model was also unexpectedly consistent across mice of different ages and sexes, and from several different pet stores. "For pretty much all the things we've looked at, there were high levels of reproducibility," Jameson said.

Jameson noted another big difference between clean and dirty mice was that the clean animals lacked memory T cells residing in non-lymphoid tissues such as the uterus and salivary gland, which are important for front-line immunity to pathogens for many infections.

"There was the realization that when we look in our lab mice, there are hardly any cells in those barrier tissues unless you've actively infected the animals repeatedly, whereas in healthy humans, there are plenty of immune cells in those sites," Jameson said.

FIGHTING DIRTY

The two studies also tested how the mice performed after immune challenges.

In the *Nature* study, exposure to microbes from outside of the lab helped mice fend off pathogenic infections. Three days after infection with *Listeria monocytogenes*, the pet store and co-housed mice had more than 10,000-fold lower bacterial burden than the laboratory mice. In a model of *Plasmodium berghei* infection, the pet store mice also showed lower levels of parasitemia than laboratory mice after five days.

> "A big question is, how do you control dirty?"
>
> Tiffany Reese, UT Southwestern Medical Center

However, in the *Cell Host & Microbe* study, mice exposed to the four model pathogens had less potent long-term antibody responses to a yellow fever virus vaccine than naïve controls.

Reese said the sequential viral and helminth infections could have impacted the vaccine response in many ways. For example, the infections could change the levels of antigen presentation or the activation state of immune cells.

Jameson thinks it's possible that prior exposure to microbes makes immune systems better at defeating certain pathogens, but paradoxically worse at building lasting responses to vaccines, which may get cleared too quickly to mount an effective immune response.

That's consistent with the standard practice of immunizing infants with immature immune systems against preventable diseases. But for vaccine developers targeting adults, the results suggest using clean mice can give misleading results.

"If someone has a really mature, prepared immune system, then a vaccine could get eliminated quickly, and ironically they may get less benefit," said Jameson.

However, he believes the dirty models could have relevance beyond infections and vaccines. In particular, they could be

useful for modeling cancer immunotherapy responses, where they can produce more realistic populations of the tissue-resident lymphocytes present in tumor microenvironments.

"If you have populations of lymphocytes occupying these tissue before cancer arises, do they influence the control of the cancer? On top of that, how would that immunological experience influence responses to cancer immunotherapies?" he said.

Janssen's Suri agreed that the tissue-resident cells are an important feature of dirty mice, and said the models could be important in understanding autoimmunity studies. "If one could track an experienced immune system, maybe one could tease apart particular triggers for the breakdown of tolerance."

> "For vaccine developers targeting adults, the results suggest using clean mice can give misleading results."

KEEPING DIRTY CLEAN

According to Jameson, there is growing interest among academics to study mice with more "adult" immune systems, although the stringent isolation protocols in animal research facilities produce inconvenient hurdles.

"These mice that my kids could have in their bedrooms, we had to have them in a facility where we have to gown up and be very careful," he said. "If any of these microbes get into a regular mouse colony, they would wreak havoc."

Reese thinks a standardized "dirtying" treatment could make studies of experienced immune systems more routine.

"A big question is, how do you control dirty?" she said. "We wonder if there's a set of particular pathogens that you could give mice that would be sufficient to change the mouse immune response such that you could model the humans better," she said.

Taconic's Seiler told BioCentury that giving microbial cocktails to clean mice would be more practical than maintaining colonies of dirty mice, which would invert the field's "test and exclude" approach to microbial exposure, in which mice are screened to keep out unwanted bugs.

"This would be flipping it on its head and saying, how do we have greater inclusion, but still maintain some homogeneity across scaled production, and maintain rigor to ensure consistency?" said Seiler. "I don't rule it out, but at this point I think it's premature to build that into our production schedule."

Neither team stated plans to commercialize their findings, but Jameson told BioCentury his group is open to collaborations to test therapeutic compounds in their mice.

COMPANIES AND INSTITUTIONS MENTIONED

American Association of Immunologists, Rockville, Md.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Taconic Biosciences Inc., Hudson, N.Y.
University of Minnesota, Minneapolis, Minn.
University of Texas Southwestern Medical Center, Dallas, Texas
Washington University in St. Louis, St. Louis, Mo.

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