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What Is the Predictive Value of Animal Models for Vaccine Efficacy in Humans?

Reevaluating the Potential of Mouse Models for the Human Immune System

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Much of what we understand about immunology, including the response to vaccines, come from studies in mice because they provide many practical advantages compared with research in higher mammals and humans. Nevertheless, modalities for preventing or treating disease do not always translate from mouse to humans, which has led to increasing scrutiny of the continued merits of mouse research. Here, we summarize the pros and cons of current laboratory mouse models for immunology research and discuss whether overreliance on nonphysiological, ultra-hygienic animal husbandry approaches has limited the ultimate translation potential of mouse-derived data to humans. Alternative approaches are discussed that may extend the use of the mouse model for preclinical studies.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

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Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

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The production and use of germ-free and specific-pathogen-free (SPF) laboratory animals continues to gain momentum in most countries, despite the fact that some members of the older school of medical research workers consider that the results of tests carried out with SPF animals may be misleading when extrapolated to man, who cannot be regarded as "pathogen-free." Our efforts must surely be directed to the task of ensuring that uniform and defined animals become more freely available. Only thus will variation between groups of animals be reduced, and so animal experimentation will, to a greater degree, become more and more qualitative.

—J.S. Paterson and R. Cook, 1971

It has become fashionable for researchers to bemoan the inadequacies of laboratory animals—especially mice—as models for the human immune system. At the outset, it is important to realize how much studies on mice have contributed to immunology research. Without the reliability and reproducibility of data obtained with inbred mouse strains maintained under stringently defined housing conditions, it is doubtful that we would have anywhere near our current understanding of the physiological role of major histocompatibility complex (MHC) molecules, the manner and mechanism of lymphocyte development and differentiation, the basis for immunological tolerance and immune memory, etc., with a dramatic impact on numerous aspects of modern medicine. Without the opportunity to characterize and immunize genetically homogenous inbred mice, there would, at a minimum, have been a substantial delay in these discoveries. And instances in which inbred mice "fail" to yield consistent results have been equally important—the finding that there is an altered frequency of Th17 populations in genetically identical C57BL/6 mice obtained from different vendors (Ivanov et al. 2009) sparked a deeper understanding of the specific role played by commensal microbes in immunity. Work on human identical twins has also revealed an impact of the environment on immune reactivity, but interpretation of such studies can go little beyond saying that nonheritable features matter (Brodin et al. 2015). From these perspectives, it could be argued that the mouse model for immunology research does not get enough credit, rather than being oversold. However, current mouse models certainly have limitations—the essential question is whether these can be improved by relatively simple changes. The benefits of building on existing mouse models, rather than dispensing with them all as passé, are numerous. In this article, we consider the strengths and weaknesses of immunology research on laboratory mice, and the way in which more natural microbial exposure may improve the utility of such models for key aspects of the human immune system. As indicated by the quote above (Paterson and Cook 1971), this idea is something of a reversal of the trend that has, justifiably, dominated the field for more than 50 years: As we argue, perhaps now is the time that the "old-school" approach can be reevaluated in a modern context.

THE GOOD AND THE BAD ABOUT LABORATORY MOUSE MODELS FOR THE HUMAN IMMUNE SYSTEM

The laboratory mouse has become the go-to model for mechanistic studies on the mammalian immune system. Several reasons lay behind this. Selective breeding of "fancy mice" eventually led to genetically homogenous inbred strains, which, although unable to capture the diversity of outbred populations (including humans), were essential for identifying quintessential features of the adaptive and innate immune system. Core elements of the mouse immune system correlate with those in humans, permitting tremendous strides in characterizing the dizzying complexity of cellular and molecular immunology, defining genetic bases for immunodeficiencies and autoimmune disorders, and testing the efficacy of vaccines and immunotherapies for infectious diseases and cancer. Beyond general immunology, some studies with mice have led to specific approaches that have translated into powerful therapies in the clinic: A notable example is development of checkpoint blockade for tumor immunotherapy (Sharma and Allison 2015). In the context of vaccines, it is interesting that the only malaria vaccine to prove efficacious in humans (Seder et al. 2013; Richie et al. 2015) uses the method-

ology developed decades earlier in laboratory mice (Nussenzweig et al. 1967).

On the other hand, there are certainly differences between the mouse and human immune systems, and multiple reviews highlight the despondent perspective that mice are an intrinsically limited model for human immunological and inflammatory diseases and, hence, an unreliable predictor of translatable therapeutic interventions (Mestas and Hughes 2004; von Herrath and Nepom 2005; Payne and Crooks 2007; Davis 2008; Rivera and Tessarollo 2008; Rice 2012; Koff et al. 2013; Seok et al. 2013). Aside from frustrating physician scientists intent on novel treatments, misleading preclinical studies can have disastrous consequences, as exemplified by the anti-CD28 "superagonist" antibody TGN1412, which produced life-threatening cytokine-release syndrome during a phase I clinical trial—responses that were not predicted by preclinical studies in mice (or nonhuman primates) (Hunig 2016).

These limitations have prompted a call for expanded use of "humanized mice" (immunodeficient mice carrying human immune cells) and studies of the immune response in humans themselves (Davis 2008; Koff et al. 2013). Although laudable goals, this faces numerous ethical and practical barriers. For example, recent discoveries have revealed that memory T cells that reside in nonlymphoid tissues (including barrier sites such as the lung, skin, and gut) mediate critical sentinel and alarm functions for protective anamnestic responses (Schenkel and Masopust 2014; Carbone 2015). These resident memory T cells are thus an objective for potent vaccines. However, because they are not, by definition, sampled in the blood, characterization of these cells in humans would require extensive, invasive biopsies. The options and opportunities broaden with humanized mice, but this system is cumbersome. The popular NOD SCID γC (NSG) model is already genetically complex, and further refinements (e.g., gene manipulation correcting cytokine/cytokine receptor species incompatibility) must, laboriously, be crossed to this background (Rongvaux et al. 2014). Although advances in gene manipulation technology will make such ventures easier, it will be some time, if ever, before humanized mice faithfully mimic key components of the human immune system.

But, before giving up on current laboratory mouse models, it is worth digging deeper into why they fail to replicate features of the human immune system. Previous reviews have explored this issue by highlighting the impact of evolutionary distance between the species, the differences in life span, and the difficulties in converting vaccination approaches from mice to humans (see Table 1) (Mestas and Hughes 2004; von Herrath and Nepom 2005; Payne and Crooks 2007; Davis 2008; Rivera and Tessarollo 2008; Rice 2012; Koff et al. 2013). Another difference that, perhaps surprisingly, was not listed in those reviews is the microbial exposure of humans and laboratory mice before immune challenge. That will be the focus of this discussion.

THE IMPACT OF PREVIOUS MICROBIAL EXPOSURES ON IMMUNE RESPONSE

There can now be little doubt that microbes in the environment dramatically shape the innate and adaptive immune systems. Pioneering human twin studies have revealed that a major element in variability within the immune system is driven by acquired rather than heritable factors (Brodin et al. 2015), whereas the contribution of commensal microbes themselves is illustrated by mouse studies that show the development and function of numerous immune cell populations is compromised or altered in the absence of a diverse microbiota (Belkaid and Hand 2014).

Likewise, an animal's infectious history may substantially impact subsequent immune responses. "Heterologous immunity," arising as a result of cross-reactive T-cell recognition of epitopes from distinct pathogens, can substantially alter the immune response (Welsh and Selin 2002). Previous infection with one microbe may improve the animal's capacity to control a different pathogen, although in some cases prior antigen exposure can exacerbate or degrade an immune response—as illustrated by "original antigenic sin" in the immune response to successive strains of Dengue virus (Mongkol-

Table 1. A comparison between clean and dirty mice as models for the human immune system

Criteria	Humans	"Clean" laboratory mice	Dirty mice
Genetics	Outbred	Inbred, in vivo manipulable	Outbred or inbred in vivo manipulable
Costs	Free (for researcher) to generate/house; requires large group sizes to address heterogeneity	Expensive to generate and house, but cost-effective due to moderate group sizes	Expensive to generate and house and may sometimes require large group sizes to address heterogeneity
Barriers to in vivo studies	Substantial logistic and ethical hurdles	Minimal, provided ethical treatment of animals	Minimal, provided ethical treatment of animals
Pace of discovery	Slow, cumbersome	Fast, efficient	Fast but cumbersome
Relevance of discoveries to human health	Axiomatic	Contentious	Promising

sapaya et al. 2003). Prior infectious history can also provoke sustained changes in the innate immune system: Chronic herpes virus infections in mice can enhance control of subsequent bacterial infections (Barton et al. 2007), whereas infection with helminthic parasites can lead to latent virus reactivation (Reese et al. 2014). Because chronic viral infections are widespread in the human population (Virgin et al. 2009), such effects may contribute substantially to baseline immunity. Systems biology studies reach similar conclusions for humans, finding that human cytomegalovirus (HCMV) infection enhances the immune response to influenza vaccination (Furman et al. 2015). Hence, an infection may modify the immune response to unrelated microbes in complex ways.

One aspect of this idea is encapsulated in the hygiene hypothesis, which proposes improved sanitation and subsequently reduced exposure to microbes has raised the incidence of allergic and autoimmune diseases in industrialized countries, whereas regular exposure to foreign antigens (including infections) derails induction or maintenance of pathological Th1 and Th2 responses (Strachan 1989; Blaser and Falkow 2009; Loke and Lim 2015). This idea has caught on, such that some individuals deliberately inoculate themselves with chronic but curable helminth infections to reap the proposed benefits of this immunological experience (Loke and Lim 2015). Very recent studies suggest more subtlety to this concept—studies of rural farmers revealed that exposure to the "right kind of dust" (from Amish but not Hutterite farms) protected children against asthma, an effect that was mimicked in mice (Stein et al. 2016), whereas other mouse studies suggested that periodic exposure to lipopolysaccharide (LPS) blocks asthma induction (Schuijs et al. 2015).

THE MANTRA OF LABORATORY MOUSE RESEARCH—CLEANER IS BETTER

However, although our understanding about the physiological impact of microbial exposure on the immune response has grown over the years, our methods of laboratory animal husbandry have taken the exact opposite tack, leading to the establishment of animals with less and less microbial exposure. Use of germ-free (axenic) mice, or mice with defined microbiomes (gnotobiotic animals) was critical to establish the effect of microbial colonization and diversification on numerous physiological processes, including the immune system (Belkaid and Hand 2014; Spasova and Surh 2014; Blanton et al. 2016). But, it is essential to recognize that these animals are not the natural "baseline," but rather the abnormal situation in which microbial exposure has been artificially thwarted. Antigen-free mice, in which exposure to all complex foreign antigens is prevented, take this to a new extreme (Jiang et al. 2004; Kim

et al. 2016). Again, such studies are tremendously important to define the impact of natural exposure to nonself (Kim et al. 2016), but do so at the expense of modeling physiological exposures to antigens that humans undergo with each breath and every meal.

Use of germ- and antigen-free mice represents a small fraction of studies on laboratory mice. Instead, the vast majority of mouse immunology studies involve specific pathogen-free (SPF) mice raised in barrier facilities. SPF colonies were first raised on a substantial level in the 1950s (in parallel with the much more demanding germ-free colonies), as investigators recognized the vulnerability of inbred mouse strains to some mouse pathogens, and the corresponding benefits to colony viability, life span, and fecundity by systematically eliminating these pathogens (Foster 1959, 1962; Foster et al. 1963; Festing and Blackmore 1971). Widespread adoption of SPF housing—which is difficult, expensive, and time consuming—took decades, and one could argue is still incomplete, because the list of offending pathogens is somewhat arbitrary. Indeed, "SPF" is a pragmatic description, which competed with terms like "disease-free" in early work with such animals (Foster 1959). Still, as currently practiced, SPF housing proved essential for immunology studies for diverse reasons, including the frequent use of immunocompromised mice (with heightened vulnerability to infection) and the dominant view that immune responses need to be studied in the context of an immunological "blank slate."

But, do we throw out the baby with the bathwater when we commit to an exclusive focus on SPF animals for immunology research? Have we lost the very context that is needed to study a physiological immune response, and hence lost a critical element in translation to human studies? If we break those artificial SPF barriers, does this make laboratory mice a more accurate model for the human immune system?

BUCKING THE TREND: "DIRTY" MICE

We recently examined these issues, initially studying feral mice that had completely natural exposure to natural pathogens or mice raised in commercial pet stores without use of barrier housing. Examination of blood and tissue leukocytes in these "dirty" mice revealed numerous changes compared with "clean" SPF mice (Beura et al. 2016). Most importantly, many differences in immune populations (including peripheral blood mononuclear cell [PBMC] gene-expression profiles) suggested a better correlation between dirty mice and adult humans (while SPF inbred mice aligned better with neonatal humans). Although genetic differences between inbred and outbred mice and other aspects of being raised outside a laboratory environment (e.g., differences in nutrition and climate) could underlie these differences, we found that most of the same immune characteristics could be induced in inbred laboratory mice by cohousing with pet store mice and that this coincided with acquisition of SPF-excluded pathogen infections (Beura et al. 2016). Parallel studies by Virgin and colleagues showed that many of the changes in blood cell gene expression were induced in inbred mice subjected to deliberate infection with a small group of laboratory strain mouse pathogens (Reese et al. 2016).

The nature of the immune cell alterations observed in dirty mice is instructive (Beura et al. 2016). In contrast with SPF mice, dirty mice (including cohoused inbred mice) had high frequencies of effector memory-phenotype CD8 T cells in the blood and lymphoid tissues, an abundance of resident memory T cells in nonlymphoid sites, more myeloid cells with an activated phenotype, and an increased expression of interferon-regulated genes in PBMC. From the perspective that these changes are probably driven by infection with easily spread mouse pathogens, many of which are viruses, these changes are predictable. But it is important to emphasize that SPF mice are not germ free—they have a highly diverse microbiota, including numerous bacterial and viral species—so the data imply that natural transmission of SPF-excluded pathogens (plus, no doubt, microbes not screened by SPF testing) is sufficient to drastically affect the immune baseline. But, whereas viral infection might be expected to induce a strong Th1 response, we found CD4 T cells with Th1, Th2, Th17, and

Treg characteristics, and a massive increase in serum IgE typically associated with Th2 responses. These suggest that diverse microbial experience induces complex changes in immune status.

Relevant to how these animals can be used to better model human vaccination, it is important to consider how broader immunological experience in dirty mice impacts new immune responses. We examined how immune reactivity toward viral, bacterial, and parasitic infections was altered in dirty inbred strain mice. Responses to all of the pathogens were altered, but perhaps the most dramatic effect was on the short-term control of *Listeria monocytogenes*, which was almost as effective in unimmunized dirty mice as in vaccinated SPF mice (Beura et al. 2016). Although one can interpret such results in many ways, it is perhaps most informative to consider that the outlier in the analysis is the SPF mouse—these are the animals maintained in artificially clean barrier facilities, although "dirty C57BL/6" are closer to what one would expect to see in a hypothetical feral colony of this strain. This raises an important issue concerning the impact of heightened immunological readiness on vaccine efficacy. Although rapid pathogen control is clearly an advantage for the animal, it is less clear that it is a good thing for the vaccinologist. A potential problem with implementation of potent vaccines in humans is that the (laudable) goals of safety and practicality trump the underlying goal of efficacy; this has led to situations in which a vaccine is withdrawn even in situations in which the risk/benefit ratio might justify its use (Bhan and Green 2011) or to hesitation in development of complex live vaccines in favor of more simple but less effective subunit vaccines (Hoffman et al. 2015). In contrast, live vaccines studied in mice are often used at just a little lower than a lethal dose, as defined for that mouse strain. This would clearly be unacceptable and, given our genetic diversity, unpredictable among humans. But, if the immune status of dirty mice makes them more resistant to infection, this may potentially degrade the efficacy of subunit or attenuated live vaccines.

It is also useful to speculate on the effects of diverse immunological experience on cancer immunotherapy and vaccines. Studies have shown that the presence of tumor-resident T cells and type-I interferon (IFN) responses are critical for immune-mediated tumor control (Gajewski et al. 2013), and it will be interesting to see how tumor immunotherapy is altered in dirty mice. Interestingly, studies conducted many years ago by Rubin and colleagues suggested that breaking the SPF barrier substantially enhanced antitumor immunization (Rubin and Gairin 2011), although whether such changes related to acquisition of SPF-excluded pathogens or some other aspect of altered housing is unclear. The limitations of SPF mice may also underlie the failure to predict the cytokine storm induced by CD28 superagonist antibodies. Hunig and colleagues speculated that the lack of effector memory T cells in SPF animals (in contrast to normal humans) leads to an artificial imbalance in stimulation of proinflammatory and regulatory T cells (Gogishvili et al. 2009). In contrast, dirty mice have an abundance of effector memory T cells (including cells in the circulation and tissues), and it will be interesting to see whether these mice present a more faithful model of cytokine release syndrome.

So, although species-specific differences between mice and humans can never be fully reconciled, recent studies suggest that broadening the immunological experience of mice may enhance their utility as a model for the human immune response (see Table 1). At the same time, there are still many unresolved questions about this model: Does infection with certain pathogens produce a better match with (average) humans, or is any substantial immunological experience sufficient? How does microbial experience impact the magnitude and characteristics of the immune response to common vaccines and tumor immunotherapy? And, at a very practical level, how can we overcome the logistical problems (of which there are many) with establishing "dirty" colonies at institutions where, often at the behest of resident immunologists, there is strict adherence to barrier facility housing?

FINAL COMMENTS

Opening the door (more or less literally) to nonbarrier housing will, inevitably, lead to some heterogeneity in infectious exposure. This issue could be overcome by the sequential infection models described earlier, although one could argue that this just substitutes one set of restrictive criteria (SPF housing) for another (scheduled infection with an arbitrary set of SPF pathogens). Permitting natural animal-to-animal exchange of pathogens is unnervingly uncontrolled for mouse immunologists, but it is the reality that human immunologists have faced for decades. Variability in the human immune system is accepted as being part of the territory, with the expectation that key fundamental characteristics of the immune response will emerge despite the "noise" of real life. Perhaps now is the time for mouse immunologists to step out of the mindset that equates homogeneity with accuracy and accept that heterogeneity is physiology.

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