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Article

Livestock Models in Translational Medicine

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Abstract

This issue of the *ILAR Journal* focuses on livestock models in translational medicine. Livestock models of selected human diseases present important advantages as compared with rodent models for translating fundamental breakthroughs in biology to useful preventatives and therapeutics for humans. Livestock reflect the complexity of applying medical advances in an outbred species. In many cases, the pathogenesis of infectious, metabolic, genetic, and neoplastic diseases in livestock species more closely resembles that in humans than does the pathogenesis of rodent models. Livestock models also provide the advantage of similar organ size and function and the ability to serially sample an animal throughout the study period. Research using livestock models for human disease often benefits not only human health but animal health and food production as well. This issue of the *ILAR Journal* presents information on translational research using livestock models in two broad areas: microbiology and infectious disease (transmissible spongiform encephalopathies, mycobacterial infections, influenza A virus infection, vaccine development and testing, the human microbiota) and metabolic, neoplastic, and genetic disorders (stem cell therapy, male germ line cell biology, pulmonary adenocarcinoma, muscular dystrophy, wound healing). In addition, there is a manuscript devoted to Institutional Animal Care and Use Committees' responsibilities for reviewing research using livestock models. Conducting translational research using livestock models requires special facilities and researchers with expertise in livestock. There are many institutions in the world with experienced researchers and facilities designed for livestock research; primarily associated with colleges of agriculture and veterinary medicine or government laboratories.

Key words: cattle; genetic diseases; genomics; infectious diseases; metabolic diseases; sheep; swine; translational medical research

This issue of the *ILAR Journal* focuses on livestock models for human diseases. It builds upon a previous issue on "Naturally occurring diseases in animals: contributions to translational medicine" (Lairmore and Khanna 2014). Inbred rodents have been very important models for research to understand fundamental mammalian biology and disease processes. The ability to use syngeneic mice and to manipulate their genetic traits has produced major advances that underpin modern medicine. However, small groups of inbred rodents should not be expected to mimic the complexity of genetically heterogeneous human populations. Nonhuman primates have been powerful models for translational studies for human application, however, their availability is limited and there are many ethical questions surrounding their acquisition and use.

Livestock models of selected human disease present important advantages as compared with rodent models when it comes to translating fundamental breakthroughs in biology to useful preventatives and therapeutics for humans. Livestock reflect the complexity of applying medical advances in an outbred species. In many cases the pathogenesis of infectious, metabolic, genetic, and neoplastic diseases in livestock species more closely resembles that in humans than does the pathogenesis of

rodent models. Livestock models also provide the advantage of similar organ size and function and the ability to serially sample an animal throughout the study period.

A dramatic improvement in our understanding of human biology began with completion of the sequencing of the human genome (International Human Genome Sequencing Consortium 2004). The original primary goal of the human genome project, providing tools and resources to identify genes and variants causing monogenic human disease, has been completed, and finding a gene variant causing a genetically simple disease is now trivial given access to patients and controls. Further, many examples of gene variants associated with complex diseases also have been reported. Interestingly, excellent examples of such identifications often arise from analysis of domesticated animal populations that have well-documented phenotypic records (Andersson 2013). This enlightenment of the genotype–phenotype association has accelerated with the functional decoding of many DNA elements (ENCODE Project Consortium 2012) in the human genome, although such information is scant for livestock species. A recent initiative is focused on improving this situation: the recently announced Functional Annotation of ANimal Genomes (FAANG) project (FAANG Consortium 2015) proposes to create ENCODE-type data for those domesticated species with a well-developed genome assembly. Coupled with the already available phenotypic resources on these species, the complex relationship between natural genotypic and phenotypic variation could be explored at unprecedented scales. Such data will further establish these species as models for human disease, as well as develop valuable practical information for agriculture. In addition, with the rapid technical advances in precision genome editing (Cox et al. 2015), it is now possible to quickly and specifically modify any gene in the genome. In many cases, it is possible to predict the effect of such mutations on phenotype based on a detailed understanding of the expression patterns of the gene and the predicted function of its product; deep annotation of genome component function will expand this capability. As depicted in several papers in this special issue, the creation of a model often involves a genetic manipulation to mimic the defect in patients. Such manipulations require molecular genetic information, and often a detailed understanding of the physiological and molecular pathways involved. The last 10 years have seen an explosion of such genomic information on a range of vertebrates, several of which have served as models in translational medicine. These genome sequences provide an opportunity for more systematic and detailed modeling at multiple levels: molecular, cellular, metabolic, and organismal (Tuggle et al. 2011). Such modeling depth will be a necessary component of any models in the future, as data-driven analysis of human systems continues to expand (Carter et al. 2013). Thus the fledgling FAANG project, which aims to create for domesticated species deep functional information similar to that currently available for humans, will add substantially to the value of these species as translational models.

Pigs have proven to be an especially important model in translational research, particularly in medical device development, xenotransplantation, and therapeutics due to the similar size, anatomy, and physiology of human and pig organs (Judge et al. 2014). The ability to produce transgenic pigs with selected human genes enhances their potential to serve as organ donors (Ibrahim et al. 2006). The similarities of the innate (Fairbairn et al. 2011; Kim et al. 2014) and adaptive (Butler et al. 2009) immune systems of pigs and humans has led to the use of pigs as translational models for the study of various infectious diseases and in vaccine development (Meurens et al. 2012). A recent sequence-level comparison of human and porcine genes involved in immunity demonstrates their close similarity (Dawson et al. 2013). Transgenic pig models have been established for neurodegenerative diseases, cardiovascular diseases, and diabetes mellitus (Aigner et al. 2010). Genetically modified porcine models of cystic fibrosis have been generated that develop lung disease similar to human cystic fibrosis (Rogers et al. 2008), an important deficiency of mouse models (Judge et al. 2014). Pigs are also increasingly being considered as the model of choice for preclinical toxicologic testing of pharmaceuticals (Swindle et al. 2012).

Livestock Translational Models in Microbiology and Infectious Disease

In this issue, examples are presented of basic research in livestock translating into improved prevention and therapeutics not only for humans but also for the livestock species. Many infectious diseases have co-evolved in livestock and humans due to their close interaction for thousands of years. This similarity allows the study of early pathogenesis and interventions in the livestock model. For important zoonotic diseases such as bovine tuberculosis, swine influenza, bovine spongiform encephalopathy, brucellosis, leptospirosis and many others, the livestock species may serve as the reservoir host, or amplifying host. Therefore, research on these naturally occurring diseases not only leads to important advances in translational medicine, but also leads to improved detection and prevention in livestock reducing exposure of humans and contributing major benefits to public health (Roth 2011).

Transmissible spongiform encephalopathies (TSEs) are an excellent example of research in livestock having multiple benefits for livestock health, food production, and public health as well as translational research as a model for TSEs in humans. Drs. Justin and M. Heather Greenlee summarize research advances in two TSEs of livestock: bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep and goats. Basic research on BSE and scrapie has made major contributions to the understanding of the biology and pathogenesis of prions. All TSEs share pathologic features and infectious mechanisms, but have distinct differences in transmission and epidemiology. TSEs can be acquired through exposure to infectious materials, but inherited and spontaneous TSEs also occur. BSE can be transmitted to people through ingestion of infectious material and can cause variant Creutzfeldt-Jakob disease. Research on BSE was essential to break the transmission cycle between cattle and to people.

Mycobacterium bovis is a zoonotic infectious agent of livestock and wildlife, which can produce severe disease in people. There is a long history of mutually beneficial research leading to improved understanding of tuberculosis in both livestock and people. Drs. Waters and Palmer summarize advances in detection and control of human tuberculosis resulting from livestock and wildlife research on *M. bovis* and discuss advances in understanding the pathology and immunology of *M. bovis* infection based on advances in *M. tuberculosis* research. Advances in diagnostic testing and vaccines for prevention of tuberculosis in people have been tested with *M. bovis* infection in cattle and refined before translated for use in people. Recent advances in understanding the immunology of mycobacterial infections are summarized in this review and current efforts to develop safer and more effective vaccines using the *M. bovis* model are discussed.

Rajao and Vincent make a compelling case for the use of swine as a model for influenza A virus (IAV) infection and

immunity in people. The same subtypes of IAV are endemic in both species and there has been repeated exchange of viruses between these two hosts. There is a similar distribution of IAV receptors in the respiratory tract, and the clinical manifestation and pathogenesis are similar. The ability to control maternal antibody transfer to newborn pigs through ingestion of colostrum enables experimental designs with groups receiving no maternal antibodies or maternal antibodies with defined antibody titer and specificity. This is a powerful tool for elucidating the role of passive antibody in immunity, in interference with vaccine responses, and in enhancement of respiratory disease. The swine model has convincingly demonstrated the benefits of modified live intranasal vaccines for inducing heterosubtypic immunity and allowed the investigation of immune mechanisms responsible. It has also allowed the discovery and characterization of vaccine-associated enhanced respiratory disease (VAERD) when inactivated vaccines are used that are not closely matched to the hemagglutinin protein of the challenge strain of virus. There is still more to be learned about the pathogenesis and immunology of influenza in people. The swine model is an important tool for understanding mechanisms of virulence and protective immunity, and for developing more efficacious vaccines and other forms of prevention and treatment.

Gerdts and colleagues discuss efforts to reduce and replace the need for animals in human vaccine research and regulatory approval; however, some use of animal models for development and regulatory approval of human vaccines is essential to mimic the complexities of the immune response. They make the case that no single animal model provides all of the information needed for advancing a novel vaccine through the preclinical phase of development and that large animal models are often better than rodent models for predicting vaccine outcomes in humans. They review advantages and disadvantages of selected livestock models of infectious diseases that have proven to be valuable for preclinical evaluation of vaccines for human disease. Advances in reagent availability for characterizing the immune response of livestock are improving the ability to use livestock models for human vaccine evaluation.

There is growing recognition of the important role of the gut microbiota in maintaining health and in disease pathogenesis. Donovan and colleagues review the use of human microbiota-associated (HMA) swine in translational research. The high degree of similarity in anatomy, physiology, immunology, and brain growth between swine and humans make pigs a better model than rodents for studying the influence of the microbiota on human gastrointestinal, immune, and brain development. HMA piglets have been established using inocula from infants, children, and adults. Importantly, *Bifidobacterium* spp. and *Bacteroides* spp., predominant bacterial groups of the infant gut, have been established in the HMA piglets. The HMA pig model may be valuable for investigating how the gut microbiota composition changes in response to environmental factors, such as age, diet, antibiotic use, and infection. The HMA pig also promises to be a useful model for screening the efficacy of pre- and probiotic interventions and for elucidating microbe–host interactions in health and disease.

Livestock Translational Models for Metabolic, Neoplastic and Genetic Disorders

Both technologies and developed models for specific metabolic, neoplastic, and genetic disease are also described in this issue. These include the development of technology to test induced pluripotent stem cells (iPSC) and spermatogonial stem cells (SSC), as well as models for studying lung cancer, muscular dystrophy, and wound healing.

Models for preclinical testing of stem cell therapeutics are described by Michael Roberts and colleagues, with a focus on the use of pigs in development and testing of iPSC. The authors detail a need for pig models to test three necessary aspects of iPSC-based therapy:

- **safety**: iPSC can form teratomas or carcinomas if cells are not differentiated;
- **efficacy**: iPSC can function to cure or alleviate disease symptoms; and
- **stability**: as the transferred iPSC can be rejected by the host.

Large-animal models are highlighted, as the current success of translation of preclinical results past Phase II trials is less than 20% (Arrowsmith and Miller 2013). The authors point out that the principle of homology to humans is important in selecting a model, and that while the mouse as an animal model has economic and infrastructural advantages, new technology for genetic modification may increase availability of useful large animal models with higher biological homology to humans. A lack of research data on a range of practical questions on development of such models will continue to slow progress, however.

Roberts and colleagues provide a history of research on pluripotent stem cells from livestock species, culminating in partially successful reports on creating iPSC. Currently there is no clear demonstration that iPSC can contribute to the germ line, likely due to incomplete reprogramming of the iPSC. However, they also discuss parallel studies on recent work showing human iPSC transplantation into pigs. For example, there is a growing body of data that pigs may be useful preclinical models for testing heart muscle repair protocols using human iPSC-derived cardiomyocytes. While some early mutations have been achieved that document the value of the pig model in testing safety and efficacy, more work lies ahead before cures are achieved. New approaches to creating hosts that are tolerant to xenogenic iPSC are also described. Severe combined immunodeficient (SCID) pig lines have been described that are tolerant because they are severely deficient in adaptive immunity. SCID pigs have been created by mutagenesis or by identification of natural mutations. Specifically, the Roberts group has reported that pigs with mutations in RAG2 were tolerant of human iPSC; however, the transferred cells formed teratomas in the recipients. The authors also discuss interesting possibilities in the use of stem cells in xenotransplantation, outlining creation of chimeric pigs with a pancreas formed exclusively from differentiated human pluripotent donor cells as a source of human stem cell-derived organs for transplant. They also discuss the technologies available, as well as those still needed, for in vitro production of meat from myogenic derivatives of iPSC.

The study of male gametogenesis and its relationship to translational medicine is summarized by Gonzalez and Dobrinski. A description of the utility of collecting sperm for conservation of endangered or valuable germplasm is followed by a summary of methods for the study of spermatogenesis, with detailed information on gene cell (GC) isolation and analysis. A critical area of study is identifying gene markers for undifferentiated and potentially self-renewing stem cells. Several candidate markers have been proposed for several species, including cattle, dog, horse, pig, goat, and sheep. In parallel with these molecular studies, transfer of GC and successful production of offspring derived from transferred GC has been reported for mice and several large-animal species. The efficiency of these procedures is still

low, and attempts to both improve purity of the GC preparation as well as modify the recipient environment to increase success rates are described. The authors point out the striking differences in immunotolerance between mouse and large-animal species, where unmatched donor and recipient GC transfer can be successful only in the latter. The authors complete their review by providing examples of the practical uses of spermatogonial stem cells (SSC), which includes the study of spermatogenesis itself, and the preservation of unique lines of animals for maintenance of biodiversity or storing superior genetics. They comment in detail on the use of SSC in generating transgenic animals, where SSC have some advantages over other methods in rapidity in passing on transgenes from males to offspring. While long-term culture of SSC has not been achieved in large animals, rapid mutagenesis techniques may overcome these roadblocks.

Lung cancer is the most common human cancer worldwide, and while the environmental insult of tobacco smoke is clearly a major factor in the incidence of such cancers, Youssef and colleagues discuss the fact that the incidence of lung cancers is increasing among nonsmokers. Interestingly, tumors from smokers and nonsmokers have distinct features, including diverse causal genetic mutations. After summarizing types of animal models for lung cancers, the authors focus on the ovine pulmonary adenocarcinoma (OPA) model in sheep. Although OPA is caused by infection with a specific virus, jaagsiekte sheep retrovirus (JSRV), the resulting cancer phenotype is quite similar to human bronchioloalveolar cancer, which is a form of non-small cell lung cancer in humans. The authors recount the common signaling pathways affected in human adenocarcinomas and OPA, including the PI3K-Akt-mTOR and RAF-MEK-ERK1/2 pathways, and then describe lines of evidence that document similarities at the gene response and cellular levels between human cancers and the OPA model. Several experimental platforms are used in the OPA model, including an in vivo lamb disease model. This model has strengths including the ability to precisely control tumor development through JSRV infection of very young lambs at specific times and lung regions and the possibility of using different viral genotypes as an infectious clone of JSRV. In addition, the lamb model enables the study of early stages of tumor progression, which is very difficult to identify in human patients, who are usually in late stages of the disease when it is diagnosed. Current limitations include the inability to titer virus accurately, as permissive cell lines are not available to grow JSRV. The authors also discuss an in vivo cancer model in mice where infection with adeno-associated virus expressing the tumor-causing Env protein of JSRV can model adenocarcinoma as well; responses observed in the lamb infected with JSRV are similar to those seen in this infection in mice. Beyond the early disease model, naturally infected older animals can also be identified and studied to gain insight into latter stages of the disease. The authors also provide an early glimpse into new in vitro models using lung tissue slices, which can be infected with JSRV and maintained for 3–4 weeks in culture to study early events in a model that bridges the in vivo lamb model and late-stage natural infections. Response to infection in the lung tissue slice model includes activation of the Akt and ERK1/2 pathways, and shows the model appears to reflect in vivo responses. The authors conclude by posing the question as to the cause of human cancers in nonsmokers: could there be a viral cause? They recount that while there is no epidemiological evidence of transmission of JSRV-related adenocarcinomas to people in close proximity to infected sheep, JSRV can infect human cells in vitro, and antibodies to JSRV proteins are reactive to an unknown epitope in human cancer tissues. Thus, while verification of a possible viral etiology in human lung adenocarcinomas awaits more definitive analyses, the experimental OPA model in lambs is established for studying early events in development of these lung cancers.

Selsby and colleagues provide a summary of the devastating effects of Duchenne muscular dystrophy (DMD), which is caused by mutations on the dystrophin gene. Dystrophin encodes a large protein that is critical for transfer of actin-myosin contractile force from muscle to the skeleton. The lack of functional dystrophin causes a loss of calcium homeostasis, inflammation and eventual protein degradation and muscle loss. The authors also describe Becker muscular dystrophy (BMD), which is a less severe form of the disease caused by inadequate dystrophin function. They argue that therapies for DMD that currently show promise in animal trials are likely to be partially effective, predicting that patients so treated would exhibit a BMD phenotype, and thus preclinical models for both DMD and BMD are needed. The authors then describe advantages and disadvantages of the mouse mdx model, a mainstay of dystrophy research; the golden retriever, hypertrophic feline, and Zebrafish models; and two newly generated rat models. They then turn to their central topic, the creation and development of porcine dystrophy models, first providing general advantages of the porcine species as a model, focusing on the high similarity of pig and human hearts, as cardiomyopathy is a component of dystrophy and a growing cause of death in muscular dystrophy patients. A description of several genetically modified porcine models is provided, including published and unpublished lines of pigs that reportedly can mimic significant aspects of the DMD phenotype. In addition, the authors describe a spontaneous porcine dystrophinopathy model, which through genetic analysis was shown to be due to a novel nonsynonymous mutation (R1958W) in the dystrophin gene. The phenotype is one of 70% decreased dystrophin expression in muscles. Affected pigs show skeletal muscle necrosis at 2 months of age, and foci of necrosis in heart tissue at 12 months of age. Abnormalities in the electrocardiogram were also observed in these pigs. The authors also discuss future analyses on respiratory and cardiac function in this promising BMD-like model of dystrophin insufficiency.

The healing of skin wounds is universally important in human and veterinary medicine. Seaton and colleagues discuss the use of the porcine model for the study of skin wound healing. The authors identify anatomical, structural, and physiological as well as protein distributional similarities between human and pig skin. While some differences exist, a comparison of multiple studies found that porcine skin is most concordant with human compared with several other models, which are no better than concordance of in vitro studies to humans (Sullivan et al. 2001). Seaton and colleagues discuss the different metrics to assess wound healing, as well as the methods to create specific types of injuries and emphasize that the depth of the wound is a critical factor in type and success in healing. A major category is chronic nonhealing wounds, which are caused by a number of factors. Of these, an important factor is diabetes, as this is increasing in prevalence in the United States and thus is an increasing problem in the healing of wounds. A pig model of diabetic wound healing processes using streptozotocin treatment has been developed. Several groups have used this model to investigate blood and growth factor secretion characteristics, as well as cell-based therapeutics and the effect of concomitant burns or bacterial infection on the rate and type of wound healing. The authors also discuss additional burn wound models, including sepsis as a complication, and the effects of debriding wounds and timing of treatments such as skin engraftments. In many of these experimental approaches, the pig model provides insights that could

allow more focused clinical trials. One wound healing defect highlighted is hypertrophic scarring, in which excessive collagen-based fibroproliferation increases the size of and pain associated with the resulting scar. A Red Duroc model has been developed by the authors, and has been shown to be an appropriate model for humans with hypertrophic scars. Comparisons of the Red Duroc pig with the Yorkshire pig, a nonhypertrophic scarring breed, has shown different patterns of growth factors and expression of collagen and MMP2, TIMP and other genes in skin. It was shown that these phenotypes have a genetic basis, as they were intermediate in offspring of the Duroc × Yorkshire cross. Because of the structural similarity of human and pig skin, the pig has also been used to test products designed to improve wound healing. While the costs and complications of working with such large-animal models are recognized, the authors conclude that these models have provided increased understanding of the molecular events during wound healing and further model development is justified.

Welfare and Ethical Considerations

An area of growing concern is the ethical and welfare considerations of using animal models in translational research. There is a concerted effort to reduce, replace and refine the use of animal models. For example, there has been some success in reducing the use of animals for potency and safety testing of both human and veterinary vaccines (Isbrucker et al. 2011; Kulpa-Eddy et al. 2011; McFarland et al. 2011). Animal welfare is a concern anytime that animals are used in research, especially whenever the research protocol leads to pain, physical discomfort, or mental distress. Livestock have been domesticated and are therefore more accustomed to contact with humans and to some levels of confinement as compared with primates or wild animals. Humane use and welfare are high-priority concerns when using any animals in research. This is addressed in the manuscript on "IACUC considerations on livestock models in translational medicine" by Thulin and Underwood. As mentioned above, the translational research often also benefits the livestock species, which partially addresses the ethical concerns.

Conclusions

In spite of the advantages discussed in the papers in this special issue, livestock models have been underutilized in translational research. This has been partially due to the slow realization of their advantages and value and to the perceived expense and difficulty of using livestock models. The use of livestock models is not difficult for institutions with appropriate facilities and investigators with experience in livestock research. There are many such institutions in the world, primarily associated with colleges of agriculture and veterinary medicine or government laboratories. These institutions have typically focused on research for the benefit of livestock health, food safety, and food production. These institutions and investigators have the capability to be more engaged in translational research for the benefit of human health. The increased cost of livestock models as compared to rodent models needs to be weighed against the added value they may provide in translational research.

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