

Gene Therapy in Large Animal Models of Human Genetic Diseases

John H. Wolfe

A major challenge to the medical sciences is the large number of disorders that are primarily genetic in origin or that involve a genetic predisposition to disease-producing factors in the environment. These disorders include a wide variety of debilitating and fatal illnesses for which few effective methods of treatment or prevention are available. Among the most common diseases in which genetic abnormalities play a role are congenital malformations, metabolic disorders, and cancer. Genetic variations also influence individual responses to pathogens and drugs. The structure and function of most genes in animals are homologous to those in humans, thus many genetic diseases that occur in humans also occur in animals. Gene mutations usually cause similar biochemical dysfunctions, resulting in pathologic changes at the molecular, cellular, organ, and organism levels. For these reasons, the knowledge gained through research in animals can be of direct benefit to human as well as animal health.

There have been dramatic advances in the science of genetics in recent years. The techniques of molecular biology have made it possible to understand the underlying mechanisms of genetic disease at the level of DNA, RNA, and protein molecules. The completion of the genome sequences of the human, mouse, dog, cat, and a growing list of other species as well as advances in genomics and related sciences represent the beginning of a new phase of understanding in which it will be possible to delineate the entire genetic program of individual types of cells and ultimately of whole tissues. Furthermore, these advances offer the prospect of correcting genetic defects and disease susceptibilities in animal and human patients through gene replacement therapy, manipulation of genetic pathways, engineering of stem cells, and delivery of recombinant proteins.

The realization of practical benefits to animal and human health will depend on a great deal of future research. The

increasing number and variety of transgenic, induced mutant, and naturally occurring animal models of genetic disease are vital for identifying new genes that cause disease, for understanding the cellular and molecular mechanisms of genetic diseases, and for elucidating the genes involved in diseases with complex inheritance patterns. A major use of such models is translational—they help move gene therapy strategies from proof of concept in cells and rodent models toward human clinical trials by enabling the investigation and solution of problems in scale-up and treatment of individual animal patients. The participation of veterinarians in these investigations is critical because they have the knowledge and skills in animal physiology, pathology, medicine, and surgery that enable them, with the proper scientific training, to effectively investigate important aspects of the pathogenesis and treatment of genetic and gene-influenced diseases in animal homologues of human disorders.

Selection of Topics and Organization of This Issue

This issue of the *ILAR Journal* focuses on the use of large animal models of human genetic diseases for gene therapy research. The term “large animal” in this context refers to species other than the commonly used laboratory rodents.¹ As is evident in the accompanying reviews, there are enough large animal models of human genetic diseases and published experiments to fill an issue.

The articles that follow illustrate the variety of models that are available and that have supported gene therapy research. The diseases span many pathogenic mechanisms and organ systems. Investigators are just beginning to explore the use of gene therapy methods to manipulate pathogenic processes in nongenetic diseases, as well as alternative therapeutic approaches such as stem cells, in large animal models of human disease, and the results will undoubtedly warrant further reviews in the future.

John H. Wolfe, VMD, PhD, is director of the W.F. Goodman Center for Comparative Medical Genetics at the University of Pennsylvania School of Veterinary Medicine and Stokes Investigator at the Children's Hospital of Philadelphia.

Address correspondence and reprint requests to Dr. John H. Wolfe, 502G Abramson Research Center, Children's Hospital of Philadelphia, 3516 Civic Center Boulevard, Philadelphia, PA 19104 or email jhwolfe@vet.upenn.edu.

¹This definition is in contrast to the usual meaning in veterinary medicine of pets vs. food, fiber, and work animals. However, it is not possible to draw clear lines according to species, as dogs are used as work animals and horses may be pets.

There were a number of potential ways to organize the articles. My coeditor Mark Haskins and I felt that the most useful division was by organ system (eye, muscle, heart, serum, blood and immune cells, nervous system), which tends to delineate the diseases. However, a number of genetic diseases affect multiple organs, thus there are reviews of lysosomal (Haskins 2009) and metabolic disorders (Koeberl et al. 2009) as well. The choice of subjects also depended of course on the availability of well-characterized models for scientific investigation.

Scope of Genetic Disease in Large Animals

Genetic disease can occur naturally in any species. The University of Sydney maintains a searchable database for animal Mendelian traits (OMIA) similar to the Online Mendelian Inheritance in Man (OMIM), and the two databases can be cross referenced. OMIA is accessible online directly (omia.angis.org.au) or through the National Center for Biotechnology Information (NCBI). Because some of the traits listed (e.g., coat color) are not diseases, there is a separate list of potential disease models, with, as of this writing, 1029 such models in more than 25 mammalian species. Most of the models (721, or 70%) occur in six species: the dog, cat, horse, cow, pig, and sheep. Among these six, 31% of the diseases fall into just five categories—congenital heart disease (63), lysosomal storage disease (57), dwarfism (38), inherited bleeding disorders (33), and inborn errors of metabolism (31)—three of which are the subject of reviews in this issue (Bauer et al. 2009; Haskins 2009; Sleeper et al. 2009). Other important groups of human diseases for which there are excellent large animal models are the muscular dystrophies and neurological disorders (Gagliardi and Bunnell 2009; Wang et al. 2009).

The suitability of each disease model for biomedical studies depends on the availability of both breeding stock to produce the disease and the amount of pathophysiological, biochemical, and genetic information about the model. For genetic disease models to become useful as laboratory animal models of disease, it is usually necessary to capture them in breeding colonies. Most genetic diseases in large animals require identification through medical diagnosis by veterinarians and subsequent laboratory tests to determine the type of disease and biochemical abnormalities. The diagnostic process is essentially the same as in human medicine and, because most genetic diseases manifest symptoms or signs at a young age, typically occurs in the pediatric population.

An important consideration in using animal models to evaluate therapeutics for translation to human use is the fidelity of the model to the human disease. Genetic diseases occur naturally in large animals and have been discovered by clinical ascertainment of the phenotype and recognition of its similarity to a human disease. These disorders are thus true homologues of the human disease rather than models—a homologous disease generally involves a mutation in an orthologous gene that causes similar biochemical, cellular, and organ abnormalities and results in clinical manifestations similar to those of human patients (Patterson et al. 1988).

Mouse models are enormously useful for laboratory experimentation, but a number of induced genetic mutations in the mouse do not manifest the disease phenotype and thus are less useful for translational medicine investigations. Recently, researchers have produced a new generation of highly sought disease models in large animals—a monkey model of Huntington's disease (Yang et al. 2008) and a pig model of cystic fibrosis (Rogers et al. 2008)—by transgenic and nuclear cloning technologies. These methods should be especially useful for creating models of important human diseases that have not been discovered naturally in animals.

Utility of Large Animal Disease Models

The frequency of occurrence of individual genetic diseases in animal populations is rare, as in the human population. However, with animals, identification of at least one parent enables the establishment of a colony for further study. Mutations have been captured by breeding a single obligate carrier to a normal animal, then mating the F1 generation with the carrier parent (backcross breeding) until the mutation re-emerges. For many diseases biochemical diagnosis can distinguish between carrier and normal amounts of a protein (e.g., enzymatic activity levels) to assist in identifying the potential carriers. After identification of the gene and mutation, PCR assays enable tracking of the carriers of the mutation.

There are a number of advantages to studying gene therapy approaches in breeding colonies of large animal models of human diseases. Body and organ sizes in large animals are much closer to those of humans than are rodent models. For blood disorders such as the hemophilias, the blood volume is proportional to body weight; for example, a blood level of normal clotting factor from a transferred gene in a 30 to 40 kg dog closely approximates the therapeutic level for humans (Øvliisen et al. 2008). Similarly, a number of genetic diseases that affect the retina occur in dogs (Acland et al. 2001), and the architecture and large size of the canine eye provide excellent models both for the pathobiology and for surgical approaches. Researchers have developed clinical gene therapy trials for one form of retinal degeneration as well as for hemophilia A and B directly from experimental studies in these dog models (Kaplan 2008; Murphy and High 2008; Nichols et al. 2009; Stieger et al. 2009).

Testing experimental therapies under actual disease conditions provides information on the responses of the affected organs. In the brain, for example, genetic diseases typically result in lesions throughout the central nervous system, requiring global distribution of the gene vector or therapeutic protein. Large animal brains provide a more accurate model of the conditions present in human neurodegenerative diseases (Vite et al. 2005) because (1) the cat and dog brain are 100 and 200 times, respectively, larger than a mouse brain, but only 10 to 30 times smaller than a human brain, depending on age (Pierson and Wolfe 2005); and (2) rodents have a smooth cortex whereas the brains of larger animals have a

sulcated cortex and an overall structure that is much more similar to those of the human brain. Large animals also provide better models for testing with noninvasive imaging modalities (such as magnetic resonance imaging [MRI] and spectroscopy of disease processes, and positron emission tomography [PET] imaging of reporter gene activity) because they are suitable for use with human clinical magnets and apparatus (Wolfe et al. 2006).

Another advantage of large animal models is their treatment and evaluation as individual patients, facilitating assessments of the range of success and failure. At the same time, the production and evaluation of large statistically significant cohorts of affected animals and normal controls permit the study of variations in age at treatment and analysis, class of gene therapy vector, route of administration, and other experimental variables. It would be virtually impossible to assemble the statistically significant cohorts, or to treat and evaluate normal controls, in human genetic diseases. In addition, human genetic disease populations are small but often harbor a variety of mutations, whereas genetically diseased animal breeding colonies typically have a single mutation. Large animals in a colony thus provide a uniform genetic mutation that causes the disease, but at the same time their genetic background is relatively outbred compared to inbred strains of mice. Another advantage for translation to clinical trials is that the large animal species live much longer than rodents, enabling studies on long-term effects—in terms of both efficacy and adverse results—of gene therapy or other experimental treatments.

The major limitations to the use of large animal models for biomedical studies are high vivarium costs, long reproductive cycles, lack of some reagents, and some physiological differences. For these reasons, some of the most effective uses have been in conjunction with murine disease models (e.g., for hemophilias, lysosomal diseases). In these cases, the mouse model permits relatively fast progress to develop optimal vector designs and test proof of concept before testing in a large animal model of the best strategies for gene delivery to determine whether they produce similar outcomes, which can accelerate translation to clinical trials.

Strategies, Methods, and Vectors for Gene Transfer

There are two basic types of gene delivery systems: *in vivo*, which involves direct vector injection into the body; and *ex vivo*, which involves genetic modification of cells in culture followed by transplantation. Most gene delivery strategies involve the former and use vectors developed from viruses, which have evolved to efficiently deliver their own genes into host cells (see bibliography for reviews). Synthetic vectors, formulated wholly from chemical and biochemical elements, are desirable because they can be made without cell systems. But despite the significant amount of ongoing research on them, they have not been developed into practical gene delivery systems yet, thus the use of viral vectors in

translational experiments and clinical application remains more likely for now.

The most widely used viral vectors are based on retroviruses (RV), lentiviruses (LV), adenoviruses (Ad), adeno-associated viruses (AAV), and herpes simplex viruses (HSV). The RV and LV vectors have RNA genomes, require a reverse transcription step, and integrate into the host cell genome; the Ad, AAV, and HSV vectors have DNA genomes. The RV vectors require dividing cells to productively infect target cells, while the others can infect nonmitotic cells. The RV, LV, and HSV vectors have envelopes derived from host cell membranes and are relatively fragile, whereas Ad and AAV vectors are nonenveloped and more stable. In all cases, the viral vectors attach and enter host cells by interactions between surface proteins on the virion and cell surface moieties. It is possible to change the host range and cell tropism of most vectors by substituting alternate viral surface proteins, a feature that has greatly improved the delivery specificity and range for some viral vectors.

The second strategy (*ex vivo*) for gene delivery is transplantation of cells that have been genetically engineered *in vitro* either to express proteins capable of correcting pathologic cells or to deliver a secreted protein. The goal of *ex vivo* gene therapy is to genetically engineer somatic cells derived from the affected individual to express the deficient enzyme in order to avoid transplantation barriers. This concept was originally formulated for the hematopoietic system (bone marrow stem cells), which has the natural properties of regeneration of cells and migration throughout the body. With the discovery of stem or progenitor cell populations in other tissues, the concept was extended to other organs, notably the brain and liver. The necessity of harvesting cells and manipulating them in culture before retransplanting them in the same host was cumbersome, but more importantly *ex vivo* culturing often resulted in the loss of stem cell properties. The ability to genetically modify cells by direct injection eliminated these drawbacks and thus has been the subject of intense study over the past decade. However, with the recent development of induced pluripotent stem (iPS) cell methods from easily accessible sources (e.g., skin fibroblasts or keratinocytes), the need for *ex vivo* gene transfer approaches has reemerged. By correcting the genetic deficiency with the patient's own cells, the problems of heterologous cell transplantation become moot.

There are a number of important limitations in using viral vectors. Integration into the host cell genome has resulted in insertional mutagenesis and oncogenic transformation in clinical trials for X-linked severe combined immunodeficiency disease (Nienhuis et al. 2006). Host immune responses to vector proteins or to the transferred gene product (protein) have been a significant obstacle to achieving therapeutic levels of gene transfer and expression in some cases, notably the hemophilias (Murphy and High 2008; Øvliisen et al. 2008). The immune system may also prevent repeat administration of a vector, resulting in only short-term gene correction. Yet despite these obstacles, large animal models have shown that long-term (years) expression of therapeutic levels of a missing gene is indeed possible.

The Promise of Large Animal Models for Translation to Clinical Trials

Large animal models of human genetic diseases have been invaluable in bringing novel treatment approaches to clinical trials (the bibliography lists several reviews of gene therapy studies in large animal models of various organ systems and diseases). Most genetic diseases do not have alternative therapies, thus it will be important to continue developing treatments for individual diseases even if they occur only rarely. In addition to genetic diseases, many other disorders may benefit from improved knowledge of how to genetically manipulate cells for therapeutic goals. Recent advances in stem cell biology, particularly the reprogramming of individual patient somatic cells, have created a great deal of speculation about their potential use for therapy of many diseases. However, the excitement needs to be tempered by a realistic understanding of both the potential for and the limitations of such treatments. Many of the important translational questions can be best addressed in large animal models under actual disease conditions. We hope the articles in this issue are informative and useful for the reader.

Acknowledgments

I thank the numerous postdoctoral researchers, graduate students, clinical fellows, and technicians who have worked in my laboratory; my faculty colleagues; the veterinary students who care for the animals in the research colonies; and the National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Center for Research Resources (NCRR), and private foundations that have supported our work.

Dedication

Mark Haskins and I dedicate this issue to Dr. Donald F. Patterson, who founded the study of medical genetics in domestic animals, at the University of Pennsylvania School of Veterinary Medicine. His vision recognized that human disease could be appreciated in animals if a deliberate search for them was undertaken. He was an academic and intellectual mentor to numerous research veterinarians who continue fascinating studies into the causes and cures of genetic diseases that afflict both humans and animals.

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