# Perspectives Series: Molecular Medicine in Genetically Engineered Animals

## Animal Models of Human Disease for Gene Therapy

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Gene therapy is in its formative stages with the scientific principles requisite for its success only beginning to be defined. The process of discovery and development necessary to make gene therapy a reality differs from that of traditional drug development in several important ways. The basic concept of gene therapy is so fundamental, i.e., modification of gene expression for therapeutic gain, and dependent on an understanding of basic biological principles, that one can expect rapid evolution of the field in directions that will be difficult to predict. Critical advances will emerge from research that focuses on basic biology of the vector systems and target cells.

Animal models of human diseases have been emphasized in the early development and evaluation of gene therapy. This has been particularly evident in the evaluation of gene therapy protocols for clinical trials by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH). Investigators are advised in the "Points to consider" of the RAC to "Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the models chosen are the most appropriate" (1). It has been five years since the first human gene therapy trial was initiated with greater than 120 others having achieved RAC approval. During this time, tremendous progress has been made in the development of animal models of human diseases through directed germ line manipulation of the mouse. In this short review, I have attempted to assess the impact animal models have had on the early development of the field. This discussion is limited to the use of genetically modified animals in assessing efficacy of gene therapy and defining the basic biological principles relevant to its successful development. I do not address the critical importance of animal models for determination of safety which are traditionally viewed in the context of toxicology.

*Models of disease pathogenesis*

The early use of animal models for gene therapy emphasized those which simulated inherited disorders thought to be appropriate candidates for early human trials. The goal was to use these models to evaluate the potential efficacy of a genetic intervention by evaluating its impact on meaningful surrogate or clinical endpoints. This is particularly important in the early stages of the field where "genetic reconstitution" is unlikely to be efficient and appropriately regulated.

The experience, in general, has been disappointing in that the animal models were not always available and when generated by germ line interruption in the mouse have not been particularly useful. Two inborn errors of purine metabolism con-

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sidered early candidates for gene therapy illustrate this point (2). A deficiency of the X-linked enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT)[1] leads to a devastating behavioral/neurological syndrome called Lesch-Nyhan syndrome, which was considered an early candidate for human gene therapy (3). Uncertainties regarding the primary pathogenesis of the disease and the role of central nervous system–directed versus systemic gene transfer made the availability of an animal model compelling and necessary for the rational development of a genetic therapy (4). The first genetically engineered mouse made by targeted interruption of a gene was created in the context of an animal model for Lesch-Nyhan syndrome (5, 6). Unfortunately, this HPRT-deficient animal manifested none of the neurological or behavioral features of the human disease, making it useless in facilitating the development of a human gene therapy. The second and more celebrated example is severe combined immune deficiency caused by deficiency of adenosine deaminase (ADA). Patients with this disorder are essentially normal except for a virtual absence of T cell-mediated immunity (7). A clinical trial of gene therapy for ADA deficiency was proposed by a group of investigators at the NIH who argued on theoretical grounds that gene transfer directed to hematopoietic stem cells or lymphocytes should be effective (8). An important point of discussion regarding the appropriateness of the trial was the absence of experimental validation in an authentic animal model which did not exist at the time. Clinical trials based on gene transfer to lymphocytes and hematopoietic stem cells were approved and initiated. Preliminary results indicate engraftment of genetically modified cells for prolonged periods of time, although true clinical efficacy has not been consistently demonstrated (9, 10). Long after the clinical trials were begun, an ADA-deficient mouse was created by germ line interruption (11). The phenotype of the mouse differs substantially from that of ADA patients in that it is lethal in the prenatal period due to failure of many organ systems, except lymphopoiesis which, curiously, appears normal.

While the initial experience with animal models in gene therapy was disappointing, subsequent studies have identified some very useful models including those representing spontaneous mutations such as the mouse model of mucopolysaccharidoses type VII, which is deficient in β-glucuronidase (12), and the hyperammonemic model of ornithine transcarbamylase deficiency (13). Animal models generated de novo by germ line interruption have been more plentiful and with some exceptions less useful. Notable exceptions include the murine model of familial hypercholesterolemia (FH) deficient in LDL receptor (14) and the Gaucher's mouse deficient in glucocerebrosidase (15), both of which manifest biochemical and clinical abnormalities similar to that seen in the corresponding human syndromes.

An important lesson from these initial studies has been the impact of environment and genetic background on phenotypic expression of the germ line mutation and the potential to generate a phenotype more useful for gene therapy by manipulating these variables. This is best illustrated in the development of a mouse model of cystic fibrosis (CF). Several groups have interrupted or incorporated specific mutations into the CF transmembrane conductance regulator (CFTR) gene of murine embryonal stem cells (16–18). The resulting CFTR-deficient animals have not been useful in evaluating lung-directed gene therapies because they do not develop pulmonary pathology at the time of their death, which occurs soon after weaning due to intestinal manifestations of the disease. Subsequent environmental and genetic modifications in the model have increased its utility. Whitsett and colleagues prevented lethal gastrointestinal manifestations of the disease by introducing into the germ line of a CF mouse a transgene encoding human CFTR expressed from a gut-specific promoter (19). The animals selectively express transgene-derived CFTR in gut epithelia sparing them of bowel obstruction without confounding the biology of the lung. In addition, animals have been removed from their pathogen-free environment and exposed to CF-associated bacterial pathogens such as pseudomonas aeruginosa and staphylococcal aureus to more accurately simulate the unprotected environment in which CF patients reside (20). Under these conditions, the CF mice developed pathological abnormalities of lung similar to what is seen in patients. The recent discovery of an alternative Cl channel found in mouse but not human lung may also explain the mild phenotype in CF mice and suggests another target for site-directed disruption that together with the CFTR knockout may enhance the utility of the model (21).

*Assessing host-vector interactions*

The natural evolution of the field of gene therapy has been toward in vivo approaches, with recombinant adenoviruses leading the way in many early animal and clinical models. These experiments have identified immune responses of the recipient to the vector and genetically corrected cells as confounding problems (22). It is my belief that many of the problems encountered with adenoviruses will be relevant to other vector systems. An understanding of the mechanisms responsible for these potentially limiting immune responses will be useful in designing strategies to overcome them. The foundation on which this research is based is basic principles of T and B cell biology which are being defined in exquisite detail through the efforts of multiple investigators interested in fundamental immunology. One factor that has contributed to the recent progress in basic immunology is the availability of genetically defined strains of mice in which immune regulatory genes have been incorporated into or deleted from the germ line. These same tools have been used to define immune responses to in vivo gene transfer with recombinant adenoviruses.

Mice ablated in specific lineages and cytokines, through germ line interruption and antibody depletion, have been used to identify both cellular and humoral immune responses to adenovirus-mediated gene transfer in a variety of organ systems (23–25). Loss of transgene expression, which has characterized the use of E1 deleted adenoviruses, is caused in part by cellular immune responses to both viral antigens and the transgene product. Antigen-specific activation of cytotoxic T lymphocytes and T helper cells of the $T_{H1}$ subset are necessary for the recipient to effectively extinguish transgene expression. Activation of T helper and B cells to viral capsid proteins produces neutralizing antibodies that prevent effective readministration of virus. An understanding of these mechanisms has suggested revisions of current strategies based on transient immune

1. *Abbreviations used in this paper*: ADA, adenosine deaminase; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; FH, familial hypercholesterolemia; HPRT, hypoxanthine-guanine phosphoribosyltransferase.

blockade and modifications of the vector to minimize viral protein expression.

Realizing the importance of host immune responses to in vivo gene therapy, it seems prudent to assess the utility of available models. The reality is that basic studies of immunology are more easily performed in mice and humans. How predictive will the mouse studies be in the actual implementation of gene therapy in humans? Difficulties in modeling human vaccines that are based on recombinant viruses in animal models would suggest important differences may emerge. Data generated in the initial human experiments will test concepts that are based on experiments in mouse models and will help focus subsequent murine studies. One approach for improving the utility of mouse models in the study of human immunology is to replace murine immunoregulatory genes with human homologues using transgenic technologies. This will be particularly useful when evaluating therapeutic proteins such as cytokines, antibodies, and soluble receptors, aimed to modulate the immune responses to the therapy.

*What have we learned?*

One aspect of the human population that will be impossible to simulate in animal models is the tremendous environmental and genetic diversity that is present. This will be particularly important in the context of immune responses to gene replacement therapy where several factors may profoundly contribute to outcome, such as nature of mutations in the disease gene (i.e., null versus a partial defect), major histocompatibility genotype, and previous exposure of the recipient to the delivery vehicle in the context of naturally acquired infection. Therapies developed in the context of inbred colonies of animals will likely yield more consistent results than what will be observed in the corresponding human populations.

Early experience in the treatment of patients with genetic diseases supports this hypothesis. The two ADA-deficient patients treated with transfusion of autologous, genetically corrected lymphocytes have realized significantly different outcomes under apparently similar circumstances with substantially better immune reconstitution achieved in the first patient (9, 10). Ex vivo liver-directed gene therapy in the rabbit model of FH accomplished significant and prolonged improvement in serum cholesterol in 5/5 animals (26), whereas only 2/5 FH patients responded to ex vivo gene therapy with similar improvements in serum lipids despite the presence of genetically corrected cells in liver of all five patients (27, 28). Finally, adenovirus-mediated gene transfer to airway epithelial cells for the treatment of CF has been consistently demonstrated in a variety of animal studies (29, 30) while the experience in humans has been quite variable (31 and Wilson, J.M., unpublished results).

The design of clinical trials based on "proof-of-concept" experiments in animal models should anticipate substantially greater heterogeneity in the response to gene therapy in humans. The consistent demonstration of successful gene therapy in the diverse human population will represent a far more rigorous test of the utility of gene therapy than what emerges from experiments in inbred strains of animals.

*Future directions*

Animal models of human disease will play an increasingly important role in the development and evolution of human gene therapies. The initial limitations of many murine models may be overcome through defined environmental manipulations and more complex germ line manipulations which bring together a composite of genetic modifications. The most useful strategy integrates extensive studies in relevant animal models with focused, informative human pilot experiments.

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