Comment

The Relevance of Genetically Altered Mouse Models of Human Disease

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Summary — The impetus to develop useful models of human disease and toxicity has resulted in a number of large-scale mouse mutagenesis programmes. This, in turn, has stimulated considerable concern regarding the scientific validity and welfare of genetically altered mice, and the large numbers of mice that are required by such programmes. In this paper, the scientific advantages and limitations of genetically altered mice as models of several human diseases are discussed. We conclude that, while the use of some such mouse models has contributed considerably to an understanding of human disease and toxicity, other genetically altered mouse models have limited scientific relevance, and fewer have positively contributed to the development of novel human medicines. Suggestions for improving this unsatisfactory situation are made.

Key words: *disease, genetically altered, mouse, mutant, Three Rs, toxicity.*

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Introduction

Advantages of using the mouse

For almost a century, mice have been used as models of human physiology, disease and toxicity. In fact, mice are used more extensively than any other mammalian species in research and testing, for several reasons. Firstly, mice are relatively easy to breed and maintain economically in captivity. Secondly, they have short life-spans, so their long-term, post-experimental care is normally not an issue. Thirdly, mice are amenable to genetic analysis and manipulation. Lastly, they share many physiological features, body systems and developmental and cellular processes with humans. There is, as a result, an enormous amount of information available on the biology, physiology and genetics of the laboratory mouse.

The completed draft sequence of the mouse genome was published in 2002 (1). A comparison of the mouse and human genomes (2) suggests that they are of comparable size (around 3 billion base pairs), encode similar numbers of genes (around 24,000), and are 99% identical, at least in terms of the presence of homologous genes (equivalent genes). The two genomes are also 95% syntenic, with the same genes clustered on equivalent chromosomes in both genomes. These observations have added further impetus to efforts to generate and characterise strains of genetically altered (GA) mice, in order to develop models that improve our understanding of human physiology and disease.

Methods of creating GA mice

GA mice are created by using targeted mutagenesis (as used in reverse genetic research), which gives rise to genetically modified (GM) mice, or by exposure to mutagens (as used in forward genetics approaches), referred to as GA mice. There are currently more than 7000 publicly-available mouse strains (3), and the ease with which mice are genetically manipulated is reflected in the fact that they are the only mammalian species which has been genetically modified by homologous recombination (whereby a mouse gene is substituted with a human gene). Since the rationale for generating such strains is the similarity between the mouse and the human genomes, it is assumed that mouse and human genes that are homologues and occupy similar positions in the genome, have similar functional roles. Thus, it is commonly assumed that when the function of a gene is altered, any changes in phenotype or physiology that result are indicative of the

roles of that specific gene in the mouse and hence, by extrapolation, in humans.

International cooperation

Several major international consortia are involved in generating GA mice, with the collective aim of isolating and characterising at least one mouse GA line for every gene in the mouse genome. These consortia include large-scale mouse mutagenesis programmes at RIKEN, Japan (4), the Baylor College of Medicine (5) and Jackson disorders mutagenesis (6) projects, and the National Institutes of Health (NIH) programmes (7) in the USA, and the European ENU mutagenesis programme (Eumorphia; 8. See also Table 1).

According to the Home Office statistics (9), between 1990–2005, there has been a progressive rise in the proportion of animal procedures that involve GM animals within the UK, and, by 2005, one third of all procedures conducted under the *Animals (Scientific Procedures) Act 1986* involved the use of GM animals, mainly mice. If this trend persists, it is likely that, within the next few years, the use of GM mice will increase the levels of animal experimentation in the UK to beyond the pre-1986 levels.

These statistics already understate the extent to which GA mice are used, because of a difference in the way that GM animals are defined legally and by the UK Home Office. The Royal Society has suggested the following definition: "an organism whose genetic material has been altered in a way that does not occur naturally or as a result of mating and/or natural recombination of its gene" (10). This definition includes transgenic and knock-out mice, as well as mutant mice resulting from random mutagenesis caused by exposure of parent mice to radiation or mutagens, or mice created by inbreeding. However, for statistical purposes, the latter two categories of GA mice are not classified as GM mice, unless the genetic defect has been defined. For the purposes of this report, in its consideration of all GA mice, the latter two categories are classified as non-GM GA mice (GA mice), while the categories of mice that fall within the Home Office definition of GM mice are referred to as GM mice.

Limitations and problems

The generation of a single GA mouse strain can require the use of very large numbers of animals, because: a) the mutagenesis methods are inefficient; b) there is poor germline transmission of gene mutations; and c) genetic modification can adversely affect fecundity and survival. These problems are so acute that, in many studies, a large proportion of the animals are incidental to the work, since they are merely used for breeding or are offspring that fail to carry the desired mutation or lack a novel and detectable phenotype.

Also, even if the desired GA mice are created, they are not always good models of human disease, for several fundamental reasons. These include the fact that humans are about 3000 times larger than mice; they possess a much greater number of cells, some of which have to communicate over long distances; and the lifespans of mice and humans are vastly different. Moreover, there is a lower likelihood that errors within the mouse genome will eventually result in chronic diseases such as cancer, due to the greatly reduced number of cell divisions that occur in the body of this species over its lifetime, as compared with humans (see *Mouse models of cancer, DNA repair disorders and their use in toxicity testing*).

We have stated earlier that the mouse and human genomes have similar gene clusters and mice possess gene homologues to many human genes. However, despite this, and despite the fact that the average similarity between mouse and human genes is 85%, with similarity ranging from 70–95% for specific genes, a single nucleotide difference can dramatically alter the function of the protein expressed. Such subtle changes can have important phenotypic consequences for a species, affecting physical characteristics, as well as biochemistry, physiology and pharmacology, leading via variations in temporal and spatial protein expression to species-specific metabolism, immune responses, sensory perception and endocrine functions. Therefore, assumptions about the functional equivalence of homologous genes in mice and humans can be erroneous.

Objectives of the review

The aim of this paper is to retrospectively assess the relevance of a number of GA mouse models of human disease and their treatment, making reference to specific animal welfare problems associated with the ways these models are created and used. The intention is not to extensively review the welfare implications of mouse mutagenesis-based research, but to discuss specific welfare-related issues.

One or more mouse models of haematological, hormonal and metabolic or cardiovascular function, allergy and infection, sensory or central and peripheral nervous systems, behaviour and cognition, cancer, and bone, skeletal or muscle defects are considered, alongside their value in the development and safety testing of new therapeutics. These form some of the main areas of interest of the major mouse mutagenesis programmes (Table 1).

For consistency, human genes are given in upper-case and italic and mouse homologues in lower case and italics.

Generating GA Mice by Forward and Reverse Genetics Approaches

ENU mutagenesis

GA mice can be generated by forward genetics, a phenotype-driven approach first described in the 1970s. It involves correlating changes in phenotype with the underlying genetic alterations. This method can involve the exposure of male or female mice or, preferably, isolated embryonic stem (ES) cells, to radiation or chemical mutagens, in order to increase the frequency of spontaneous mutation. A commonly-used approach involves injecting *N*-ethyl-*N*-nitrosourea (ENU) into the abdominal cavities of approximately 12-week-old male mice. The ENU causes single base pair mutations by alkylating nucleic acids that may eventually lead to the incorporation of the incorrect base into DNA following replication. However, like many chemical mutagens, ENU can also induce temporary sterility or fatal tumorigenesis, since mice lack many of the antineoplastic mechanisms present in humans (11). Assuming that the animals survive the ENU treatment, sterility is generally reversed by the time the mouse is about 17 weeks old. The male can then be mated with untreated females. The mice in the resulting generation are phenotypically characterised and those displaying novel or useful traits are selectively bred. The frequency of mutation at any given locus is only about $^1/_{500}$–$^1/_{1500}$ of the G1 offspring (12). However, less than 2% of the resultant offspring display desired phenotypes, due to the high incidence of recessive traits, poor germline transmission and poor progeny survival. Thus, when using forward genetic studies, very large numbers of animals are used and produced, in order to obtain a few useful GA animals

The main advantage of this technique is that it is not necessary to make any prior assumptions about the potential functions of genes, since all genes are potentially susceptible to random modification by a mutagen. Furthermore, in theory, many GA mice can be generated by a single mutagenic treatment, including animals with mutations that result in congenital, biochemical, immunological and other complex traits. Nevertheless, identifying the genetic basis of the phenotype can prove difficult, especially when gametes, rather than ES cell-based methods, are used, and particularly where there is little or no information about the biochemical changes that accompany a novel phenotype. Furthermore, the majority of ENU-based mouse mutagenesis studies are geared toward identifying dominant traits, with few studies being undertaken to obtain recessive mutants, since it can be difficult to identify recessive carriers for subsequent breeding. It is also common practice to selectively screen for particular phenotypes, so large numbers of animals can be wasted, because a useful trait is not detected. Consequently, it is likely many potentially relevant mouse models have not been identified and isolated, since their phenotypes would only be evident during specific life stages. However, of particular concern is the fact that mutated animals may display only very subtle changes in phenotypes that nevertheless are able to adversely affect their welfare in unpredictable or undetected ways.

Knock-out mutagenesis

The second main way to generate GA mice is by reverse genetics, sometimes known as the genotype-driven approach. This involves targeted manipulation of the genome, to generate GM mice which display a desired phenotype or carry a specific genetic alteration. Gene knock-out can involve altering a gene's function by inserting a segment of non-coding DNA or marker protein DNA, or by using an antisense oligonucleotide or RNA interference to disrupt gene expression. In each case, the expression of the targeted protein is disrupted and a loss-of-function mouse strain is generated. The NIH Knock-out Mouse Project (KOMP) is aimed at producing a knock-out mouse for each gene within the mouse genome (13). This equates to a minimum of approximately 24,000 knock-out mouse strains. However, since each affected protein could have multiple physiological roles or could play a key role in development, knock-out and other loss-of-function mutagenesis commonly results in lethal fetal abnormalities, without necessarily allowing any clues to be discovered as to the function of the targeted gene in question.

Transgenesis

Knock-in mutagenesis involves the introduction of one or more foreign genes — or transgenes — into the mouse genome, to permit the study of the roles of normal or mutant forms of mouse or human genes, or genes ordinarily absent from the mouse genome. Virus-based transgenesis, the earliest developed of the three main transgenic methodologies, involves the use of viruses to deliver the required transgene into sperm and eggs. However, it is difficult to control the number of copies and the precise location of insertion of the transgene when using forward genetic studies. This is particularly problematical, since it is now known that many non-coding

Table 1: A selection of mouse mutagenesis programmes and consortia

Research programme	Type of mutagenesis	Disease or genetic components	Website
Examples of programmes aimed at producing GA mice			
Baylor College of Medicine	ENU	Developmental defects; endocrine defects; neurological anomalies; blood defects	http://www.mouse-genome.bcm.tmc.edu/ENU/MutagenesisProj.asp
Baylor College of Medicine	Genetic manipulation	Chromosome 11 engineering	http://www.mouse-genome.bcm.tmc.edu/ChrEng.asp
Baylor College of Medicine	Gene trapping	Reproductive mutants	http://www.mouse-genome.bcm.tmc.edu/bartmice/home.asp
Centre for Modelling Human Disease, Toronto	ENU/genetrap	Genetic control of bone mineral density	http://www.cmhd.ca/genetrap/index.html
Centre for Modelling Human Disease, Toronto	ENU	Neurological and behavioural research; haematopoesis research; cardiovascular research	http://www.cmhd.ca/enu_mutageneis/index.html
Harwell Mutagenesis Programme	ENU/targeted mutagenesis	Genome-wide	http://www.mgu.har.mrc.ac.uk/
Jackson Laboratory	Mutagenesis/breeding	A wide variety of disorders including neurological, heart, lung, blood and sleep disorders	http://www.jax.org/ http://www.jax.org/nmf/
McLaughlin Research Institute	ENU	Genome-wide; recessive screen	http://www.montana.edu/mri/enump.html
National Institutes of Health Initiative	Knock-out mutagenesis	Genome-wide gene function study	http://www.nih.gov/science/models/mouse/knockout/komp.html
Oak Ridge National Laboratory Mammalian genetics & genomics	ENU	Regional/chromosome specific screen	http://lsd.ornl.gov/mgd/mutagenesis/
RIKEN	ENU	Genome-wide; includes screen for recessive mutants and both early and late-onset diseases	http://www.gsc.riken.jp/Mouse/main.htm

Table 1: continued

Research programme	Type of mutagenesis	Disease or genetic components	Website
Producers of gene-trap ES cells that may be used to create mutant mice			
Baygenomics	Knock-out	Cardiovascular and pulmonary diseases	http://baygenomics.ucsf.edu/overview/welcome.html
European Conditional Mouse Mutagenesis Programme	Conditional mutagenesis; knock-out; transgenesis	Genome-wide	No current web address — new initiative
German Genetrap Consortium	Transgenesis	Genome-wide	http://genetrap.gsf.de/
International Gene Trap Consortium	Transgenesis	Genome-wide	http://wwwgenetrap.org/
Omnibank	Gene trap knock-out	Genome-wide for drug discovery	http://www.lexicon-genetics.com/discovery/omnibank.htm
Sanger Institute	Knock-out	Genome-wide	http://www.sanger.ac.uk/PostGenomics/genetrap/

sequences regulate gene expression, so introducing a foreign gene can disrupt normal patterns of expression for other genes. Therefore, it is not surprising that many of the "mosaic" offspring that result from transgene insertions are non-viable. This technique has been largely supplanted by a method in which the transgene is incorporated into the pronuclei of embryos or ES cells. When transgenes are inserted into embryos in this way, the efficiency and survival of embryos to birth is increased to around 4–6%. However, since pronuclear injection does not overcome the problems of multiple and random transgene insertion, targeted gene insertion into ES cells has become the method of choice. By using this method, it is possible to flank the transgene with DNA sequences that correspond with those of the desired insertion site, and then to select engineered stem cells for propagation and injection into blastocysts destined for uterine implantation. A similar technique has been developed for engineering sperm stem cells, followed by their reintroduction into testes lacking germ cells. A detailed review of the methodology can be found elsewhere (14).

The main concern with transgenic research is that, even if the transgene is expressed, the result might not be sufficiently informative about the role of the targeted gene, since its expression may not be regulated in the same way as it is in humans. Furthermore, although tissue-specific or temporal expression can be achieved by placing transgene expression under the control of tissue-specific or inducible promoters, respectively, there is no guarantee that the protein following expression of the gene is folded, targeted or regulated in the same manner in mice and humans. This can give rise to unexpected and significant animal welfare problems.

The Relevance of GA Mouse Models: Case Studies

GA mice as partial replacement models

In a small number of cases, GA mice might eventually supplant the use of other animals in research and testing. One example is the use of transgenic TgPVR 21 mice expressing the human polio virus receptor for the neurovirulence testing of the oral polio vaccine (OPV), which could obviate the use of primates in such tests. The use of this model is supported by the World Health Organisation (15), since not only do these mice display histological and physical signs of motor neuron degeneration associated with human forms of the disease that can be monitored by paralysis scoring, but also because tests of OPV lots can be conducted in 2 weeks rather than in 1.5–2 months, the time taken to conduct primate-based neurovirulence testing. Where there is a need for the routine animal testing of vaccines for the presence of infectious viral particles, it is perhaps more ethical to use transgenic mice than primates, since this also avoids serious logistical issues, such as containment of infected animals or the need for the long-term care or re-homing of laboratory animals with longer life expectancies.

Are GA mice relevant and useful?

GA mouse models of human disease often lack relevance in the case of complex multigenic disorders. Indeed, some studies in GA mice have been less informative than the corresponding investigations with less-complex organisms and cell culture systems. This is particularly true for mouse models developed by using forward genetics, where an undefined number of mutations may have contributed to an overall phenotype which resembles a human disorder, but which may share few, if any, of the underlying biochemical or genetic causes of the respective human disorders. The relevance of many transgenic mouse models can be questioned on the basis that, even if a species gene homologue has the same function and expression patterns and levels in humans and mice, all the remaining components of the biochemical pathway must be equally represented in the surrogate animal, if relevant mouse models of human diseases are to be created within a laboratory setting.

It is not feasible to consider the scientific relevance of every GA mouse model. For this reason, the limitations, including those described above, of using GA mice as models of human diseases will be illustrated by reference to models of specific human diseases and to groups of diseases that share fundamental features.

Mouse models of haematological diseases: sickle cell anaemia

Sickle cell anaemia (SCA) is one of several types of inherited blood disorder that can affect the shape and haemoglobin content of red blood cells, their half-life in the blood, and their ability to pass through small vessels. In unaffected individuals, 95–98% of normal haemoglobin content is haemoglobin A, which is composed of two α and two β chains; a small proportion of haemoglobin is composed of two α and two δ chains (haemoglobin A2), or of two α and two γ chains (fetal haemoglobin). In SCA, the red blood cells contain mostly haemoglobin S, because of mutations in the β chain protein-encoding gene (β^s mutations).

Transgenic mouse models of SCA were first created by replacing the mouse α and β^s haemoglobin genes (*hbA* and *hbB*, respectively) with the equivalent human genes (*HbA and HbB*). The resultant mice express haemoglobin chain proteins that comprise the major form of haemoglobin, haemoglobin A (16).

These mice demonstrate limited red blood cell sickling. Further genetic engineering of the β^s gene has therefore been performed, in order to create SAD mice that contain three point mutations, namely the β^s Antilles β23Ile and D Punjab β121Gln mutations in the *HbB* gene (17). These mice exhibit enhanced sickling, a feature that has been used to examine the effects of potential anti-sickling agents. However, these mice fail to display the haemolytic anaemia characteristic of the disease in humans.

Other mouse models of human SCA have been created by deactivating the mouse *hbA* and *hbB* genes by using homologous recombination. These models include Berkeley mice, which express the full complement of human SCA globin α and β genes, including the mutant β^s genes (genotype: Tg[Hu-miniLCR$\alpha 1^g\gamma^A\gamma\delta\beta^S$]$Hb\alpha^o//Hb\alpha^o Hbb^o//Hbb^o$). Yet Berkeley mice still display only some, but not all, features of the human disease (18). The differences between the Berkeley mouse model and the human disease have been reviewed (19). Rather than an increase in red blood cell haemoglobin levels, as seen in human SCA, Berkeley mice tend to have reduced levels of normal haemoglobin content in their red blood cells. This is presumably because mice express very low levels of fetal haemoglobin, a protein that is able to protect against reduction in haemoglobin levels in human individuals with SCA. Indeed, the variation in the levels of fetal haemoglobin is directly related to the severity of SCA seen in these individuals — those with relatively high fetal haemoglobin levels exhibit milder forms of the disease. Such variation is not seen between individual Berkeley mice. Furthermore, the red blood cell volumes are much smaller in mice than in humans. Presumably, these features of the Berkeley mouse together contribute to the increased severity of disease symptoms in the mouse model in comparison with those that develop in humans. Furthermore, differences in the main site of haematopoiesis (the spleen in the Berkeley mouse and the bone-marrow in SCA patients) might contribute to the apparent splenic hypertrophy in 1–6 month old mice, whereas in humans, initial hypertrophy is followed by atrophy of the spleen and almost total loss of spleen function in adult patients. Nevertheless, since many of the same organs are affected by SCA in the mouse model as are involved in the human disease, the Berkeley mouse is likely to be useful in the study of some features of the human disease.

Mouse models of hormonal and metabolic function: diabetes

Type 1 diabetes

Human diabetes is characterised by an inability to regulate blood glucose levels. However, each of the various types of diabetes has specific aetiologies and treatment regimes. Human type 1, insulin-dependent diabetes, for instance, has been attributed to an autoimmune condition. It affects children and young adults, and occurs when the body makes little or no insulin.

Mouse models of insulin-dependent type 1 diabetes have been created by gene knock-out and gene manipulation, principally to increase understanding of the role of the immune system in the aetiology of the disease (20). The non-obese diabetic (NOD) mouse, however, was created by the selective inbreeding of a specific mouse strain. These mice spontaneously develop insulin-dependent diabetes, but, unlike the analogous situation in humans, female NOD mice develop diabetes at a higher incidence than males (21). Currently, there is little understanding of the role of other pathways in the pathogenesis of diabetes. Yet, despite its limitations, the NOD mouse has been used to examine the complex and multifactorial changes in the expression and activity of a number of proteins and genes, and also provides some useful insights into the human disease. This is because, despite the differences between diabetes in the NOD mouse and the human disease, there appears to be considerable similarity between the roles of the components of the major histocompatibility complex II and cytokines in the mouse model and human conditions (22).

Type 2 diabetes

Maturity onset diabetes of the young (MODY) and adult type 2 diabetes are no less difficult to study, since there appear to be several mutations in different genes that can contribute to the development of these disorders. Several GM mouse models have been generated for studies on individual components of type 2 diabetes. Knock-out mice have not been useful for deciphering the mechanism of type 2 diabetes. For instance, despite the supposed roles of the equivalent proteins in human diabetes, loss-of-function mutations in the insulin receptor gene (resulting in poor insulin sensitivity), combined with the absence of a K_{ATP} channel (which regulates insulin secretion), were insufficient to trigger diabetes in mice carrying both genetic defects (23). Furthermore, it seems that, while loss-of-function mutations in the human K_{ATP} channel protein, SUR1 — the receptor for sulphonylureas used to close the K_{ATP} channels in type 2 diabetes, and thus to reduce insulin secretion — cause hyperinsulinemic hypoglycaemia, *Sur1* gene knock-out mice, despite dysfunctional K_{ATP} channels, are able to regulate insulin secretion. This appears to be due to the presence of a second insulin regulatory mechanism that is found in mice, but not in humans (24). This suggests that

the usefulness of GA mice in the development of therapeutics that target K_{ATP} channel activity is likely to be severely limited.

The phenomenon of ectopic expression, the expression of a protein in a tissue where it is not normally expressed, has been used to create other models of type 2 diabetes. In the case of ectopic expression of dominant mutants of agouti protein (a natural ligand for melanocortin receptors that helps to regulate appetite) in numerous tissues (25), GM mouse models have not helped in determining the mechanisms of insulin resistant diabetes and obesity. Instead, studies in these mice have simply confirmed that agouti protein has a number of roles within mice, many of which may only superficially resemble the roles of the protein in humans. One such agouti mutant strain that expresses the agouti transgene hemizygotically, *Tg*/–, starts to develop diabetes-like symptoms when the mice are only 4–5 weeks old, in contrast to the more delayed onset in human patients. Furthermore, hyperglycaemia in mice appears to occur only in males, which is not the case in humans. This mouse model continues to produce insulin at higher levels than normal mice, such that the model is only potentially relevant for studying one type of insulin resistant diabetes.

Mouse models of infection and susceptibility to infection

Cystic fibrosis

Perhaps the best studied human disease is cystic fibrosis (CF). In the 1980s, CF was linked to mutations within the gene encoding a chloride channel, which is now referred to as the CF transmembrane conductance regulator (CFTR; 26). The CFTR normally facilitates the movement of chloride ions into and out of cells within the lungs and other organs. *CFTR* gene mutations can impair CFTR expression, structure or function, resulting in the accumulation of thick, sticky mucus and other secretions that increase the individual's susceptibility to infections, impair cellular secretion and/or transport, and contribute to early death. Some 70% of CF sufferers carry a deletion of three nucleotide bases corresponding to the loss of a single phenylalanine residue in the encoded protein. However, about 1300 further frameshift and site-specific mutations have been detected within the human *CFTR* gene or the upstream promoter regions (27).

CF is an autosomal and recessive disease, so two copies of a defective *CFTR* gene are needed before the disease manifests itself. This represents the first limitation of the mouse models, since not all *CFTR* gene mutations will have the same phenotypic consequences, and the likelihood that a person would inherit identical mutant alleles from both parents is low.

The most commonly-used CF mouse model was generated from ES cells, in which the mouse gene thought to be homologous to the human *CFTR* gene, *cftr*, was inactivated by gene targeting. The resultant *cftr (–/–)* homozygous mice exhibited some of the features of human CF (28). However, there are important differences between this and other mouse models of the disease and CF in humans. Some of these differences stem from the fact that the so-called mouse CFTR is now thought not to be a species homologue for the human protein (29). The most important difference is the fact that *cftr (–/–)* mice do not develop excessive amounts of mucus in the lungs, a very common complication in human CF. This is because mice have fewer mucus-secreting cells in their lungs than are present in humans. In fact, whereas lung infections are the major cause of death in CF humans, *cftr (–/–)* mice tend to die mainly as a result of gastrointestinal obstruction, which is not a feature of the human disease (30). Furthermore, while pancreatic disorders that arise due to an inability to secrete pancreatic enzymes are seen in around 85% of CF patients, pancreatic dysfunction is rarely seen in *cftr (–/–)* mice. Moreover, when it does occur, its onset is delayed in mice. A high proportion of CF patients also exhibit loss of fertility, and some develop liver cirrhosis. Again, neither of these symptoms is seen in *cftr (–/–)* mice. In fact, only by considering the contribution of other proteins to fluid composition within cells of the respiratory tract to the accumulation of thickened mucus in the lungs, were some of these important differences between the human disease and mouse models of the disease resolved (31). This suggests that CF is unlikely to be a monogenic disorder, but instead, is dependent on the expression and function of other proteins, such as protease-activated receptor-2, found in alimentary and respiratory membranes, where it regulates mucus secretion (32), and on sodium ion transport. Indeed, by overexpressing an epithelial sodium ion channel under the control of an airway-specific promoter, a more useful model of airway mucus hypersecretion has recently been created. The resultant mice show increased sodium ion absorption and higher mucous concentrations in their lungs, resulting in many of the features of CF lung disease in humans (31).

Thus, to date, GM mice have largely proven useful only in terms of identifying the differences between the human disease and mouse models of the disease. Such studies have therefore revealed some of the underlying mechanisms of the human disease. However, the relevance of CF mouse models to the development of therapies is somewhat limited, due to the lack of correspondence between CF in the mouse models and human CF.

Vaccine development and testing

GM mice have been developed to study infections caused by pathogens such as the human poliovirus (33), to develop vaccines, and to bioassay pathogens, such as BSE (bovine spongiform encephalopathy; 34). Challenge with a pathogen itself can have severe implications for the welfare of mice used in these studies. However, in some instances, the use of GM mice represents a refinement in the context of the Three Rs, particularly for HIV/AIDS research, where mice have partially replaced the use of primates (see *Mouse models as partial replacements*). The latter have a much longer life expectancy, and often fail to develop symptoms of infection, in addition to being difficult to naturalise or re-home, once they have been exposed to HIV. However, the immune systems of mice and humans are far from identical (35), and this limits the relevance of some mouse models, not only for modelling human autoimmune disorders such as type 1 diabetes and some aspects of neurodegenerative disorders, but also for modelling pathogenic infections.

Xenografting of immune-compromised mice

In an attempt to overcome some of these limitations, severe combined immune deficiency (SCID) mice have been developed by genetic engineering, initially by the chance mating of two recessive carriers of a mutant form of the *SCID* gene. Homozygous *SCID*/*SCID* mice lack T-cells and B-cells. Another SCID mouse, the *SCID*/*beige* mouse, additionally lacks natural killer activity. These mouse models have been used as a recipient for human tumour cell xenografts (see *Immune-compromised mice in cancer research*) and to study diseases such as hepatitis B and hepatitis C (36), Epstein-Barr virus infection, and HIV infection (37). A recent example of such an application is the use of SCID mice that are also homozygous for the urokinase plasminogen activator (denoted (*uPA)-SCID* mice) as hosts for primary human hepatocytes. Once the human hepatocytes had been transplanted into the *(uPA)-SCID* mice, the animals were infected with hepatitis B virus or hepatitis C virus, which afforded a well characterised model of human hepatitis infection. However, the mice are immune-compromised, so this model is not able to take account of any influence of the host immune system on the aetiology of the diseases being studied (38).

A better approach would appear to be the generation of trimera mice. Such animals are created, for instance, by the total body irradiation of BALB/c mice to destroy the haematopoietic system, followed by its replacement by engrafting bone-marrow samples from SCID mice. Human peripheral blood mononuclear cells (PBMCs) are also introduced into the mice, so they are capable of mounting primary and secondary cellular and humoral immune responses specific to a number of infections, including hepatitis B, hepatitis C and HIV (see 39 and the references therein). The availability of such models has provided a means of studying infectious diseases such as HIV, which have largely relied on far from ideal studies in non-human primates.

The fact that SCID mice are immune deficient, raises concerns about their welfare. Furthermore, due to the need for the containment of possible pathogens such as HIV and the hepatitis viruses, these animals require special conditions, in order to avoid the risk of contamination of co-housed, immune-compromised animals. Infection rates are minimised by using ventilated cages with filtered air, and by sterilising the cages, bedding and nesting material, and food and water. Attempts have been made to create other useful models that retain immune-competence, but which can still be useful to study the same range of diseases. An example of this is the gp120 mouse, which expresses the HIV genome in astrocytes (40). The main drawback of this model is that HIV does not replicate in human astrocytes, so it is difficult to determine whether this mouse model is suitable for studying HIV-associated effects on the human brain. This limitation has been overcome by engineering HIV, to create a chimeric virus which encodes viral coat glycoprotein, gp80, from murine leukaemia virus, instead of the corresponding coat protein, gp120, for HIV. The resulting engineered virus, EcoHIV, encodes HIV packaged within a murine viral capsule. This virus was used to successfully infect several strains of mice without the prior need to resort to creating GM mice or using an immune deficient model (41). Many of the major characteristics of HIV infection in humans were reproduced. Furthermore, since the engineered virus is only able to infect rodents, cross-species infection is not an issue.

Mouse models of sensory function

Using GA mice to map the roles of genes involved in sensory perception is particularly difficult, since evolutionary changes have resulted in species-specific gene repertoires for olfaction, vision and taste. Thus, olfactory receptors, for instance, are encoded by the largest mammalian gene superfamily (comprising more than 1000 genes). However, while the olfactory gene repertoires of rodents and primates are similar in size, the proportion of functional olfactory receptors in rodents and new world monkeys is higher than in old world monkeys and apes, with humans possessing the lowest number of olfactory receptors (42). In fact, although there is syntenic clustering of genes that encode these receptors in the mouse and human genomes, many of the human orthologues are pseudogenes, which

never result in the expression of functional proteins, but may regulate the expression of other genes or, as recently suggested, may have contributed to the acquisition of trichromatic vision specifically by humans and other apes (43). The mere fact that the functional gene repertoire may be expanded in mice can mean that, as in the case of olfaction, the ability of one gene or several genes to functionally substitute a gene that has been inactivated, can severely limit the use of functional genetic studies in mice to assign roles to human genes.

Deafness

A number of GM mice have been used to study the sensorineural and conductive forms of human deafness. The former type of deafness is associated with defects in the inner ear (mainly the cochlea) that impair the auditory processing of a signal, while the latter is related to structural defects in the external or middle ear that result in impaired sound conductance down the auditory canal. *The atp6b1* null mutant mouse, which cannot express the B1 subunit of H^+-ATPase, was developed as a model of human sensorineural deafness. However, this GM mouse displays distal renal tubule acidosis (44) but normal hearing (45), even though the same genetic defect in humans has been related to autosomal recessive sensorineural hearing loss as well as distal renal tubule acidosis (46). As a result, this mouse model has been more useful as a model of distal renal tubule acidosis than of human deafness.

The *shaker1* mouse (47), on the other hand, displays profound hearing loss, and was one of the first GA mouse models of human genetic deafness. Once the mouse gene underlying the natural defect in the *shaker1* mouse had been identified, the location of the corresponding gene in the human genome could be determined. It was found that the *shaker1* locus was encoded in mice by the myosin VIIA gene, *Myo7a*. As the name suggests, *shaker1* mice show hyperactivity, head-tossing and circling activity, in addition to hearing loss. It was subsequently demonstrated that mutations in the *Myo7a* lead to hearing loss in humans, symptomatic of non-syndromic forms of deafness and Usher's syndrome.

Half of all human individuals who are both deaf and blind suffer from Usher's syndrome. In humans, some mutations in the myosin VIIA gene (*MYO7A*) can also lead to a specific form of Usher's syndrome (type 1b syndrome), in which both hearing loss and the retinal degenerative disorder, retinitis pigmentosa, occur at around seven or eight years of age. Yet none of the *Myo7a* mutations in mice cause blindness in mice, even in very old mice, despite the fact myosin VIIA is normally expressed in the retinal pigment epithelium and photoreceptor cells of both humans and mice. This may be a reflection of the short life-span of the mouse, which prevents the retina from receiving sufficient exposure to light to elicit pathological changes (48). However, since high intensity light appears to reduce the size of a and b waves in young *shaker1* homozygotes and, to a lesser extent, in *shaker1* heterozygotes (49), the *shaker1* mouse might act as a suitable model for the early stages of retinitis pigmentosa, although not the later stages of the human disease.

Visual defects

There are also a number of specific GM mouse models of human retinopathies. Autosomal dominant retinitis pigmentosa (ADRP), for example, has been studied in rhodopsin (the visual pigment) knock-out (*rho –/–)* mice and transgenic mice that carry equivalent rhodopsin mutations to those related to human forms of the disorder (50). Extensive studies on both naturally occurring hereditary mutations in genes that influence visual perception, as well as on artificially produced GM mice, have provided much information about the visual process (51).

Nevertheless, several important differences exist between the organisation of the mouse and human visual systems, which derive from the fact that mice are nocturnal animals. The most significant of these are differences in visual acuity due to the substantially lower density of cones (for colour vision) and the absence of a fovea (for visual acuity) in the mouse retina, compared with the human retina. The mouse retina is dominated by rod cells for improved night vision. The presence of two, rather than three, colour pigments in mice may also have a bearing on the relevance of certain mouse models, since, while the absorption maxima of human red, blue and green pigments are 564, 429 and 534nm, respectively, the absorption maxima of the two mouse pigments are 360 and 510nm (52). The latter wavelength represents a considerable overlap with the absorption maximum of rhodopsin found in the rod cells of humans and mice, so there is a strong possibility that mouse colour pigments can partly compensate for visual defects that result from loss-of-function mutations in rhodopsin in a way that is not possible in the human visual system. This may explain why the rhodopsin mutants expressed in some mouse models do not result in retinopathies of the same severity as those seen in humans.

Mouse models of nervous and muscle systems

Muscular dystrophy

Duchenne Muscular Dystrophy (DMD) in humans is an X-linked recessive disorder due to mutations in the dystrophin gene (*DMD*). This gene encodes a

protein that ordinarily provides structural support for muscles. In humans, DMD is characterised by the rapid degeneration of muscles early in life, usually with onset before the age of five. Because the disorder is X-linked, it mainly affects males.

The most obvious problem with mouse models of DMD, such as *dy*/*dy*^{2j}, is that the genetic defect is not X-linked, but is instead a recessive autosomal trait of the mouse dystrophin gene homologue, *dy*. The first sign of DMD in *dy*/*dy*^{2j} mice, muscle weakness in the hind limbs, is evident within 2 weeks of birth (53), with affected individuals generally not surviving beyond 6 months. Thus, the onset of the disease appears to be earlier in the mouse model than in human patients, even taking into consideration the difference between the lifespans of mice (2–3 years) and humans (70–90 years). Indeed, histological examination suggests that the muscle membrane is more extensively damaged in *dy*/*dy*^{2j} mice than in human patients, partly because of the lower extent of fat replacement in the skeletal muscles of affected animals. In fact, human DMD is thought to be myogenic in origin, whereas in the mouse DMD model, it is believed to have a neurogenic origin, arising as a result of impeded nerve conductance due to the lack of nerve myelination.

A second mouse model of DMD, the *mdx* mouse, mimics the X-linked recessive nature of human DMD. This model arose as a spontaneous mutation in the mouse dystrophin gene, but this model also fails to exhibit many of the features of the human disease. In fact, although young *mdx* mice exhibit muscle necrosis, there is a rapid recovery due to a high rate of phagocytic infiltration and rapid muscle regeneration, such that the mice are essentially disease-free by the age of 5 weeks (54).

Together with differences between the anatomical positioning of muscles in humans and mice (55), these observations have limited the usefulness of mouse models of DMD in determining the mechanistic details of the disease and therefore in drug development. Nevertheless, using the *mdx* mice, and also a knock-out mouse that lacks the expression of dystrophin and the related muscle architectural protein, utrophin (the *dko* mouse model), potential problems of using dystrophin gene therapy to treat DMD patients have been identified, including difficulties with delivering large DNA molecules (56). This has led to considerable interest in developing alternative therapies, such as the use of minigenes and antisense oligonucleotides.

Neurodegenerative disorders

Predisposition to neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease (AD), is commonly dependent on mutations in several genes. For instance, mutations in genes encoding β-synuclein, parkin and α-ubiquitin hydrolase have all been linked to Parkinson's disease, and mutations in genes encoding β-amyloid, presenilin and tau proteins, have all been linked to human AD. Furthermore, many of these diseases are age-related and typically display slow onset, which is suggestive of a complex interplay between several disease susceptibility genes and non-genetic components, such as infection and age. As illustrated below, this can complicate the development of relevant models of these diseases.

Alzheimer's disease

AD in humans is an age-related disease associated with progressive loss of cognitive function and memory, and the development of amyloid plaques (APs) and neurofibrillary tangles (NFTs) in the brain. These mainly comprise β-amyloid protein and hyperphosphorylated tau protein, respectively. The human disease is also characterised by the loss of neuronal synaptic density.

GM mouse models of AD have, until relatively recently, been used to examine the individual roles of the two predominant forms of β-amyloid protein, the amyloid protein precursor (APP) and proteases that act on APP or β-amyloid proteins or, alternatively, on the expression and activity of tau or apolipoprotein E (a serum protein that regulates cholesterol levels; 57). The resultant mouse models display some, but not all, of the features of the human disease. However, the development of the *3xTg-AD* transgenic mouse model has greatly improved the situation. The *3xTg-AD* mouse was generated by using the homozygous presenilin 1 mutation (PS1M^{146}V) knock-in mouse (*PS1-KI* mice) as a starting strain. This transgenic mouse contains a mutated form of the *presenilin 1* gene, which results in the expression of a mutant form of presenilin 1 that causes the rapid development of APs in transgenic mice that also carry the *APP* double mutant K^{670}-N/M^{671}-L (APPswe) gene (58).

The resultant *PS1M*^{146}*V*/*APPswe* mice displayed limited neuronal depletion and no sign of NFTs. By contrast, while APs were not seen in a transgenic mouse model expressing the human P^{301}L tau protein, NFTs were evident in this model (59). Neuron-specific CNS expression was achieved by placing the expression of P^{301}L tau protein under the control of *Thy1.2*, a neuron-specific mouse promoter.

The rationale for creating the *3xTg-AD* model was to get the transgenes for PS1M^{146}V APPswe and P^{301}L tau proteins expressed simultaneously in the same model, in order to represent all the main features of the human disease. To achieve this, the genes encoding human K^{670}-N/M^{671}-L APP (APPswe) and human P^{301}L tau proteins were inserted into *Thy1.2* cassettes, to drive CNS expression of mutant preselinin 1 and tau proteins. The *3xTg-AD*

mice were then created by injecting these constructs into single cell embryos from *PS1-KI* mice.

This approach meant that fewer animals were used to generate the *3xTg-AD* mice than are required for conventional step-wise transgenic approaches, where one transgene is inserted into the genome at a time and its expression is confirmed prior to the introduction of subsequent transgenes. The resultant *3xTg-AD* mice exhibited the progressive, brain region-specific neuropathological features characteristic of the human disease, with synaptic dysfunction preceding AP formation, which, in turn, preceded NFT formation. This mouse model is potentially the most promising one for developing therapies for AD, since, unlike many AD mouse models, these mice are not behaviourally abnormal from birth, despite exhibiting subtle differences in the extent and type of DNA damage that occurs in mice and humans.

It has recently been discovered that keeping genetic mice in a pathogen-free environment may limit the development of NFTs and APs characteristic of human AD, which suggests a role for pathogen-induced inflammation in AD (60). This implies that attempts to elucidate the function of genes or model human diseases could fail, due to an absence from laboratory environments of environmental factors that ordinarily would be present, and not because of the genetic alteration itself.

Welfare of animal models of neurodegenerative diseases

Several animal welfare implications arise specifically as a result of creating mouse models of neurodegenerative diseases. These include the distress and suffering caused by neurological impairments, including tremors and ataxia (loss of full control of bodily movements), confusion and disorientation. The loss of neurological capacity may also influence general behaviour, including the ability to interact with other animals. These symptoms often have substantial consequences for the fecundity and viability of the animals, and their occurrence requires careful monitoring throughout their lifetimes. This is more problematic when neurodegenerative phenotypes are induced as a result of exposure to mutagens, rather than by genetic modification. This is because, in the former case, all or some of the relevant features of the altered phenotype arising from any mutation may not be detected. The existing models of neurodegenerative diseases are nevertheless of great interest. Hence, we advocate the greater use of temporal and spatial gene expression coupled with histopathology, imaging and electrophysiological techniques, which allow the progression of neurodegenerative disorders in mouse models to be mapped with better regard for animal welfare than is possible by using the more traditional, invasive approaches, and at the same time are more reflective of the onset of these diseases in humans.

Development and congenital defects

Cleft palate

Cleft palate is the most common form of congenital bone dysmorphia in humans. It develops within the first trimester of pregnancy. A number of genes and external factors may play a role in the aetiology of this disease, so its study in GA mice has been difficult. Mouse models of cleft palate have been generated by ENU mutagenesis and by targeted mutagenesis. A large proportion of ENU mouse strains exhibit cleft palate, in addition to other phenotypes, presumably because of random and undefined mutations within the homeobox genes that regulate the expression of other genes (6, 61). While this has contributed to the widely-held belief that cleft palate is a multifactorial disorder caused by point mutations in one or more genes, it does not circumvent the problem that the chances of developing a preventative cure for cleft palate are limited. The fundamental question is whether cleft palate should be modelled at all, in the absence of a tangible human therapeutic benefit from this kind of research. This is particularly important, given the animal welfare implications. As a result of cleft palate, GA model offspring may not be able to feed properly, or survive to adulthood or to a stage at which the underlying mechanisms of cleft deformation can be studied. This is exemplified by the case of the homozygous *MSX-1* –/– null mutant mouse. *MSX-1* is a homeobox gene that is expressed in a number of developing organs in vertebrates. *MSX-1* –/– neonates fail to survive, because of severe craniofacial abnormalities, including secondary cleft palate and abnormal tooth development (62).

Apert syndrome

Recently, a transgenic model of Apert syndrome was generated (63). In humans, Apert syndrome is an autosomal dominant disorder characterised by skull, limb and visceral defects. Two-thirds of the affected individuals carry a single $S^{252}W$ mutation in fibroblast growth factor receptor 2 gene (*FGFR2*). The transgenic Apert mouse model was generated by homologous recombination to replace a segment of the normal mouse *fgfr2* gene with a segment carrying a mutation equivalent to $S^{252}W$ in the human protein. Heterozygous $fgfr2^{+/S252W}$ mice die within 36 hours of birth, due to defects of the palate and respiratory system, whereas most human sufferers of the disease survive past this early postnatal stage. Nevertheless, since the

fgfr2$^{+/S252W}$ mouse model shares many features with the human disease, it might serve as a useful model of the disease, provided that its limitations are carefully taken into account.

Down's syndrome

Human Down's syndrome (DS) is classified as a congenital disorder characterised by different levels of mental retardation. It is caused by three genetic variations, the most common of which is the presence of an extra copy of chromosome 21 in all the cells of the affected individual's body (trisomy 21). Mosaic trisomy 21 is similar, except that the extra chromosome 21 is only present in some of the cells of the affected individual. The third form of DS is caused by translocational errors that result in repeats within chromosome 21.

Trisomy (Ts) mouse models were first isolated in the 1970s, including the *Ts16* mouse model that possesses an extra copy of mouse chromosome 16 (64). The *Ts16* mouse represents a naturally occurring trisomy in mice, but its relevance as a model of DS is questionable, since the human chromosome 21 and mouse chromosome 16 share only 80% of the same genes, and human and mouse chromosomal numbers and gene arrangements differ. Even more problematical is the fact that *Ts* mice display developmental cardiovascular defects that are not evident in human DS (65), but which resemble symptoms associated with DiGeorge syndrome in humans (66). Furthermore, these mice do not survive beyond birth, prohibiting the study of CNS development and of the age-related diseases associated with DS.

More recent mouse models of DS include the *Ts65Dn* mouse, which carries an extra copy of part of mouse chromosome 16 which corresponds with the critical region of human chromosome 21 (67) involved in the aetiology of the human form of the disease. *TS65Dn* mice are able to survive to adulthood, and display some of the characteristics of human DS, including delayed postnatal development, muscular trembling, male sterility, skeletal malformations and abnormal cholinergic function (68). However, although there appears to be some evidence of elevated levels of amyloid protein in the CNS of 6 month-old mice, the animals do not display the characteristic signs of the later stages of DS such as the APs and NFTs that predispose DS patients to AD (69).

The most recent attempt to create a mouse model of DS involved the addition of an almost entire copy of human chromosome 21 to the mouse genome, resulting in the *Tc1* mosaic mouse, which contains human chromosome 21 DNA in some of its cells (70). Each individual mouse potentially contains a different number of cells with the extra chromosome, more closely mimicking mosaic trisomy 21, rather than trisomy 21, the predominant form of human DS. *Tc1* mice exhibit several features of human DS, including changes in cardiac function that were not accurately modelled by the *Ts65Dn* mouse (71). Since these Ts mice are able to survive past birth, there is now considerable scope for studying aspects of the development and function of the nervous system and premature ageing associated with the human disease.

The most obvious problem with mouse models of DS stems from the fact that none of the predisposing factors of the human disease, such as maternal age, are represented. Furthermore, the insertion of human genetic material into mice may have unforeseen effects which do not reflect the human condition of the disease. Whether studies in these mice models will lead to the development of gene therapies able to treat all the major symptoms of DS, is also debatable. This is because DS is a collection of health and developmental problems, resulting from an extra copy of a chromosome containing some 225 genes, and there is evidence to suggest that additional genes might also be involved in its aetiology (72). Hence, as there are already treatments available for some complications of the disease, such as heart defects, resources would perhaps be better focused on developing treatments for other complications arising from DS.

Mouse models of cognitive function and psychiatric disorders

Understanding the roles of genes involved in certain inherited psychiatric disorders by using studies in GA mice is particularly difficult, since, in humans, the onset of these disorders can be triggered by non-genetic factors. Indeed, simply carrying a genetic defect may not necessarily mean that the disease will ever develop. Furthermore, GA mice may not necessarily develop neuropsychiatric effects that can readily be extrapolated to human diseases. GM mice have also been screened for subtle behavioural changes, to assist in the identification of genes that may be implicated in complex behavioural disorders in humans, such as anxiety or schizophrenia. Again, behavioural defects are likely to manifest themselves in very different ways in mice and humans.

Schizophrenia

Schizophrenia in humans is believed to be a polygenic disorder, and has been attributed to mutations in genes on chromosomes 2, 13 and 15. Its development can be triggered by external factors, so a positive genetic diagnosis does not necessarily mean that the disease will ever develop. In humans, the various forms of schizophrenia all cause dys-

functional social behaviour. Several GM mouse models of schizophrenia recreate some of these symptoms, but none of them have successfully recreated all the symptoms associated with any of the forms of the human disorder. This may relate to the fact that many of the human patients who suffer from these disorders do not inherit them through simple genetic determinants, since environmental factors play an important role.

CADASIL

A second example of how human behavioural defects can be displayed in very different ways in mice, concerns the mutant *NOTCH 3* gene knock-in mouse model for stroke and dementia syndrome, which has been given the acronym, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; 73). In humans, CADASIL has been attributed to a missense mutation in the *NOTCH 3* epidermal growth factor receptor gene, resulting in impaired post-translational processing and trafficking of the expressed receptor. This causes degeneration of vascular smooth muscle, resulting in clinical signs such as recurring strokes and dementia in humans between the ages of 30 and 50 years. It has been suggested that the absence of the CADASIL-like phenotype in heterozygotes ($CADASIL^{R142C/+}$) or homozygotes ($CADASIL^{R142C/R142C}$) could reflect differences in one or more of: a) the spatial or temporal expression of species gene homologues; b) gene regulation; c) the organisation of the mouse and human brains; and d) life-span (because the short lifespan of the mouse does not allow the disease time to develop). What is clear is that these mice are not adequate models of the human disease.

Interpretation of information from studies in GM mice

GM mice suffer from the further limitation that, although mouse proteins may appear to have identical functions in humans, many genes and their encoded proteins play vital roles, not only in early development, but also in the aetiology of diseases later on, due to changes in protein expression in specific tissues. Thus, mice in which the NR1 subunit of the NMDA receptor is deleted, die shortly after birth (74). However, mice in which *NR1* gene expression in the hippocampus was conditionally knocked-out by using the *CRE-LoxP* system, survived and showed signs of impaired spatial learning, consistent with the predicted role of the hippocampal NMDA pathway in learning (75). Similarly, it is possible to mimic temporal changes in expression by using cell-specific promoters, such as the tetracycline-responsive element, *tTA*, to drive the protein expression of a downstream gene. This can be achieved by supplementing the diet of transgenic mice with tetracycline and allows the effects of changes in protein expression on cognitive function at various life stages to be monitored more precisely.

Mouse models of cancer, DNA repair disorders and their use in toxicity testing

Carcinogenicity testing

Since the late 1980s, a number of transgenic mouse strains have been developed that are of interest in mutagenicity and carcinogenicity testing. Models such as the *Eμ-pim-1* transgenic mouse, the *rasH2* transgenic mouse, the *p53*+/– heterozygous knock-out mouse, and the *Tg.AC* transgenic mouse, have been used in carcinogenicity testing. The *Eμ-pim-1* transgenic mouse, which contains the activated *pim-1* oncogene, has a low spontaneous tumour frequency, yet develops cancers upon exposure to even low levels of mild genotoxic carcinogens or to normal levels of mild genotoxic carcinogens that target lymphatic tissues (76). The *rasH2* transgenic mouse contains a mutation in the *p21 Ha-ras* oncogene, which enhances susceptibility to neoplastic induction — an early marker of tumorogenesis (77). This model is useful for assessing a variety of genotoxic and non-genotoxic carcinogens, and has been used to obviate the need for conventional 2-year rodent bioassays, since carcinogens can be identified during 26-week studies (78).

p53+/– heterozygous knock-out mice contain only a single functional copy of the *p53* tumour suppressor gene. Up to 36 weeks of age, the spontaneous tumour frequency in these mice is very low. Although, unlike the *rasH2* model, *p53*+/– heterozygous knock-out mice are not suited to the assessment of non-genotoxic carcinogens, when these animals are exposed by almost any route of exposure to certain classes of genotoxic agents (reviewed in 79), they develop cancers. In contrast, the *Tg.AC* transgenic mouse, which carries the activated *v-Ha-ras* oncogene fused to a mouse globin promoter, must be exposed topically to the chemical in order to trigger the development of papillomas. Indeed, the US Food and Drug Administration have suggested that the *p53*+/– mouse can be used for assessing genotoxic chemicals, except in the case of dermal drugs, where the *Tg.Ac* model is preferred. On the other hand, the *rasH2* model is considered to be more suited to the assessment of non-genotoxic chemicals. The *rasH2*, *p53*+/– and *Tg.AC* models have been used since 1997 in Europe for rapid carcinogenicity testing (< 1 year), in order to reduce the need for 2-year studies in rodents. This has resulted in considerable benefits to animal wel-

fare (80), but it should be remembered that these assays have yet to be successfully validated for regulatory use. The most obvious advantage is that, since these GM mice are more susceptible to cancer than normal mice, fewer animals should be needed in each study to achieve statistically significant data.

Mutagenicity testing

Another example of how the use of GM mice can have a positive impact on animal welfare is the use of reporter mice for mutagenicity (or genotoxicity) testing. The Organisation for Economic Cooperation and Development (OECD) is considering the benefits of using the *LacI* BigBlue® mouse and *LacZ* Muta™mouse transgenic models for mutagenicity testing (81). The mutagenic target of Muta™mouse is a bacterial *LacZ* gene, and the target of Big Blue is the *LacI* repressor gene. Both of these transgenes act as reporter genes. The *LacI* repressor binds to the lac operon and prevents the expression of β-galactosidase from the *LacZ* gene. Hence, mutations in the *LacI* repressor gene result in changes in the levels of β-galactosidase expression, whereas mutations in the *LacZ* gene result in the expression of mutated forms of the enzyme. This means that exposure to a mutagen is, in theory, able to cause mutations which can be detected by analysing β-galactosidase activity. This is done by extracting genomic DNA extracted from the tissues of exposed mice, packaging the DNA into a bacteriophage, and using it to infect *Escherichia coli* in culture. These bacteria express β-galactosidase, the activity of which is related to the expression level of the gene and whether it has been mutated (Figure 1). The latter is determined by monitoring the ability of infected *E. coli* colonies to catalyse the formation of a coloured product. The TSG-p53/Big Blue mouse (Taconic, New York, USA) has the *LacI* transgene integrated into every cell, and is hemizygous for the endogenous p53 tumour suppressor. Thus, this model shares features of both the *p53*+/– and *LacI* BigBlue® mice, and is suited to the more-rapid screening of mutagenic and tumorigenic compounds.

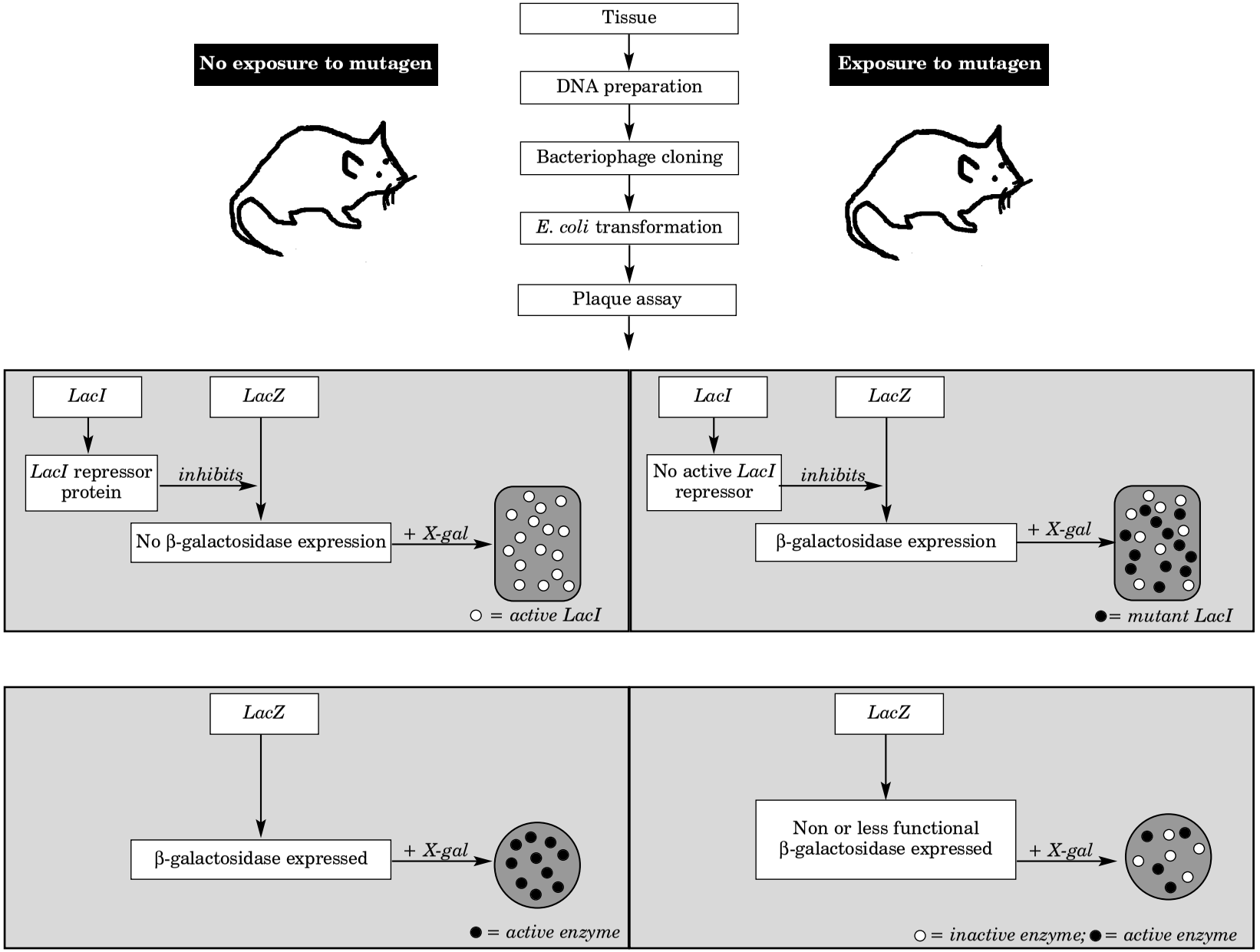
The use of such models for mutagenicity testing has the potential to provide a means to screen mutagens which may have escaped detection in simpler assays, such as the mouse spot test and mouse bone-marrow micronucleus test, since it is possible to monitor the ability of mutagens to trigger mutations in more than one type of tissue, without using large numbers of animals (81, 82). Indeed, despite differences between the endpoints, tests based on the use of these transgenic models and the mouse spot test or *in vivo* micronucleus test have similar predictabilities. However, tests in these transgenic mice are not able to detect mutagens that cause chromosomal aberrations. Hence, a strategy that takes into account the information which can be reasonably obtained by using *ex vivo* and cell-based assays, such as the bacterial reverse mutation test (Ames test; OECD Test Guideline [TG] 471), the *in vitro* mammalian cell chromosomal aberration, gene mutation, sister chromatid exchange and micronucleus tests (TGs 473, 476 and 479, and draft TG 487, respectively), should be applied before tests are conducted in mice, in order to minimise *in vivo* studies, whether or not they employ GM animals. Indeed, as is discussed below, there are reasons why human risk cannot necessarily be accurately estimated from studies in mice alone.

The relevance of using mice to study human cancers

The incidence of cancer in humans has a strong correlation with age and genetic damage. This is consistent with the theory that the greater the number of cycles of cell division, the greater the risk of cancer. This relationship is less obvious in laboratory mice, where, despite similar rates of cell division and 10^5 times fewer cell divisions during the lifetime of a mouse, susceptibility to cancer is higher than in humans. In fact, around 30% of rodents develop cancers during their 2-year to 3-year life-spans. Admittedly, in relative terms that take into account the difference between the life-spans of the two species, the incidence of cancer in the two species is similar, but because, in comparison with humans, mice are relatively deficient in antineoplastic defence mechanisms (11), inferences as to the underlying mechanisms of carcinogenesis are subject to considerable debate. Higher metabolic rates occur in mice than in humans, and differences in detoxification between mice and humans will undoubtedly also contribute to species differences in the types, sites and incidences of cancer.

Most human cancers arise in epithelial cell layers. This is not usually the case in mice, although the types of tumours that occur in *p53* mutant mice do more closely resemble the epithelial cancers found in humans (83). This seems to be related to the fact that cells from these mice contain chromosomes with more similar telomeres to human telomeres, the latter of which appear to be more susceptible to end-to-end chromatid fusion and non-reciprocal translocation of chromatid DNA, resulting in abnormal karyotypes — a common feature of human (particularly epithelial) cancers, but not mouse cancers (84). This suggests a role in cancer research for the *p53* +/– heterozygous mouse and transgenic mice that contain mutants within the mouse or human *p53* genes. Indeed, these mice have been used to establish a number of cancer models for drug development and basic cancer research (76–80, 85), as well as models for DNA repair-related diseases (see below).

Figure 1: A schematic showing the *LacI*/*LacZ* and *LacZ* reporter gene systems



Immune-compromised mice in cancer research

Another group of mice that have featured heavily in cancer research are mouse models based on the xenotransplantation of human tumours and tumour cell lines into immune-compromised mice. Early models involved the use of the nude mouse (86), a hairless and athymic mouse resulting from spontaneous mutations in a homeobox gene. This mouse carries two copies of a mutated forkhead box N1 (FoxN1) transcription factor. The fact that the nude mouse lacks a thymus gland also rendered it unable to produce T-cells, essential components in foreign tissue rejection (6). Therefore, the nude mouse can be used to host human tumour cells, so that some forms of human cancer could be studied in the context of a live animal. Indeed, with the more recent advent of engineered tumour cell lines that express bioluminescent or fluorescent reporter proteins and high resolution imaging techniques, the growth and metastasis of human tumours and their regression in response to treatment can readily be monitored in these animals by non-invasive methods and over extended periods of time. Although the cell immortalisation process will almost certainly affect the characteristics of the tumour cells, so information from such studies must be interpreted with great care, this not only means that fewer animals are needed for each study, but also that studies can be terminated when the tumour reaches a size at which it is likely to cause the animal pain or distress. Indeed, the use of magnetic resonance imaging (MRI) or positron enhanced tomography (PET) means that it is now possible to monitor the fate of a single cancer cell within a mouse (87). Such studies are far less reliant on the development of large tumours, so the animals can be euthanised before they suffer detectable distress or pain.

However, such studies do not circumvent other animal welfare problems in the use of immune-compromised mice. For instance, as a result of this homozygous mutation, 55% of nude mice die within two weeks of birth, and the surviving mice display poor hair growth and thin skin, low body weights, retarded growth and small body size. This means that the mice are often unable to maintain a steady body temperature. Nude mice also have reduced fertility, due partly to abnormal gamete development, and can exhibit signs of metabolic disorders. These features not only affect the welfare of the animals that survive to adulthood, but also mean that the strain is difficult to maintain. Similar concerns are raised by more-recent immune-compromised mouse models, such as *SCID* mice and nude/*SCID* mice, as well as the even more immune-compromised *SCID-beige* mice that also lack natural killer cell function, which are increasingly being used because of the lower incidence of tumour rejection in these mice (see *GA mouse models of infection*). These animals must be afforded the highest quality of animal husbandry, if inadvertent infections are to be avoided.

DNA repair disease models

Human DNA repair diseases have also been modeled in GA mice. Diseases such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS), are genetic disorders due to specific defects in nucleotide excision repair (NER; 88).

In XP, mutations in one or more of eight *XP* genes (*XPA-XPG* and *XP*-variant), normally *XPA, XPC, XPD* and/or *XPF*, result in distinct patterns of defective DNA repair. However, the most striking characteristics of all forms of XP are sunlight-induced pigment changes in the skin, erythema, dry skin, extreme sensitivity to sunlight, and photophobia. Affected individuals may also suffer from CNS disorders such as sensorineural deafness and movement problems, as well as from immunological defects. Continued exposure to sunlight can give rise to a variety of skin cancers. These additional symptoms are often due to loss-of-function mutations in the *XPA* gene which encodes a zinc finger protein, which participates in photoproduct recognition and DNA binding.

While *xpa (–/–)* knock-out mice are susceptible to sunlight-induced cancers (89), they do not exhibit visible signs of erythema, nor do they display the characteristics of immunological defects or neurological disorders. Nevertheless, the fact that the *xpa* (–/–) knockout mice, and also *xpa/p53*+/– double knock-out mice, are both susceptible to skin cancers, and given the spontaneous incidence of tumours is low in mice of less than 11 months of age, these models are potentially useful for genotoxic carcinogenicity testing. This is particularly true of the double knock-out mouse, the more sensitive of the two models.

In CS, mutations in the CSA and CSB proteins impair transcription-coupled DNA repair. Patients with the syndrome can exhibit signs of developmental defects, including severe physical and mental retardation, microencephaly and limb and visual defects. They are also sensitive to sunlight. However, while they develop a severe rash, they differ from XP sufferers, in that they do not manifest any signs of pigment changes nor hypersensitivity to sunlight-induced skin cancers. The disease is inherited as an autosomal recessive trait, and CS type 1 can result in death during early infancy. *csa* (–/–) and *csb* (–/–) knock-out mice display a similar phenotype to control mice, but with an increased incidence of carcinogenesis when exposed to UV light (90), despite this not being a feature of the corresponding human disease.

Discussion

The value of GA mice to human medicine: summary

We have considered the scientific merits and limitations of a range of available GA mice models that are used to model human diseases and toxicity. The results are summarised in Table 2. These particular models were selected, since they are pivotal to ongoing projects to develop GA mouse models.

Although we initially set out to address the question of whether research using GA mice as models of human diseases has directly resulted in the development of new human medicines or treatments, this is almost impossible to determine without first considering the entire history of how each disease has been studied. We have, therefore, selected a limited number of GA mouse models of specific diseases, with at least one example for each of the major biological systems, and considered whether the mouse models have: a) increased understanding of the human disease; and b) been useful for assessing the safety and efficacy of existing and new therapeutic agents.

In our opinion, the current extent to which GA mice are used cannot be justified on the basis that they are vital for the development of human medicines, since few human medicines have so far been developed which were largely or exclusively based on the use of GA mouse models. This unsatisfactory situation is despite nearly four decades of studies in GA mice — the first knockout, transgenic and trisomy mice were all produced in the 1980s. Indeed, the inability of many GA mouse models to recapitulate all the features of a human disease has often resulted in several mouse models being created, for studies on different aspects of the disease in question. Together with problems of differences in the genetic backgrounds of the mice used in these mutagenesis studies, this has confused the interpretation of the information provided, and has potentially slowed, rather than expedited, the development of new medical treatments.

One of the first two knock-out mice were null mutants for the *hprt* (hypoxanthine-guanine phosphoribosyl transferase) gene which is apparently homologous to the human gene associated with Lesch-Nyhan syndrome (LNS; 91). Despite the early link between the gene and the corresponding human disease, only some of the symptoms of the disease can be treated and there is no standard or specific treatment for the neurological symptoms of LNS. Similarly, the first transgenic mouse contained a mutated form of the metallothionein-1 (*MT-1*) gene homologous to the human gene associated with Menkes disease (92). Again, despite over 20 years of research, the main way of managing this disease is by injections of copper supplements, and there is still no specific therapeutic treatment. Finally, trisomy mouse models have still not provided a route for developing realistic treatments for DS (93).

This, albeit limited, set of examples highlights three questions that need to be considered before any new GA mouse models are generated: a) will studies in GA mice further our understanding of the disease in question; b) how much would the new information provided by a GA model facilitate the development of new treatments for this disease; and c) would any such new treatment be better than any existing treatments or preventive measures for the disease?

In the case of disorders such as cleft palate and DS, GA mouse models have permitted some of the features of the diseases to be studied. However, it is not clear whether this will assist with the management or prevention of the diseases themselves. In particular, there is intensive debate as to whether treating cleft palate and DS as deformities is degrading to individuals affected by these conditions. This could mean that, even if effective therapies could be devised, their use might not be ethically acceptable. Certainly, the existence of such complex disease aetiologies somewhat limits the scope for gene therapy, although they can be addressed by surgical and symptomatic treatments. While it is clear that some GA mouse models have proven useful in terms of providing relevant information about specific human diseases and biological processes, many more of the models are severely compromised with regard to their relevance, and some can even produce misleading information. This is partly due to the fact that there are many, often undefined, differences in the anatomy, physiology, metabolism, disease susceptibility and patterns of behaviour characteristic of humans and mice. Also, human diseases can arise because of specific combinations of alleles of different genes and mutations that are induced within genetic or mitochondrial DNA, as a result of environmental factors, injury or infection, in ways that cannot be faithfully reproduced by using GA mice kept in controlled environments.

We have concluded that many GA mouse models that carry well-defined genetic modifications underlying a particular disease phenotype are unsatisfactory for developing effective therapies. Therefore, it follows that models (such as those produced by ENU mutagenesis), which share few, if any, of the underlying biochemical mechanisms of a disease, are likely to be even less suitable for the above purpose. A further problem with such models stems from the fact that ENU preferentially alters A–T base pairs (12), so some parts of the mouse genome are more susceptible to ENU mutagenesis than others. Since ENU induces 1 base mutation every 100,000 to 1,000,000 bases,

each mutant animal could, in theory, carry thousands of mutations within its genome, many of which will be within regulatory elements and highly susceptible genes, and which will remain undetected, unless full genomic sequencing is undertaken. This means that the genetic background of every animal could potentially be different, with the result that a direct link between a gene and its function is extremely difficult to establish in ENU mutant mice. Moreover, ENU tends to cause loss-of-function mutations, resulting in a high incidence of *in utero* and early *post partum* death. Therefore, it is questionable whether further ENU-based mutagenesis screens will yield any new and relevant models.

A case for using alternative models of human diseases

In view of the limitations of GA mice in the modelling of human diseases in ways that have resulted in many effective cures, and because some of the genes involved have been conserved in evolution, the possibility of undertaking at least preliminary studies in organisms such as *Caenorhabditis elegans* (a nematode) and *Drosophila melanogaster* (an insect) deserves more serious attention. Indeed, the higher gene density, often higher natural mutation rate, and relative ease with which the genomes of such organisms can be manipulated and studied, emphasises the importance of a thorough investigation into how studies in less sentient organisms can be used to curb the increasing animal welfare burden inherent in using GA mice. Of course, these invertebrate organisms do not display the complexity of the human body. However, the above attributes, together with the much lower gene duplication rates in these lower organisms, means that functional abnormalities due to gene mutants or knockouts of the target gene will have a reduced likelihood of being rescued by other genes. This simplifies the process of assigning gene function, which, in mice, is complicated by the prevalence of gene duplication and gene compensation. Furthermore, since lower organisms reproduce more rapidly and some, like *C. elegans*, have transparent bodies, they are extremely well-suited to the study of evolutionarily conserved developmental genes and processes. Hence, such organisms might be more useful than GA mice, where the aim is to develop a preventive treatment that stops the disease from developing, thereby obviating the need to develop medicines to treat symptoms. Many such human diseases result in prenatal, postnatal or premature death and are inherently difficult to model using mice.

Where there are problems with establishing the relevance of studies in lower organisms, and indeed using GA mice, the most practical way forward is to utilise cell culture methods and population genetics to inform the development of clinical and cell-based research into human diseases. Although these topics are beyond the scope of this paper, we hope that more emphasis will be placed on finding a way to utilise information that is already available from such studies to implement a move away from the large scale generation and use of GA mice.

Scope for the Three Rs: recommendations

The use of GA mice as models of human disease warrants specific attention, since the induction of symptoms of human diseases in laboratory animals unavoidably involves the potential to cause them considerable pain and suffering.

There should be an extensive reassessment of the value of GA mice as models of human diseases that takes into account:

1. The failure of many GA mice to adequately model human diseases;

2. The prospects for using the information from the studies to design new treatments for human diseases; and

3. The availability and relevance of alternative methods.

Physical and physiological differences between humans and mice need to be considered in detail, prior to the generation of new models of human diseases. Where these differences are likely to compromise the relevance of models for their stated purpose, this information must be considered carefully before project licences are granted for their generation.

The prospects of assigning functions to individual genes by methods other than by using GA animals should be further investigated. Examples of such methods include the use of human population genetics information and the more extensive use of genomic analysis, genetically engineered cells and lower organisms to identify genes that may then go on to be studied by targeted mouse mutagenesis.

If prior evidence indicates the functional non-equivalence of mouse homologues of human disease-related genes, or if the expression patterns of the true species homologues differ in mice and humans, alternative investigative strategies should be devised. This is particularly important, since some genes, such as the CFTR gene, that were once thought to encode mouse homologues for human proteins, have later been shown not to be functionally equivalent. Thus, it is not acceptable to assume that a close evolutionary relationship between mice and humans can guarantee functional homology between genes from the two species.

Table 2: A summary of the scientific and animal welfare considerations involved in using GA mice in medical research and toxicity testing

Area of research	Relevance to human health benefit	Scientific limitations	Animal welfare implications
Vaccine testing	This is a partial replacement that makes it possible to conduct some tests without using higher mammals	Relies on other components of the biochemical pathway being present endogenous to the mice	Has the capacity to cause extreme suffering unless humane endpoints are used
Cystic fibrosis	Limited, because of the substantial differences between the aetiology of the disease in humans and mice No current treatment for the disease. Symptoms are treated with generic drugs, e.g. bronchodilators, steroids, antibiotics and DNAses (to breakdown mucus). Gene therapy, despite promising outcomes in mouse models, did not prove beneficial for most patients	CFTR homologue in mice is not a strict functional homologue of the human protein Significant differences between mouse model and human disease Models have failed to generate information leading to the development of effective therapy	Mice suffer from dietary and digestive system complications
Duchenne muscular dystrophy	Potentially relevant model for development of gene therapy Currently no specific treatment for this or any other form of muscular dystrophy	The aetiology of the human disease differs from that seen in the mouse model	Some models can severely impair locomotive behaviour and survival
Down's syndrome	Currently no treatment Poor prospect of using information to develop effective treatments, e.g. gene therapy	Limited to the study of developmental and early life stage Down's syndrome	In the case of several models, mice do not survive past birth Some models develop signs that affect their behaviour and reproductive capacity, the latter with implications for the number of mice used to maintain a strain
Diabetes	Limited scope for using certain models in drug development Several existing treatments that predate or did not rely on crucial information from studies in GM mice	Species-specific differences in insulin regulation Poorly understood roles for some putative targets in diabetes	Some models of diabetes are immune compromised and prone to infection
Neurodegenerative disorders	Potentially useful for drug development. For example, new putative targets for vaccine and drug treatment have been identified for Alzheimer's Disease	Age-related and multifactorial disorders with complex aetiologies are difficult to model in short-lived species	Impaired neurological function may cause distress, alter behaviour and reduce the viability of the animals

Table 2: continued

Area of research	Relevance to human health benefit	Scientific limitations	Animal welfare implications
Sickle cell anaemia	Development of anti-sickling drugs and stem cell therapies are currently underway although there is currently no cure available	Fundamental differences in site of haematopoiesis, blood volume and red blood cell size and clinical effects	Poor oxygen carriage resulting in distress and pain due to rapid physiological degeneration
Bone dysmorphia	Limited — mainly fundamental research to understand gene regulation Questionable scope for antenatal, preimplantation screening for some diseases such as cleft palate because of ethical objections Apert syndrome has no gene therapy or drug-based treatment. All treatment is surgical	Differences in the skeletal and dental anatomy	Some mice are unable to feed, see or move normally, leading to extreme suffering, altered behaviour and early death
Sensory function	Putative targets for classical drug targeting may be identified for sensorineural deafness but not retinopathies Some stem cell-based treatments for deafness that have reversed sensorineural deafness in mice are currently being investigated but have yet to give a treatment for human deafness. Similar treatments for some forms of blindness have been successful in mice At present most stem cell therapies have yet to be proven safe and effective	The functional gene clusters in humans and mice differ There is a large degree of compensatory gene function and redundancy in sensory perception genes which make it difficult to model human sensory diseases in mice	Some mouse models exhibit rapid and debilitating loss of sensory function with severe animal welfare consequences
Cognitive disorders	Some concerns over genetic screening of humans to identify susceptible individuals based on potentially unsound assignment of gene mutations from mouse studies	Multifactorial diseases that are difficult to study in mice because of the absence of non-genetic factors, differences in behavioural effects of specific gene mutations and functional non-equivalence of some genes and the shorter life spans of mice	Unpredictable and often subtle changes in behaviour with potential problems for animal care
Toxicity testing	Human risk assessment based on relatively sensitive, short-term studies	Some problems with false positives and extrapolating short-term studies to long-term exposure	Large numbers of animals may be used in each study. The implementation of the EU Chemical policy may mean that many more mice are used in toxicity testing

Table 2: continued

Area of research	Relevance to human health benefit	Scientific limitations	Animal welfare implications
Cancer research	Useful transplantation based models for testing the efficacy of novel therapeutics Potentially useful as a means of identifying novel drug targets Some treatments, including monoclonal antibodies, first tested in mouse models are now clinically used. Some experimental gene therapies	Some problems with the lack of functional correspondence of some genes in mice and humans, the differences between the types of cancer, incidence of cancer and life-span	Immune compromised animals are extensively used in these studies giving rise to problems with infection. Some models have a low survival. Large numbers of animals may be used in each study
Infectious diseases	Some models are useful in vaccine/drug development and testing and can expedite testing traditionally conducted in primates and other higher mammals Containment is easier than for larger animals	The immune systems of humans and mice are not identical, which suggests that vaccines may not elicit a humoral response in mice	Experiments can cause a wide variety of symptoms, including paralysis, breathing difficulties, lesions and changes in body temperature Testing of vaccines requires the use of large numbers of animals

As a matter of principle, and to avoid any unnecessary wastage of animals, GM mouse disease models should, wherever possible, be created by efficient processes, so that they mimic all the key characteristics of the clinical condition as closely as possible. This has now been facilitated by the availability of conditional knock-out, tissue-specific and inducible expression systems. GM mice created by using such methods have increased survival rates, and are already proving to be highly relevant for human diseases. However, such improved models should not only be used in *in vivo studies,* but should also serve, wherever possible, as sources of cells and tissues for replacement *in vitro* studies.

While a few important models have been discovered by genome-wide mutagenesis, there is limited evidence to suggest that ENU models have contributed to the development of human medicines, with the possible exception of GM mice used in the study of infectious diseases and some types of cancer. Hence, ENU mutagenesis should only be used if equivalent studies in lower organisms and cell-based systems, and genomic and molecular interaction analysis, are unable to provide information about the roles of human genes that is necessary for the development of relevant models of human diseases. However, the screening of existing ENU and gene trap libraries might result in the avoidance of the further use of ENU mutagenesis altogether.

Conclusions

Over the past decade, there has been a dramatic shift toward the use of GA mice in research and testing, which has, in turn, prompted concerns about the welfare of the animals used. Issues such as reducing the number of animals wasted during the production of GM mice, and the effective welfare assessment of GA mice, remain causes for concern.

The main question is whether the widespread use of GA mice can be scientifically justified. Only by addressing this question objectively, and without bias, can we hope to identify the scope for replacing GA mice in research and testing. It should be recognised that the generation of GA mice has often confused, rather than improved, our understanding of the genetic basis of human diseases. The large numbers of only partly-relevant models available for many diseases have complicated the meaningful extrapolation of the information they provide to human medicine.

There is an urgent need to re-evaluate GA mice as models of human disease, to take into account the availability of alternative models based on studies on lower organisms, normal and diseased human cells, and the increasingly availability of other sources of human information of direct relevance.

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