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Overview

Considerations for Infectious Disease Research Studies Using Animals

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Animal models are vital in understanding the transmission and pathogenesis of infectious organisms and the host immune response to infection. In addition, animal models are essential in vaccine and therapeutic drug development and testing. Prior to selecting an animal model to use when studying an infectious agent, the scientific team must determine that sufficient in vitro and ex vivo data are available to justify performing research in an animal model, that ethical considerations are addressed, and that the data generated from animal work will add useful information to the body of scientific knowledge. Once it is established that an animal should be used, the questions become 'Which animal model is most suitable?' and 'Which experimental design issues should be considered?' The answers to these questions take into account numerous factors, including scientific, practical, welfare, and regulatory considerations, which are the focus of this article.

Introduction

People have been interested in the prevention and treatment of infectious diseases for thousands of years. Yet it wasn't until the midnineteenth century that scientists identified microorganisms as the causative agent. As our understanding of their virulence and the host response to microbial infection has grown, so has our ability to develop vaccines and therapeutics. The use of animal models is vital as we continue to acquire knowledge of the process of infection and methods for treating human and animal disease. Indeed, the use of animals in infectious disease research has escalated with the increases in drug-resistant organisms, the threat of bioterrorism, expanding global trade and travel, and the rapidly growing list of emerging infectious diseases.

Although some infectious disease research can be conducted with human subjects or during naturally occurring human disease outbreaks, many areas of research cannot be ethically or scientifically conducted with humans. These areas include study of agents known or suspected to cause serious, potentially fatal, or untreatable human disease; potential agents of biowarfare not normally encountered in nature; diseases of extremely low incidence within the human population; and initial vaccine and therapeutic drug development and testing. For these studies, the use of inanimate systems (for example, in vitro techniques or mathematical and computer modeling systems) and in vivo animal systems is appropriate.

The conduct of in vivo studies should be reserved to advance research that cannot otherwise be achieved through the use of alternative methods. To identify these situations, the scientific team should fully investigate alternate modeling systems through extensive literature searches and professional communications. In vivo animal studies can subsequently be used to supplement and advance areas of research that cannot be explored by alternative methods.[29,56]

The decision to develop or use an animal model should always be preceded by an assessment of the available in vitro and ex vivo tools necessary to support animal-based work. The specific in vitro and ex vivo technologies required vary by the distinctive needs of the study but may include bacterial or cell culture systems or alternative methods to maintain viable pathogen stocks. In addition, immunologic assays may be required to characterize humoral or cellular responses, to measure antigen-induced production of IFNγ or cytokine release, to characterize immunoglobulin isotype profiles or antigen epitopes, and to evaluate chemotaxis. In addition, biologic reagents are often required and may include both primary and secondary antibodies with a conjugated element for easy detection and quantification, as well as unique antigens including complete proteins and epitope arrays. Cell proliferation (stimulation or inhibition) assay systems may be required as well. Many in vitro and ex vivo technologies are available for murine models but may be scarce for less commonly used species.

In addition, during the consideration of proposed animal-based research, a harm–benefit analysis should be conducted to evaluate whether the harms anticipated to be experienced by animal subjects are justified by the potential benefits of the research.[10,34] In this context, 'harm' to an animal includes not only direct, physical insult (for example, discomfort and pain) but also other impairments of animal wellbeing, including the induction of anxiety or distress, prevention of species-typical behavior, or single housing of a social species. Both discreet harms and the cumulative harms experienced by animals throughout the entire experiment should be considered. The number of animals and species used often are considered as harms, with greater harm tied to a larger number of

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animals and more evolved species.[10] The '3Rs' principles[56] can be useful in the harm evaluation. In contrast, the potential benefits of the harm–benefit analysis include experimental outcomes that directly advance the health or wellbeing of humans (for example, development of new disease treatments) as well as broader or indirect advancements, including the expansion of scientific knowledge and improvement of the health of animals or the ecosystem. These potential research benefits can be abstract and therefore difficult to quantitate. Moreover, they are highly influenced by the quality of research design, conduct, and reporting.[10] As such, experiments that are poorly designed or that cannot be reproduced yield minimal benefit and should be avoided. In the United States, IACUC members are charged with considering the benefits of research projects relative to animal welfare.[29]

Recently, a heightened emphasis has been placed on the accurate and complete reporting of in vivo biomedical research study methods and results. This increase is at least partially in response to a large-scale review of research publications, which revealed that critical information frequently is omitted or inadequately described to allow readers to fully understand how the research was conducted or how conclusions were made.[15] As a result, the reliability and validity of these research studies might be questioned and, consequently, the financial cost of the study and the ethical cost of animal use wasted. In response, the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) published the ARRIVE (Animal Research: Reporting in vivo Experiments) guidelines to help improve the reporting of biomedical research using animals. These guidelines list 20 items that should be detailed, as appropriate, in research publications to clearly and comprehensively describe animal studies with the intent to ensure reproducibility of the research and avoid unnecessary animal use.[32]

When animal use is deemed necessary and appropriate, the research team must select the most suitable animal model and evaluate multiple aspects of experimental design, including scientific, welfare, and regulatory considerations (Figure 1). These considerations are the focus of this article. We encourage readers looking for detailed information describing how to develop and validate an animal model suitable for infectious disease research to read reference 5. In addition, we advise researchers to consult the literature relevant to their pathogen and field of interest when selecting the most appropriate animal model for their area of study.

Scientific Considerations

Defining research objectives. The first steps in the development of an infectious disease animal model are clarifying the specific study objectives and then defining the research questions. These objectives must be clearly articulated so that the chosen animal model can be developed with the understanding of its intrinsic benefits and limitations. Common objectives in infectious disease studies include determining the pathophysiology associated with the infectious disease process, identifying a pathogen's virulence factors, identifying host susceptibility or resistance factors, and then using this information to develop countermeasures to infection. A single animal model may be suitable when the research focus is narrow in scope and involves only one or a part of an objective; however, when the objectives are broad or when regulations require, multiple models may need to be identified to evaluate different aspects of disease progression or host response. For example, the study of influenza relies on a variety of models, each designed for a specific purpose: ferret and guinea pig models are preferred for the study of interhost transmission, whereas mouse models are preferred for the study of host immune response to infection.[42,61] Figure 2 provides examples of animal species, their distinguishing features, and their usefulness in the study of various pathogens.

Response to infection. After clearly identifying the scientific objectives, researchers should consider the model animal's physiologic response to infection and the similarity of disease progression compared with that in the host species (for example, humans). Morbidity and mortality are typically the first criteria evaluated, especially in the context of highly infectious diseases. Evaluating morbidity, the proportion of the infected population that develops disease, requires identification of the clinical changes that take place and the timing of their onset during disease progression. This goal is best accomplished when the scientific and veterinary teams work together. Parameters such as changes in body temperature or body weight often are useful indicators of animal illness because they are easily obtained empirical measurements. Observation of clinical signs, such as changes in attitude, appetite, or activity level after infection can also be appropriate but are subjective and require a thorough working knowledge of animal physiology and behavior. Identification of organ or tissue tropism and analysis of the pathophysiology of the targeted organs is critical to model establishment because they provide insight into the molecular mechanism of the infection, the host response to infection, and the role or function of the proposed intervention. In addition, researchers may need to use multiple animal models to study the full complement of physiologic effects caused by a pathogen. For example, Zika virus infection, despite being predominantly asymptomatic in adults, has been associated with congenital microcephaly and arthrogryposis. No single animal model has been identified that recapitulates all of the pathologies of infection, but several have been established that model individual pathologies.[7,37,38,54,70]

Intrinsic animal characteristics. ***Phylogenetic scale and species selection.*** A number of practical issues should be considered once the determination has been made that the scientific objectives require use of an animal model and an appropriate pathogen has been selected. As for any study using animals, the appropriate species lowest on the phylogenetic scale should be used as the host, in accordance with the principle of replacement from the 3Rs.[56] Some characteristics of the interaction between pathogen and host are conserved across phylogeny, allowing lower order organisms such as nematodes and insects to be useful in the study of infection. Specifically, the innate immune system occurs in various forms in all multicellular organisms,[11] and numerous studies have demonstrated the existence of what appear to be universal virulence mechanisms that are functional in many hosts.[53] For example, as an alternative to using mammals, the nematode *Caenorhabditis elegans* has been used to screen bacterial mutants of *Pseudomonas aeruginosa* for changes in pathogenesis, the fruit fly *Drosophila melanogaster* has been used to study the innate immune system after infection with *Candida albicans*, and the greater wax moth *Galleria mellonella* has been used to study pathogenic temperature-sensitive virulence.[23] In addition to the ethical advantages of using lower-order organisms, these organisms cost less, are less intensive to manage, elicit fewer welfare concerns, and are not as heavily scrutinized by regulatory agencies as are more phylogenetically advanced species.

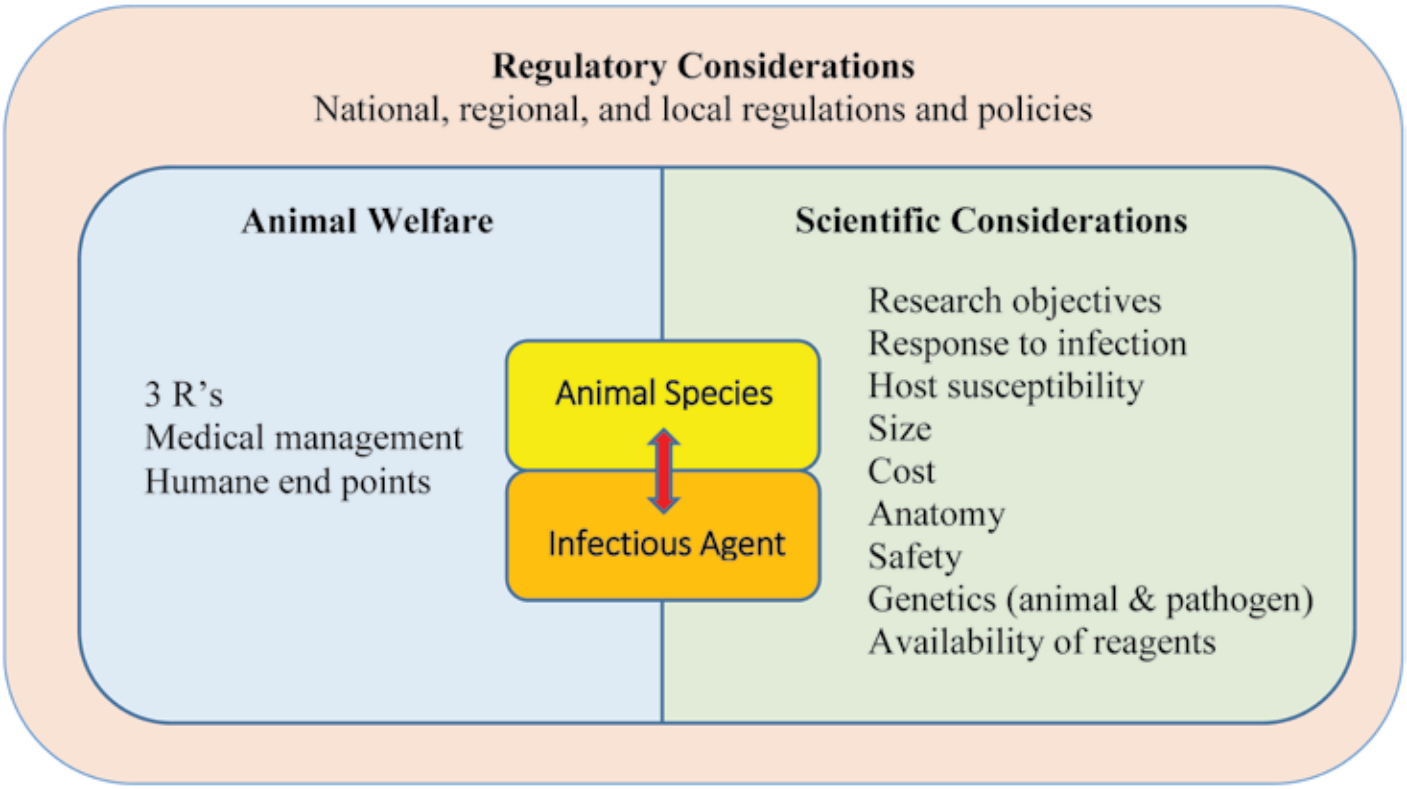

Figure 1. Design considerations for infectious disease research using animal models. Experimental design for infectious disease research using animal models requires the consideration of the inherent characteristics of the model animal and the infectious organism of interest. Clearly defined research objectives, the animal's response to infection, the similarity of disease in the model system compared with the human condition, and the availability of reagents must be considered alongside the size, cost, availability, anatomy, and safety concerns of the animal species. In addition, ethics and animal welfare must be considered, including medical management and use of humane endpoints. All of these aspects must be developed in the context of national, regional, and local regulations and policies.

Animal size. Using animals that are smaller in size for infectious disease studies has a number of advantages when compared with using larger ones. With the exception of specific, highly specialized, transgenic rodents, smaller animals typically cost less to purchase and ship than larger animals, and smaller animals require less space to house. For example, when using modern ventilated caging systems, an approximately equal amount of space is required to house 800 mice, 140 rats, 24 ferrets, or 6 rabbits.[3] This scale is significant because areas suitable for work with infectious agents (that is, ABSL2, -3, and -4) are often small and have limited housing capacity. In addition, smaller animals generally require fewer husbandry resources and, on a per-animal basis, caging to house smaller animals is generally less expensive. In addition, smaller animals usually generate less waste and require less waste management; therefore, relative to larger animals, fewer autoclave cycles are run, fewer chemicals are used to disinfect and decontaminate equipment and work spaces, and less labor is required to provide a clean environment. Finally, smaller animals require smaller volumes of test materials, such as vaccines and experimental drugs; this attribute is particularly important for experiments that necessitate the use of novel compounds that are expensive and time-consuming to produce. Conversely, use of larger animal models may be more beneficial in some circumstances, despite the potential advantages of smaller animal models. Examples include when large amounts of tissue, blood, or sera must be collected at one time; when a large number of samples must be collected over time; when an anatomic area of interest is more similar to that in humans, or when the pathogenesis of the disease behaves more similarly to that in humans.

During the experimental design phase, considering the amount of tissue that can be collected from an individual animal and the assays in which it will be used is important. The amount of available tissue can differ dramatically among animal species. Models using small rodents may require increased numbers of animals to provide enough material for analysis. This is especially important to consider when blood is required. Even the removal of as little as 10% of the total blood volume from a healthy animal can trigger cholinergic homeostatic responses, and the rapid removal of too much blood can result in hypovolemic shock.[18]

Lastly, the time required to train technical staff to work with larger animal species in an infectious disease environment often takes longer, the procedures are often more complex, and training must be done for each specific study or species. In contrast, when working with smaller animals, such as mice or rats, many of the procedures are the same between studies and require less time to master.

Anatomic limitations. The anatomic features of an animal species may affect its suitability for use in an infectious disease experiment. For example, guinea pigs have a small and narrow oral cavity, and the soft palate covers nearly the entire back of the pharynx, leaving only the small palatial ostium for access to the esophagus, making them technically difficult to gavage.[58] As a

Pathogen	Animal species	Significant features	Application
Ebola virus[44]			
	Mice	Requires mouse-adapted virus Lack of coagulation disorder	Evaluation of countermeasures
	Guinea pigs	Requires guinea pig-adapted virus Coagulopathy only at late stage of infection	Study of virulence factors
	NHP	Best recapitulation of human disease, with wildtype virus	Assessment of replication and coagulopathy
Zika virus[49]			
	Chickens (embryos)	Resembles human fetal neurodevelopment	Studies of early infection CNS infection model High-throughput screening method
	Mice	Congenital brain malformations Virus-induced conjunctivitis Most are immunocompromised	Microcephaly model Ocular infection model Preclinical vaccine trials
	NHP	Symptoms similar to those in humans Mimics human placentation, neurodevelopment, and brain structure	Placental and sexual transmission Vaccine evaluation
Plasmodium falciparum (malaria)[47]			
	Mice	Typically uses humanized mice	Studies of host–pathogen interaction Therapeutic evaluation
	NHP	Natural host for *P. falciparum* Natural disease progression	Therapeutic evaluation
Influenza virus[61]			
	Mice	Requires mouse-adapted virus Physiologic response differs from that in humans Well-characterized immune response	Virulence Species tropism Preventive and countermeasures evaluation
	Ferrets	Naturally infected by human and avian strains Symptoms similar to those in humans Both upper and lower respiratory tract disease Develops acute respiratory distress syndrome (ARDS) and multisystem organ dysfunction after infection with highly pathogenic avian strains	Virulence Pathogenesis Tropism Transmission Decreasing disease severity
	Guinea pigs	Naturally infected by human strains Less overtly symptomatic than ferrets Both upper and lower respiratory tract disease More resistant to lethal infections than humans	Transmission
Clostridium difficile[16,28]			
	Mice	Requires antibiotic pretreatment or use of germ-free mice	Host immune response

Figure 2. Notable features of animal species used to model human infection with various pathogens. The predominant research application of each model system often varies by species.

result, guinea pigs may not be as well suited as mice and rats for studies of infectious agents causing gastrointestinal disease, such as *Listeria monocytogenes* and *Clostridium difficile*, which are often administered by this route. Similarly, rabbits have a large tongue, multiple skin folds in the diastema, a limited range of mandibular opening, and prominent incisors that obstruct the placement of an endotracheal tube.[46] Due to these features, rabbits may not be well suited for studies requiring multiple intubations for the administration of an agent (for example, to induce pneumonia) or repetitive sample collection (for example, bronchoalveolar lavage fluid). When an animal species with anatomic limitations must be used, personnel should be well experienced in the relevant procedure, to prevent inadvertent harm to animals.

Personnel safety. The selection of an animal species for study must take into account personnel safety. Personnel safety is facilitated by the use of smaller animals, because they require smaller volumes of infectious agent for inoculation, thus decreasing the amount of agent to which both laboratory and husbandry personnel might be exposed. Smaller animals are housed more easily in closed or containment-style caging, and they can be manipulated more readily in a biologic safety cabinet when procedures involve the manipulation of infectious materials or when aerosols or splashes may be created (such as during necropsy). In contrast, larger animals tend to be more difficult to handle and restrain and are more likely to kick, trample, or cause crushing injuries. In addition, they tend to cause more tissue damage when they

bite. Caging used to house larger animals can weigh hundreds of pounds, and its manipulation increases the risk of musculoskeletal injury. To protect themselves, personnel may need to wear additional personal protective equipment, such as steel toe boots and gauntlet gloves, which can be difficult to work in. Finally, the disposition of the animal species can influence safety and should be considered when evaluating an animal model. Rhesus macaques (*Macaca mulatta*) are often aggressive and unsociable toward humans; long-tailed macaques (*M. fascicularis*) are more cautious and fearful; and pigtailed macaques (*M. nemestrina*), especially the males, are more sociable and less aggressive than other NHP species.[59] Furthermore, the potential of an animal species to harbor zoonotic organisms can increase risk to the worker. Although most vendors are able to eliminate the vast majority of these organisms, some agents, such as *Macacine herpesvirus* (B virus), may be difficult to eliminate completely,[33] making work with NHP subjects riskier than work with other species.

Natural animal models. Another factor to consider in choosing an animal model is the inherent susceptibility of the animal to the pathogen under study. Depending on the research objectives, it is often preferable to use animal models in which the natural routes of disease transmission, disease pathogenesis, and clinical disease development closely mirrors that of the original host (for example, humans). This model type has been called a 'natural model.' Examples include influenza in ferrets,[76] *Mycobacterium tuberculosis* in macaques,[20] rabies virus in dogs,[40,75] and *Brucella abortus* in goats.[6,31] These models require minimal artificial manipulation of the host and infectious organism to induce the disease condition. They also facilitate the study of transmission routes and factors, virulence determinants, and the host immune response including regulation of gene expression.[21,22] Furthermore, compared with other model types, these models may permit the use of a lower dose of infectious organisms thus benefiting researcher safety. However, due to their natural susceptibility to infection, infected animals may shed more organisms into the environment through the feces, urine, respiratory secretions, and aerosols, thus increasing risk to both research and husbandry staff. Studies using natural disease transmission from infected to naïve animals have inherent variability in infectious dose exposure, often resulting in a more variable outcome and requiring larger numbers of animals to obtain statistical significance.[21] In addition, animals may develop only asymptomatic illness after infection with organisms that fail to cause either significant pathologies or elicit a strong immune response in immunocompetent subjects. Although this type of model may be valuable for studying specific aspects of disease resistance, it may not be an appropriate model to study the pathogenesis of human disease when humans develop symptomatic illness.[21,22]

Surrogate animal models. In addition to using an animal species in place of the target host species, many animal model systems use alternative pathogens. Despite differences in host and pathogen species, these surrogate animal models can provide insight into infectious diseases of humans, given that disease mechanisms are often similar across select host and pathogen species.[72] Examples of surrogate animal models include SIV infection of macaques as a model for AIDS and murine norovirus infection of mice as a model of Norwalk virus infection. In addition, surrogate animal models may be used when ethical concerns, cost, biologic containment, animal availability, or limited accessibility of reagents make the use of natural models untenable. For example, chimpanzees and tree shrews, although naturally infected with hepatitis C, are not practical surrogate model species, because the use of chimpanzees in biomedical research is restricted,[60] and tree shrews are difficult to handle and breed in a laboratory setting. Instead, tamarins infected with GB virus B have been used as a surrogate model of human hepatitis C infection.[24] Similarly, an attenuated strain of a pathogen can be used in place of the more virulent wildtype strain.[45] As demonstrated by these examples, the use of an alternate pathogen may also provide the added benefit of reducing or eliminating the risk of zoonotic infection to personnel.

Yet other animal model systems require artificial manipulation of the animal, the infectious agent, or the route of agent administration to permit infection or promote the development of a disease or condition.[5] Examples include modification of the host species, such as genetic modification of mice to knockout the hemojuvelin gene to increase their susceptibility to infection with attenuated strains of *Yersinia pestis*,[52] and alteration of an infectious agent to increase its infectivity in a given host, such as with serial passage of Ebola Zaire virus, which does not naturally infect mice, for study in mice.[8] Although these models may enhance the ability to tease out specific aspects of pathogenesis or host response, using artificial routes of infection may require higher infectious doses, increasing risk to personnel handling the pathogen.[21]

Genetics of the animal host. The choice of using outbred or inbred animals should also be considered during the experimental design phase. Outbred stock animals mimic the human population in that they are genetically heterogeneous and therefore may be preferable when attempting to model the response of a population to a vaccine or drug therapy. However, due to their heterogeneity, their response will be variable, and greater animal numbers might be needed to obtain statistical significance. Inbred strains of animals are genetically uniform and therefore will have a narrower response to infection, vaccination, and treatment. For this reason, fewer animals are usually required to obtain statistical significance, making them especially useful for pathogenesis studies.[17]

Mice are often the model species of choice for infectious disease studies. In addition to their small size, availability, ease of handling, and comparatively low cost, they are available as both outbred stocks and inbred strains. Mice have a well characterized immune system that is relatively similar to humans', they can be infected with many human pathogens, and they can be genetically modified with relative ease. By comparing the extent of disease in different mouse strains, researchers can better understand a pathogen's virulence factors, identify host factors that confer resistance or susceptibility, and explore the host response to infection. For example, MyD88 knockout mice have been used to identify the MyD88 protein as a key factor in the immune response against more than 45 pathogens in mice, including gram-negative and gram-positive bacteria, viruses, mycoplasma, parasites, and fungi.[27,71] In addition, genetically modified mice can be used to mimic host conditions that can significantly alter the natural course of infection. For instance, mouse models of diabetes have been used to understand effects on the diabetic's immune system and their increased susceptibility to infection,[25,73] and a mouse model of hereditary hemochromatosis was used to examine whether increased iron load in host tissues may restore the virulence of vaccine strains of *Y. pestis*.[52] In addition, 'humanized' mice are increasingly used to study pathogens that only

infect humans or for which models that effectively recapitulate infection, pathogenesis, or immune response have not yet been developed. These immunodeficient mice have been engrafted with human hematopoietic stem cells that develop into functional human immune system components or have been engrafted with human tissues.[30] These mice have been used to study the pathogenesis of a variety of viral and bacterial pathogens, including Epstein–Barr virus, hepatitis B, hepatitis C, and *Salmonella enteric* serovar Typhi (*S. typhi*).[9] These models have been especially useful for the study of HIV1 pathogenesis, since this virus specifically targets and depletes human immune cells.[74] Overall, humanized mice are becoming increasingly valuable in translational research of infectious diseases by bridging the gap between basic science research and human clinical trials.[9]

Infectious agents. Just as it is necessary to select the most genetically appropriate animal for infectious disease research, selection of an infectious organism with suitable genetic composition is also crucial. In addition to genus and species, researchers must also consider the strain of the agent. Strains may differ markedly in their pathogenesis due to many factors, including the animal from which they were originally isolated as well as the culture and growth environments in which they are maintained. In general, laboratory-adapted strains of bacteria and viruses tend to become attenuated through successive generations of growth in an artificial culture environment,[19] whereas clinical isolates are more likely to possess the virulence determinants necessary to induce a robust infection in a susceptible model host. Therefore, when establishing new animal models, isolates obtained from clinically ill patients are preferred over laboratory-adapted strains. An example is the use of CO92, a clinical isolate of *Yersinia pestis* that was originally isolated from a human patient in the US in 1992 and is capable of inducing both bubonic and pneumonic plagues in mice,[2,35] rats,[4] guinea pigs,[50] and NHP.[36,51] The pathophysiology observed after infection by this isolate in these animal models is very similar to that in humans. However, the iron-acquisition locus directly associated with virulence can be lost at high frequency after passage in culture, resulting in attenuation.[12,13] Assuring the virulence of *Y. pestis* CO92 cultures for animal studies requires avoidance of liquid media systems, selection for the appropriate pigmentation phenotype and frequent retrieval of bacteria from the original frozen stock (provided by the repository). This scenario is in contrast to the use of the lab-adapted isolate KIMD27, which does not cause disease in these models unless they are pretreated with iron prior to infection. Maintaining the features of clinically derived isolates over time in the laboratory requires careful manipulation and tracking of serial cultures, for which the guidance of an experienced microbiologist or virologist can be invaluable.

Adaptation of an infectious organism to a model animal species can be beneficial when studying organisms that don't consistently infect the desired research animal or that induce a disease condition in the model animal that differs in severity from what occurs in humans. For instance, mice are not naturally susceptible to infection with human strains of influenza virus, but most viral strains can be experimentally adapted for mouse virulence by serial lung-to-lung passages.[55] In addition, the JSNZ strain of *Staphylococcus aureus*, a naturally mouse-adapted strain isolated from an endemically infected colony of C57BL/6J mice, was found to be a better colonizer of mice and more virulent in an intraperitoneal infection model than the human-derived strain, Newman.[26] Adapting an organism to a model animal species broadens the scope of possible research with the model system and expands it beyond what otherwise would be possible.

Availability of reagents. Before committing to the study of a specific animal model, attention should be paid to the availability of reagents necessary for ex vivo and in vitro analyses. To date, monoclonal and polyclonal antibodies against numerous proteins are commercially available or can be commercially produced as needed. Recombinant DNA and protein production technologies allow companies to offer a wide array of reagents, such as intracellular antigens, cell surface antigens, and immunomodulatory proteins. However, although many reagents are available for mice, fewer are available for other animal species. Therefore, investigators wishing to rely on nonmurine animal models should identify, early on, all the reagents that may or may not be available to them.

Welfare Considerations

Medical management The consideration of animal welfare is paramount in all animal-based research. A harm–benefit analysis should be conducted during the evaluation of each proposed animal study to ensure that animal pain and distress is minimized to the furthest extent possible yet still supports the research objectives. Nevertheless, animals may become severely debilitated or can experience significant pain or distress during the conduct of infectious disease studies. These conditions may occur secondary to the disease itself or from necessary experimental procedures, such as repeated invasive sample collections or single housing of a highly social species. However, the provision of pharmacologic interventions and supportive care intended to minimize animal pain and distress is controversial due to concerns regarding research variability and validity. Despite these concerns, both pharmacologic interventions and supportive care should be thoroughly considered during study design, both to maximize animal welfare and to optimize research outcomes.

During this assessment, researchers must determine whether animals will serve as appropriate models in the absence of pharmacologic interventions or supportive care. For example, would withholding support result in accelerated clinical disease progression or mortality not representative of human patients? Would the evaluation of potential treatments or a chronic disease condition be hindered by animal death due to treatable secondary effects (for example, dehydration, hypothermia, inability to obtain food or water)? Would diseased animals provided pharmacologic interventions or supportive care better model human patients who commonly receive aggressive, comprehensive care in modern healthcare settings? Will the expected level of pain or distress experienced by animals significantly alter their immune response (for example, in response to infection or vaccine administration)?[39] This final question is especially relevant for animal models subject to painful or distressing conditions required for model development or support (for example, surgeries) and not experienced by the target population. If the use of pharmacologic interventions or supportive care is not contraindicated, then each should be examined to determine which may best support research objectives without the induction of unacceptable research outcomes.

Potential pharmacologic interventions include the administration of analgesics, anesthetics, anxiolytics, and antibiotics. For each, a species' normal response to the drug, in the absence of disease, should be evaluated, as well as the drug's direct influence on the course of a disease. For example, morphine has been shown to markedly potentiate *Salmonella* infection and enhance

subsequent dissemination of *Salmonella* organisms in mice, thus altering survival, mean survival time, and tissue titers.[41] In contrast, buprenorphine has been shown to reduce pain and distress in mice infected with *Toxoplasma gondii* without interfering with acute infection as defined by survival.[39] Furthermore, drugs within and across drug classes should be evaluated, given that their mechanisms of action and immune system influences may differ substantially. For example, when compared with morphine, buprenorphine may provide animal subjects a comparable level of analgesia yet induce fewer significant alterations of the immune system.[57] Species-specific pharmacodynamics and appropriate dosing regimens should be considered for all drugs. This assessment is of particular importance in drug development and testing studies in which animal models must accurately reflect a drug's action when administered to humans. In addition, a drug's direct influence on animal performance relative to humane endpoint criteria must be recognized, independent of the infectious organism, because endpoint criteria may need to be refined to accommodate this influence. For example, animals treated with analgesics may exhibit decreased spontaneous movements relative to their unmedicated cohorts, regardless of disease status, therefore additional control animals that receive the same analgesic but are not infected may be required.

In addition, the use of supportive care should be carefully considered. Supportive care aids the basic physical and psychologic needs of an animal so that it can fully respond to external insults (that is, infectious organisms). Examples include the provision of a warm environment (supplemental heat or increased environmental temperatures); parenteral fluids; nutritional support such as easily-accessible and digestible, high-quality nutritional sources; and cage modifications including alternate or increased bedding or nesting materials for comfort and temperature regulation and the provision of hiding locations. The potential effect of providing supportive care should not be underestimated, and its possible influence on experimental outcomes should be critically evaluated, as is done for pharmacologic interventions.

Recognizing that any type of intervention (for example, administration of analgesics, provision of enhanced nutrition) may influence animal response to infection, studies should be designed to uniformly apply interventions across all subjects to minimize experimental variability. In addition, the anticipated benefits of all interventions should be weighed against potential indirect harm to the animal or study. For instance, repeated administration of subcutaneous fluids in rabbits may induce psychologic stress due to handling and restraint as well as pain during administration. Likewise, the administration of drugs requiring intravenous delivery may either reduce the number of patent vessels available for administration of test substances or involve a surgical procedure for placement of an indwelling catheter. As a result, when designing studies, researchers should strive to anticipate harms or sources of experimental variability likely to be induced by the provision of interventions. When appropriate, interventions that induce the least harm or source of variability should be selected. For example, a long-acting analgesic may be preferable over the use of analgesics requiring frequent administration.

In conclusion, although researchers should consider potential use of pharmacologic interventions and supportive care practices for each study, their use should ultimately be decided only after careful and knowledgeable consideration of the advantages and disadvantages to both animal welfare and research outcomes.

Humane endpoints. Many infectious disease research studies have the potential to induce considerable animal pain or distress due to the inherent needs of the study. For example, in vaccine efficacy studies, disease development in negative-control animals is required and occurs in animals given nonprotective vaccines. Similarly, evaluation of experimental therapeutic agents may require animals to exhibit significant clinical disease, similar to that observed in infected human patients, prior to agent administration. To mitigate possible pain and distress and to potentially optimize research outcomes, humane endpoints should be clearly defined prior to study initiation and continually refined as additional experience is gained with the animal model system.

The *Guide for the Care and Use of Laboratory Animals* defines a humane endpoint as "[T]he point at which pain or distress in an experimental animal is prevented, terminated, or relieved."[29] Simply speaking, humane endpoints are designed to allow the earliest removal of an animal from an experiment, with the goals of preventing unnecessary animal suffering while achieving the desired scientific objectives.

Spontaneous animal death was once a commonly used endpoint in infectious disease studies. Although this criterion may still be necessary in limited situations, the use of death as an endpoint requires substantial ethical and scientific justification. In addition to the benefits to animal welfare, the identification of earlier endpoints can improve scientific results by helping to ensure that biologic samples are minimally degraded and of limited variability due to adverse effects of severe illness (for example, inability to obtain food or water, dehydration, hypothermia).[62] Using earlier endpoints also minimizes the influence of adverse effects unrelated to the infection on disease development and animal death, thereby complicating determination of an organism's pathogenicity.

A lack or severely diminished response to external stimuli (that is, moribundity) and palpable, terminal hypothermia were commonly used criteria when the use of humane endpoints was first becoming common practice.[62] Since then, a wider range of clinical signs, physiologic measurements, and detectable biochemical changes and biomarkers have been explored to identify early predictors of disease progression.[48] Ideally, endpoint criteria are highly predictive of disease outcome, are easily obtained, do not influence animal disease response, and can be detected before animals experience unnecessary pain or distress. Development and refinement of endpoints are preferably done through collaborations between research and veterinary personnel as each may provide unique skills and observations. The selection of appropriate humane endpoints can be difficult and should not be approached casually.

Humane endpoints must be specific to each experiment and not generalized between similar studies or model systems. They should not be selected exclusively in response to the anticipated level of animal pain and suffering nor set at a premature point in the disease process. Doing so may result in statistically insignificant or inaccurate research conclusions and therefore an ineffective use of animal life. Instead, humane endpoints should reflect the combination of factors that make each experimental model unique. These factors include the genetic background of the animal (for example, species, strain, substrain) and the infectious organism (for example, wild-type, laboratory-maintained), experimental procedures (for example, routes of agent administration, surgical manipulations, biologic sample collection method

and frequency), environmental conditions (for example, animal housing room temperature, food and bedding types, provision of environmental enrichment and social housing), and animal husbandry practices. As an example, hypothermia or a decreasing body temperature is a commonly used endpoint in mouse studies. However, mouse body temperature can be affected by many variables including individual animal variation, time of day, ambient environmental temperature, bedding type and quantity, nesting material, presence of cage mates, and method and anatomic location of measurement (for example, rectal or ventral skin surface).[1,62] Therefore, to accurately interpret body temperature measurements relative to humane endpoint criteria and to minimize erroneous experimental conclusions, each of these potential variables must be held constant for a given experiment. To take into account the unique parameters of each model, we recommend performing a pilot study with a small cohort of animals to effectively determine early endpoints for a specific model or condition when a similar model or previous experience is not available.

The methods used to assess endpoint criteria must be considered, because they themselves may influence animal health or wellbeing. For example, rectal temperature measurements can induce animal stress due to repeated handling, and the monitoring of serum biomarkers requires repeated blood sampling and may involve surgical placement of a vascular catheter.

In addition, animals should be assessed for endpoint criteria at an appropriate time and frequency. For instance, spontaneous movement in rats may be best observed during the dark phase of the light cycle, when rats are normally most active. To facilitate the identification of animals soon after reaching established endpoints, the frequency of observations should increase with anticipated disease progression and should occur sufficiently often to minimize instances in which an animal's condition worsens beyond endpoint criteria. Similarly, experiments should be scheduled so that an adequate number of trained research personnel are available to assess animals when they are most severely affected and require intensive monitoring.[48] This aspect is particularly important when severe clinical disease is anticipated. Additional guidance on selection of appropriate endpoints, including information specific to infectious disease research, is available.[43,48,63]

All personnel performing endpoint criteria assessments must have extensive knowledge not only of the disease model but also of species-typical behavior and physiology so that they appropriately differentiate normal from abnormal findings. In addition, personnel must be proficient and consistent in the assessment technique to minimize intra- and interobserver variability. To decrease unintentional bias, personnel should be blinded to experimental groups, whenever possible.

Regulatory Considerations

It is not sufficient to consider only the scientific, practical, and welfare aspects of study design when developing an animal model of infectious disease. Researchers must also consider applicable regulations and the regulatory environment. Scientists performing animal research must follow national, local, and institutional regulations and policies. In the United States, regulations, policies, and guidelines specific to infectious disease research with animals include *Biosafety in Microbiologic and Biomedical Laboratories* (5th edition),[64] Select Agent regulations,[67] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*),[66] and *Occupational Safety and Health Administration Laboratory Safety Guidance*.[69]

In addition, the choice of animal model for research on human vaccine or drug development may be influenced by licensing agencies. In the United States, under what is now known as the 'FDA Animal Rule,' the FDA may grant marketing approval based on adequate and well-controlled animal efficacy studies when the results of those studies establish that a drug is reasonably likely to produce clinical benefit in humans, provided that human efficacy studies are not ethical and field trials to study effectiveness of the drug are not feasible.[68] Guidelines and model selection criteria are provided in the regulation, but ultimately, the FDA evaluates the suitability of proposed animal models on a case-by-case basis. Therefore, the FDA strongly encourages "early and ongoing communications" regarding animal model selection and study design.[65] Thus, it is critical that the scientific team understand the transition from basic to applied science and plan ahead to bridge the potential capabilities and limitations of the chosen model system.

Discussion

There are many indications for the use of animal models in infectious disease research that includes studies for which using human subjects would be unethical or impractical and for which a living model system is required. The selection and development of animal models should be guided by multiple factors including clearly defined research objectives, the inherent characteristics of the animal model species and the infectious organism of interest, the animal's response to infection, and its similarity to the human disease condition. However, to obtain accurate research results that are predictive of the human condition, researchers must recognize and appropriately manage the unique characteristics and potential limitations of the model system (that is, the animal, the infectious organism, and research procedures). In addition, researchers face the difficult task of optimizing animal welfare while maximizing research results. Furthermore, personnel health and safety must be protected from occupational hazards associated with infectious organisms and the animals themselves. And, of course, all of this must be done while remaining compliant with applicable regulations.

Developing new animal models or adapting existing ones to address new research objectives can be challenging. There is often a lack of information to help researchers determine an appropriate dose range, the rate of disease onset, and appropriate humane endpoints. In addition, special husbandry may be required to support infected animals, biosafety concerns may need to be resolved, and waste handling may need to be determined before studies can be performed on a larger scale. This lack of information can severely impair future research design, negatively affect animal welfare, and decrease safety. The conduct of pilot studies can be extremely useful in these situations by generating preliminary information that can be applied to refine experimental design and animal management and care practices, and pilot studies are highly recommended before starting new, large-scale experiments.

To fulfill these many objectives, infectious disease studies using animal models are best done as a collaborative effort. Research staff, bacteriologists and virologists, veterinarians, husbandry staff, and biosafety officers all have expertise that can not only improve study design but also may improve staff safety and

animal welfare. Working together, these subject experts can improve scientific quality and accelerate scientific advances.

However, the conduct of scientifically appropriate experiments is insufficient to truly advance scientific knowledge. Complete and accurate information must be shared with the greater research community so that the information can then be applied to subsequent areas of study. As detailed in the ARRIVE guidelines,[32] details such as the strain of pathogen, animal genetics, the experimental procedures performed, supportive care, medications, and humane endpoints should be described fully so that the utility of published research can be maximized. In addition, the description of these details enhances the reproducibility of data between studies.[14]

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