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REVIEW ARTICLE

Choosing the right animal model for infectious disease research

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Abstract

A complex biological system is often required to study the myriad of host-pathogen interactions associated with infectious diseases, especially since the current basis of biology has reached the molecular level. The use of animal models is important for understanding the very complex temporal relationships that occur in infectious disease involving the body, its neuroendocrine and immune systems and the infectious organism. Because of these complex interactions, the choice of animal model must be a thoughtful and clearly defined process in order to provide relevant, translatable scientific data and to ensure the most beneficial use of the animals. While many animals respond similarly to humans from physiological, pathological, and therapeutic perspectives, there are also significant species-by-species differences. A well-designed animal model requires a thorough understanding of similarities and differences in the responses between humans and animals and incorporates that knowledge into the goals of the study. Determining the intrinsic and extrinsic factors associated with the disease and creating a biological information matrix to compare the animal model and human disease courses is a useful tool to help choose the appropriate animal model. Confidence in the correlation of results from a model to the human disease can be achieved only if the relationship of the model to the human disease is well understood.

KEYWORDS
animal, disease, extrinsic, fidelity, infectious, intrinsic, matrix, model, research, validity

1 | INTRODUCTION

The development of safe and efficacious vaccines and therapeutics relies on a proper understanding of the advantages and limitations of animal models available to researchers. In certain cases, such as for new or emerging diseases for which human data are not available, the animal model is crucial for understanding the pathogenesis of the disease before the development of vaccines or therapeutics can even be considered. Beyond that, a well-designed animal model provides a sound basis for supporting good science and ensuring the most beneficial use of both animal and human resources. Animal models play an especially important role in infectious disease research because in many cases, the resultant disease is potentially lethal or permanently disabling and therefore does not readily lend itself to research using human subjects. Animal models are essential for scientific advancement in many areas of human health, but if they are not well characterized and understood, erroneous conclusions may be drawn, hindering scientific advancement and resulting in a waste of animal life. A well-designed animal model requires a thorough understanding of similarities and differences in the

physiology between humans and animals and incorporates that knowledge into the goals of the study. To that end, the information presented in this article provides a systematic approach to animal model development.

2 | WHAT IS A MODEL?

There are many types of biomedical models that range from biological models, such as whole animal models and ex vivo models, to models of nonbiological origin, such as computer or mathematical models.[1] Biomedical models simulate a normal or abnormal process in either an animal or a human. For infectious disease research, animal models are meant to emulate the biological phenomenon of interest for a disease occurring in humans.

In developing or choosing an animal model, it is necessary to first have an understanding of terminology commonly used when discussing animal models. Important parameters of animal models include the concepts of homology, analogy, and fidelity. Homology refers to morphological identity of corresponding parts with structural similarity descending from common form. Homologous models therefore have genetic similarity. The degree of genetic similarity required for a model to be considered homologous is variable. Analogy refers to the quality of resemblance or similarity in function or appearance, but not of origin or development. Therefore, analogous models have functional similarity. In general, animal models exhibit both of these attributes to various degrees, and so may be considered a hybrid of these. Model fidelity refers to how closely the model resembles the human for the condition being investigated.[2] Another layer of fidelity also may be a measurement of how reproducible the data are within the model itself.

Other important concepts include one-to-one modeling and many-to-many modeling. These terms refer to the general approach to the modeling process itself and not the individual animal model. In one-to-one modeling, the process that is being simulated in a particular animal has analogous features with the human condition. In many-to-many modeling, each component of a process is examined in many species at various hierarchical levels, such as system, organ, tissue, cell, and subcellular.[3] Many-to-many modeling is often used during the development of animal models, whereas one-to-one modeling is more suited for research when the animal model is already well characterized and validated for the specific biological phenomenon being investigated.[4]

Conceptually, animal models may be described in a number of ways:[5,6] Induced (experimental), spontaneous (natural), genetically modified, negative, orphan, and surrogate. However, these descriptive categories cannot be used as classifications because the descriptions are not exclusive and models may have properties of more than one of the descriptions. Furthermore, as the knowledge of the model and the disease process progresses, the descriptive category of the model may change. Each of these descriptive categories will be discussed in the following paragraphs.

Experimental animal models are models wherein a disease or condition is induced in animals by the scientist. The experimental manipulation can take many forms, including exposure to biological agents such as an infectious virus or bacteria, exposure to chemical agents such as a carcinogen, or even surgical manipulations to cause a condition. In many cases, this approach would allow the selection of almost any species to model the effect. For example, many biological toxins may be assayed for activity in invertebrates as well as vertebrates.[7,8] The model selected would depend on the needs of the researcher. However, many biological agents are selective and cause species-specific responses. This is particularly true of infectious agents including bacteria and viruses. Many infectious agents are limited in the species that they can infect and in which they can cause disease. Some are restricted to a single known host, such as human immunodeficiency virus causing disease only in humans. Thus, these models are restricted to animals that are susceptible to the induced disease or condition.

The spontaneous model is typically used in research on naturally occurring heritable diseases. There are hundreds of examples of this type of model, including models for cancer, inflammation, and diabetes. As the term "spontaneous" implies, these models require the disease to appear in the population spontaneously. These types of models are not limited to inherited disease but may also apply to inherited susceptibility to disease. For instance, susceptibility to type 1 diabetes is a heritable trait. The NOD strain of mouse also exhibits a heritable susceptibility to diabetes relative to most strains of mice and has been used as a spontaneous model for type 1 diabetes.[9] Although the appearance of diabetes in the NOD mouse is spontaneous, the occurrence of the disease is associated with environmental factors. Thus, the NOD mouse model is described as a spontaneous model because diabetes arises without experimental intervention, even though the disease is triggered by environmental factors. Although spontaneous models are typically associated with genetically inherited diseases, some of these models may represent diseases for which the inducing agent, such as virus, bacterium, or chemical, has not been identified. Once the inducing agent has been identified and actually applied by the researcher, the model would be described as an induced model. An example would be type 1 diabetes, for which it has been demonstrated that viral infections can either destroy beta cells directly or induce an autoimmune response that destroys the cells.[10] If the researcher employs the virus to induce diabetes in the NOD mouse, then this becomes an experimental model.

The genetically modified animal model is one in which the animal has been selectively modified at the genetic level. Because these models are produced from manipulation by researchers, models using genetically modified animals are actually a special example of the experimental model. In the broadest sense, genetically modified models may result from breeding or chemically induced mutations. These may also include animals that have been modified through the use of recombinant DNA — a subgroup of genetically modified animal models referred to as transgenic animal models. Such transgenic models can involve gene deletions, replacements, or additions. The development of genetically modified animal models has rapidly

expanded as technologies for genetic engineering have advanced. For example, a transgenic model for staphylococcal enterotoxin B (SEB) has been developed in mice that were genetically modified to express human leukocyte antigens DR3 and CD4.[11] These mice have an increased sensitivity to SEB and develop an immune response more similar to that of humans relative to the parent strain of mouse.

In a negative model, the agent that causes disease in humans does not cause disease in the animal. In the early stages of development of an animal model for disease, the lack of disease would often cause the animal to be rejected as a model. However, exploring why an agent does not cause disease can also provide insights into the disease process. This may be applied across species. For example, the resistance of bovines to shiga toxins relative to the sensitivity of rabbits and mice is caused by the relative levels of expression of receptors for the toxin.[12-14] Negative models are particularly powerful when differences are identified between strains of a species, thereby allowing a comparison within the same species. As a recent example, Lyons et al observed that the sensitivity of mice to *Bacillus anthracis* could vary more than 10-fold, depending on the strain of mouse tested.[15] Comparing the response to infection between these strains of mice should provide significant insights into the disease process. The use of transgenic models provides additional power to the negative model; animals may be genetically engineered to create an isogenetic change. This was applied as described earlier for SEB to create a more sensitive animal by inserting the gene for human leukocyte antigens.

Orphan models are those with no known correlation to human disease. However, as we increase our understanding of these animal diseases and human diseases, correlations may become apparent in the future. Some orphan models may have direct comparison to human disease, such as the realization that the enteritis and death caused by administering antibiotics to hamsters were related to antibiotic-associated pseudomembranous colitis in humans by facilitating the overgrowth of toxigenic *Clostridium difficile*.[16] Once an orphan model is linked to a human disease, it is no longer considered an orphan model.

A newer descriptive category is the surrogate model. In a surrogate model, a substitute infectious agent is used to model a human disease. In some cases, the substitution may be obvious, as when feline leukemia virus in felines is used to model human immunodeficiency virus,[17] *Salmonella typhimurium* in mice is used to model *Salmonella typhi* infection in humans,[18] or monkeypox virus is used to model smallpox due to the stringent restrictions on the access to or possession of variola virus and its strong tropism and resultant clinical disease only seen in humans. However, more subtle differences also apply such as a human pathogen adapted to infect the species used for the animal model. For instance, Ebola Zaire virus can infect and cause disease in mice and guinea pigs after it is serially passaged in these species.[19] The fact that the virus has to be adapted to the new host implies that the virus undergoes a change; the Ebola virus adapted to the mouse and guinea pig cannot be considered identical to the human virus and must be considered a surrogate agent.

More difficult to define are the unintentional changes that occur to a pathogen with the mere passaging of organisms in the laboratory, such as propagation of human viruses in nonhuman primate cell lines or the cultivation of bacteria in artificial media. The potential for genetic drift in the strains of organisms underscores the need to minimize the passage of strains to maintain identity to the original clinical isolate. Incumbent on the interpretation of results in the surrogate model is the understanding that not only does the animal differ from humans but the infectious agent in the animal model differs from the agent that infects humans. This adds an additional layer to the extrapolation of the results from the animal to the human disease.

An animal model can be described more than one way. For example, the mouse used to analyze SEB can be described as a genetically modified, induced model. Alternatively, a model may be described in a different way depending on the experimental design. For instance, the spontaneous mouse model for diabetes may be described as an induced model if the experimenter uses a virus to cause destruction of the beta cells.

3 | IDENTIFICATION AND DEVELOPMENT OF AN ANIMAL MODEL

Finding a model of disease depends first on identifying animals or tissues that are responsive to the agent. Then, the intrinsic factors in humans, such as pathological progression of the disease, must be related to the factors of the disease in the model to support its validity. If a disease-causing agent is novel, and no animal models are described, the researcher must identify and develop animal models. By identifying the relationship of a novel agent to known pathogens with established animal models (eg, through identification and rRNA sequencing), animals for modeling may be initially selected based on known models for the related organisms. In lieu of known models, animals for modeling will have to be identified empirically, and this selection should start with the evaluation of animals that are well supported by reagents for research (eg, mouse) and progress to less-supported animals only as needed to meet the requirement of mirroring the disease in humans.

Although this process begins with a one-to-one comparison of the pathological progression of the disease, conceptually the collective analysis provides a many-to-many perspective. As a model is selected and validated, analysis may focus on a one-to-one approach to modeling. The basic steps to identify and develop an animal model are as follows:

1. Define the research objective.
2. Define the intrinsic factors associated with the biological phenomenon under investigation, such as the pathological progression of the disease process.
3. Define the extrinsic factors associated with the biological phenomenon under investigation such as the method used to prepare the pathogenic bacteria.

4. Create a search strategy and review the literature of previous animal models.
5. Create a biological information matrix.
6. Define unique research resources.
7. Identify preliminary animal models of choice.
8. Conduct research to fill critical gaps of knowledge in the biological information matrix for the preliminary animal models of choice.
9. Evaluate the validity of the animal models of choice.
10. Identify animal models of choice.

4 | DEFINING THE RESEARCH OBJECTIVE

A single model likely will not be applicable to every situation. The model of choice is the model that best addresses the study's research aim within the research constraints. Therefore, a first step in animal model development is to define explicitly the specific question the research needs to address. The next step is to determine what specific information must be provided by the animal model to accomplish the research objective. This information is critical and will give direction to the remaining animal model development process.

5 | DEFINING INTRINSIC FACTORS

Once the experimental need has been defined, the next step in establishing an animal model is to develop the intrinsic points of reference to the human illness. Intrinsic factors are inherent factors in the interaction between the host and biological agent or pathogen. Confidence in the model will grow with increasing common points of reference between the animal model and the human. It is critical that all intrinsic factors relevant to the biological process associated with the research question be identified, so that they may form the basis for comparing the animal model to the human condition being studied.

Although the early steps in the model development process are fundamental and appear obvious, the details can be easily overlooked, potentially leading to a selection of an animal model that is not entirely appropriate for the specified research. When defining the pathological progression of infectious disease, basic steps in the progression of the disease process will be identified. From a simple linear view of events, a disease-causing agent must gain exposure to the host, bind to and enter the host, distribute within the host to the target tissue, and exert disease through a specific mode of action. Pathogens may gain access to the systemic circulation by injection (via bites from parasites such as fleas and ticks) or through abrasions. The pathogen may also interact with the mucosa, such as found in the intestines and lungs. In these situations, the pathogen may bind to specific receptors on the host cells. The pathogen then may enter the host through the mucosal cells by commandeering the host's cellular processes to take up the pathogen and enter the systemic circulation. After entering the host, the pathogen may be distributed in the body (such as by the circulatory system). During this distribution, the pathogen can target specific tissues by binding to receptors on those tissues. The pathogen can then enter the cells of the target tissue and cause disease by affecting specific biochemical processes in the target cells. Some pathogens, however, do not invade the host's body or target tissues but produce extracellular factors, such as toxins and tissue-damaging enzymes. These factors may be transported into the body and be subsequently distributed by the circulatory system to target tissues or cells. For example, if the biological phenomenon being investigated is to determine the 50% human lethal dose of an agent such as botulinum toxin, then the anticipated intrinsic factors, such as pathogenic steps in the progression of the intoxication process from absorption into the body to the toxin's effect on the neurons, might be identified as follows:

1. Toxin/agent penetration/absorption and biological stability.
2. Toxin/agent persistence in circulation and transit to target tissues.
3. Toxin/agent binding and uptake into target tissues.
4. Toxin/agent mechanism of action in target tissues.

Superimposed on this simple linear view of the disease process is a complex interplay between the host and pathogen. The pathogen will significantly change its physiology and expression of virulence factors in response to interactions with the host, and the host will also change in response to the pathogen. For example, the host cells may produce specific receptors only after exposure to the pathogen.[20] In addition, invasion by the pathogen will prompt the host's innate and acquired immune responses. The pathogen must circumvent the host's resistance, including competitive exclusion by the normal microflora, assault by host factors such as antimicrobial peptides and enzymes, and destruction by the innate and acquired immune response. In some cases, this evasion of the immune response leads to misdirection and deregulation of the immune response, resulting in the host's immune response actually contributing to the pathogenesis of the disease.

As this interaction progresses, the invading organism will typically harness the cellular processes of the host to promote its own replication and may directly cause damage to the host's cells and tissues. The ability of the host to respond to the pathogen in a manner that halts the infection determines the degree of the disease that the host will experience. Thus, virulence is not solely a property of the invading organism but, rather, an expression of the interaction of the pathogen with its host.

A model of disease attempts to mimic the host-pathogen interaction. Therefore, the combination of both the host and pathogen defines a model for a disease and collectively makes up the intrinsic factors of the model.

6 | DEFINING THE EXTRINSIC FACTORS

Other useful parameters that are not intrinsic to the host-pathogen/agent interaction, but that can affect the process, are known as

extrinsic factors. Functionally, extrinsic factors are variables that may be manipulated outside of the host-pathogen/agent relationship. Although extrinsic factors are not routinely considered part of the animal model, they are in fact a critical component. Extrinsic factors can influence the intrinsic factors as they relate to the host-pathogen interaction, which in turn defines the specific animal model. For example, results may be affected by factors affecting the pathogen, such as the means of preparing, handling, and formulating the agent. Extrinsic factors may also influence the response of the host. For instance, the bedding used for the animals, temperature and light cycles provided, and even the time of administering agents may affect the immunological response of the animal or the pharmacokinetics of therapeutic agents that are being studied. Extrinsic factors are an extension of the experimental design. As such, these must be identified and documented to allow comparison of data and to aid in the extrapolation of results to the human disease. The application of this requirement may be complicated by the reality that some of these factors may not be recognized.

The functional definitions of intrinsic and extrinsic factors are not uniformly accepted. Alternate definitions describe the animal as the only intrinsic factor and the pathogen or biological agent as an extrinsic factor that can be manipulated in the experimental design.[21] However, there is general consensus that the interaction between the host and pathogen must be considered the model for the disease, though a philosophically different opinion is held by some in the scientific community. Notwithstanding this difference of opinion, it is well accepted that the extrinsic factors influence the intrinsic factors of a model and that, collectively, these factors affect the design of experiments using animal models. An animal model of disease is the interaction between the host animal and the pathogen or biological agent. This interaction is influenced by intrinsic factors of the host and pathogen that cannot be directly manipulated, as well as by extrinsic factors that can be directly manipulated. Collectively, extrinsic and intrinsic factors are the components of the experimental design for a given animal model and must be defined to gain understanding and control of the model.

Many of the basic steps in pathogenesis may be modeled ex vivo. However, for the ex vivo models to be predictive of the in vivo pathogenic process, the model must account for the potential factors that can influence the interplay of host and pathogen as occurring in vivo. A preliminary review of the literature may be necessary to adequately define the distinct features of the biological phenomenon under investigation.

7 | CREATE A SEARCH STRATEGY

A preliminary, brief review of the literature using freely accessed information may be necessary to confirm the relevant intrinsic and extrinsic factors identified previously. The preliminary review should include previous studies using animal models and human clinical data. This review will allow for development of a detailed search strategy. If there are no previous data on animal models used for the specific condition being modeled, it is reasonable to search for animal species that have been used for modeling similar conditions. Animals with a close phylogenetic relationship to humans, such as monkeys, should be considered because it is reasonable to assume they may have a higher degree of homology and therefore may respond in a more similar manner. However, caution must be exercised because analogy does not always follow homology. This is demonstrated in monkeys, which do not develop acquired immunodeficiency when infected with the human immunodeficiency virus. Instead, the more distantly related feline infected with the feline leukemia virus is considered a more appropriate model for AIDS in humans.[22]

A comprehensive literature search strategy can be designed based on the relevant intrinsic and extrinsic factors associated with the biological phenomenon of interest that were identified by the preliminary literature review. Such a review is often an overlooked endeavor, but it is absolutely necessary to acquire a body of knowledge on which to make scientifically informed decisions during the animal model identification and development process. The search strategy should be designed to provide a comprehensive survey of the relevant information from libraries of publications and data. No single database is comprehensive, so the ideal search strategy should include a comprehensive search of all relevant informational resources. However, this may be cost prohibitive, and a tiered search strategy may be more appropriate, starting with the most relevant and free informational resources and expanding to the additional proprietary resources as needed. In addition to the electronic search for information, it is prudent to personally consult clinicians and scientists with experience of the disease or its models. If the comprehensive literature review identifies additional factors, such as pathological features, animal species, or other parameters that were not found in the preliminary literature review, then the review strategy should be changed accordingly.

8 | CREATE A BIOLOGICAL INFORMATION MATRIX

Following the preliminary assessment of the literature, a biological information matrix of the relevant intrinsic and extrinsic factors for each of the animal species can be prepared. The biological information matrix is an index of the information used to compare the factors of the models to the human disease. The biological information matrix should reveal what animal models are available and which are the most relevant for the proposed research. Depending on the organism, route of infection, or pathogenesis of the disease being studied, key matrix criteria may include the biological stability of the agent; penetrance (eg, route of absorption); in vivo persistence; mode of transit to target tissues; uptake at target tissues; mechanism of action in target tissue(s); lethality; clinical signs; epidemiology; immune response; therapeutic response; and anatomic deposition and distribution.

As the matrix is filled in with discrete data, comparative analogies can be made between the different species and the human data.

The species that most accurately reflects the human condition of study is then identified on the basis of the current state of scientific knowledge. It may become apparent at this point that more than 1 species is needed to address the research objective accurately. In addition, the best animal to model a specific component in the disease process may be different than the animal species chosen to model the entire disease process. It is paramount that the model be judged by how well it can be applied to the specific research question, rather than how well the animal models the entire array of the disease process in humans. For instance, yeasts may not be used to model central nervous system dysfunction caused by prions because they do not have a central nervous system. However, yeasts are used to model the biology of prion infection and propagation.[23]

9 | DEFINE RESEARCH RESOURCES

In addition to the biological matrix of information, there are many other considerations that must be taken into account when choosing the animal model. Because of animal availability, suitable housing, or other restrictions, some animal models may not be feasible for a particular researcher. For these investigators, only the more distantly related animals, such as mice, rats, guinea pigs, and rabbits, may be available. The researcher should prepare a list of unique resource requirements. It may be helpful to use an integrated team approach when identifying the resource requirements. The primary and secondary investigators in collaboration with the laboratory animal veterinarian and statistician would best be able to address unique requirements.[2] There are many lists cited in the literature for general considerations in choosing the ideal model. The following is a partial list of the general qualities of an ideal model, and these should be considered against the available resources of the researcher when selecting a model:[24,25]

1. Accurately mimic the desired function or disease: This is a fundamental cornerstone for extrapolation of data.
2. Exhibit the investigated phenomenon with relative frequency: The phenomenon must be readily present to lend itself to unhindered scientific study.
3. Be available to multiple investigators: The animals should be handled easily by most investigators. This facilitates leveraging of the scientific community.
4. Be exportable from 1 laboratory to another: The model should lend itself to widespread usage. This implies that the model must be compatible with available animal-housing facilities. This facilitates leveraging of the scientific community.
5. Be a polytocous species: The number of offspring produced is a limiting factor for future unrestrained availability. This criterion is especially relevant for spontaneous models for genetic disorders.
6. Be of sufficient size to allow appropriate sampling: The animals must be of sufficient size to allow for appropriate methods of data collection, such as for the sampling of multiple blood collections. This also implies being amenable to investigation with appropriate technological tools.
7. Be of appropriate longevity to be functional: The animal should survive long enough to allow for experimental manipulation and investigation.
8. Be accompanied by readily available background data: The availability of extensive background data may readily contribute to the biological information matrix and enhance interpretation of new data.
9. Be of defined genetic homogeneity or heterogeneity: This has traditionally been relevant for spontaneous and transgenetic models. This criterion is now achieving increased importance with the advances in microarray and proteomic technology.

10 | CONDUCT RESEARCH

The biological information matrix should provide information to identify potential animal models. The animal models identified at this stage are only preliminary assessments that are meant to help focus the remaining animal model development process. Optimally, at least 2 species of animals should be selected for modeling to allow for comparison of results between the models as well as to humans. Concordance between animal models increases the level of confidence in the biological response.

Research to fill all the critical gaps of knowledge in the biological matrix of information may be cost prohibitive. Therefore, because of financial constraints, only the gaps viewed to be the most important may be addressed with research. Because some agents result in rare infections in any population, the human condition may not be well documented. This makes the animal model development process much more difficult. However, this may be partially overcome by addressing the gaps in knowledge using a reductionist approach. Using the many-to-many animal model methodology, the intrinsic and extrinsic factors of the disease process or biological phenomenon under investigation should be identified and characterized with ex vivo experimentation. Technology should be explored to determine what ex vivo assays are available that may adequately reflect the factors in the disease process or biological phenomenon under investigation. These ex vivo experiments should be evaluated with both animal and human tissues or cell lines. This allows for data to be compared and evaluated for concurrence of data between the animal and human. In vitro experimentation may also be necessary to supplement the ex vivo studies. The same intrinsic and extrinsic factors in the disease process or biological phenomenon under investigation also should be evaluated using a holistic approach. This approach involves in vivo animal experimentation. It is anticipated that the in vivo study may differ from the ex vivo study because of the unique relationships and interactions of the cells within the intact animal, and these differences will need to be considered when interpreting the data.

Microarrays and proteomics have the potential to lend valuable insight for data interpretation from ex vivo and in vivo studies. These techniques are obviously limited to species that have been sequenced and for which microarrays have been developed.

However, if available, they will rapidly indicate whether the cells have similar or different responses to the agent. Additional approaches to rapidly evaluate the similarity of the mechanism of infection between the species could include using proteomics and electron microscopy to monitor stages of entry and propagation in host cells. Further, comparing the agent's effect on human cells derived from different organs/tissues to its effect on similar cells derived from the species used for the animal models can provide profiles of activity that may be used to evaluate the animal model. These approaches provide relatively rapid means to evaluate the correlation of the agent in animals to the agent in humans. Data obtained from in silico models, such as computer assimilation models, can also be evaluated by comparing the appropriateness of data as compared to the in vivo and ex vivo studies.

Ex vivo modeling may be done at the same time as animal models or may even precede the animal models if observations allow identification of target tissues. The data and conclusions from the animal ex vivo experiments should be compared and evaluated for concurrence of data from animal in vivo experiments. If there is concurrence of the data between animal in vivo, ex vivo, and in silico studies, as well as human ex vivo studies and available human case studies, then the extrapolation of data can be made with increased confidence. This process is an ongoing endeavor and should build on information learned previously.

11 | EVALUATE THE VALIDITY OF THE ANIMAL MODEL

What is required to validate an animal model, and at what point does the model become validated? Simply, a validated model is one in which a significant overlap of analogies for the intrinsic and extrinsic factors exists between the animal model and human disease. The definition of "significant" in this context is a point of contention that must be defined by the individual researcher and accepted by scientific peers. The animal model and human condition being modeled should have similar characteristics in the biological information matrix. The experimental design of the research to validate the model is similar to the research done for step 8, except that a more comprehensive approach is taken to further fill gaps of knowledge in the biological matrix of information. If there is not a sufficient amount of overlapping data between the animal model and humans in the biological information matrix, then additional experiments should be conducted to fill in the gaps. If these gaps are filled and the overlap of analogies is determined to be insignificant, then the model must be deemed invalid and another model sought.

The degree of accuracy of the animal model depends on the reliability of methods used to measure the pathological process or biological phenomenon under investigation. The techniques used for evaluation must be sensitive. A failure to accurately identify similarities and differences between the animal model and human can lead to erroneous extrapolations. Hierarchical evaluation of each factor, at the system, organ, tissue, cellular, and subcellular levels, can provide invaluable insight. It is important that this evaluation be done early in the animal model development process. The greater the sensitivity of measurements, the more reliable the validation will become.

12 | EXTRAPOLATING ANIMAL MODEL DATA TO HUMANS

Models are a copy or imitation of the study target. They will never be perfect in every instance. A thorough understanding of the model and an appreciation of its weaknesses will enable the researcher to make more accurate assessments and extrapolate results with a higher degree of confidence. What can be extrapolated and what cannot is one of the challenges of working with models. To extrapolate data directly from the animal to the human without first investigating and evaluating other sources of data such as ex vivo, in vitro, and in silico modeling and clinical case studies would not promote a high degree of confidence in the validity of the extrapolated data. The goal of an animal model is to have a high degree of valid extrapolation to the humans.

A consideration that is critical to the extrapolation process is the experimental design and methodology used to collect the data. Ideally, the experimental design and methodology should mirror the conditions being modeled as closely as possible and must consider the relevant intrinsic and extrinsic factors of the model. Infectious agent research commonly uses animal challenges via aerosol or oral exposure; however, both of these exposure routes may provide misleading data if they are not designed correctly. For example, the pattern of deposition within the animal varies by particle size in the generated aerosol. A particle size of 1 μm provides a similar pattern of pulmonary particle deposition in the guinea pig, nonhuman primate, and human, but at 5 μm, the pulmonary particle deposition is much lower in the guinea pig.[26] Similar considerations are important for oral challenges, such as the effects of stomach pH in the fed and unfed animals and between the various species, or the gastric emptying time compared to the volume of the challenge dose.[27,28] The different strains or isolates of agents, and the differences in their preparation, must also be considered when comparing data from different research experiments and making extrapolations.[29]

The time points for therapeutic intervention in animal models often require much deliberation to accurately reflect the human time course of intervention. Animals do not provide symptoms, and the progression or time course of clinical signs is not always the same. For example, the botulinum intoxication process in the guinea pig differs by time course, and the clinical signs are not always appreciable in these small species as compared to nonhuman primates and humans.[30]

In vitro models may provide a more ethical or humane course of research, and the reductionist approach of in vitro assays can offer the advantages of controlling the variables in the environment. However, in vitro studies are often limited in what can be accurately extrapolated to a more complex biological system. Mathematical and

computer models and other in silico models are constructed by data already gained from research and are therefore limited by what is already known about the disease. Cell culture studies are limited because they may not behave normally in the ex vivo setting when removed from the animal. In addition, cell lines are obtained from individual members of the species and, because of intraspecies variability in the biological response, may not accurately reflect the general population.

Animal models are limited because the conservation of biochemistry among species and the physiological differences between species are not fully defined. Any predictions based on models must be tempered by the realization that the interaction of a disease-causing agent with its host is complex and influenced by numerous intrinsic and extrinsic factors, many of which probably have not been identified. At least some of these factors may be specific to a given species, and others will vary even within a species. Factors such as nutrition, stress, and rest are known to influence animal and human response to infections. Controlling the significant intrinsic and extrinsic factors, as well as proper experimental design and statistical analysis, can normally overcome the biological variability of a model.

Human studies have the potential to provide the most accurate data. However, controlled experimental studies using humans are limited in scope or may not be possible for more virulent or untreatable disease. Human clinical case studies used to model the general population may also be limited because the data are not generated from a controlled environment. The patients may have preexisting conditions, and thus conclusions drawn may be less clear. In addition, the relatively small number of patients typically described in case studies of rare diseases may not reflect the general population, and this may affect the accuracy of the interpretation of the results. The human population is considered to be genetically limited relative to most other animals, with the members of a single tribe of monkeys demonstrating more genetic diversity than that observed for the whole of humankind. Nevertheless, there are known genetic differences in the human population that influence susceptibility to diseases. For instance, susceptibility to infection by *Plasmodium vivax* is dependent on the host expressing the Duffy blood type, which is the receptor in humans for that parasite.[31] Further, for every disease described for humans there have been survivors, but in most cases we do not know what parameters influence survival. Simply, individual responses to an agent are not uniform.

The understanding of the disease process is further complicated by the fact that there are variants for most pathogenic agents, and these variants are associated with different virulence potentials. Realizing the large array of factors that influence disease, and the limited understanding of these factors, models have still been used to determine whether a disease-causing agent follows a similar pathological progression between species and how the steps in this process contribute to the disease. When the disease processes and host's responses are similar to that of humans, the model provides a reference to allow predictions of responses in humans. However, numerous examples exist of accepted models that failed to be predictive at some level. For example, lethality of *Yersinia pestis* to small rodents is considered indicative of the virulence of the bacteria; however, strains of *Y. pestis* have been described that kill mice but do not cause disease in larger animals, including humans.[32] As another example, primates are considered to be predictive of infections with Ebola virus, yet the Reston strain of Ebola virus that causes disease and death in primates apparently does not cause disease in humans.[33] Therefore, the degree of accuracy of predictions based on animal models can only be definitively assessed by comparing to natural cases of human exposure.

13 | CONCLUSION

The significant effect that animal models have had in the study of infectious diseases is exemplified by the application of Koch's postulates early in the history of microbiology.[34] The continued use of animal models has been essential to achieving our present understanding of infectious diseases and has led to the discovery of novel therapies. Animal models have been used to provide the preliminary safety and efficacy testing for nearly all therapeutics in use today and have reduced testing in humans of potentially dangerous or ineffective therapies. The role of animal models in safety and efficacy testing has only increased with time. With the implementation of the "animal rule" by the United States Food and Drug Administration, the animal model provides the only premarketing efficacy data available for the evaluation of new therapeutics targeting diseases caused by certain biological agents.[35] This underscores the need for well-characterized animal models. Confidence in the correlation of results from a model to the human disease can be achieved only if the relationship of the model to the human disease is well understood. This article is intended to provide a systematic approach to achieve the required understanding of an animal model, so that it may be applied with confidence.

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CONFLICT OF INTEREST

None.

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