28 The Nonhuman Primate as a Model for Biomedical Research

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ABSTRACT

This chapter provides a brief summary of issues surrounding the utilization of nonhuman primates in biomedical research. Although a relatively small proportion of the total number of animals utilized in biomedical research, nonhuman primates occupy a unique position as the species most closely related to humans, and thus have the potential to provide highly relevant information regarding human health issues. Nonhuman primates are utilized across a wide diversity of research topics and examples are provided including infectious disease, neuroscience, and genomics. Pertinent information relating to ethical issues, species selection, housing, and specific pathogen-free status are provided as an overview of relevant issues associated with selection of nonhuman primate models. Selected references are provided as a reference for more comprehensive information relating to these topics.

Key Words: Nonhuman primate, Animal model, Macaque, AIDS, Neuroscience, Genomics, Specific pathogen free.

INTRODUCTION

Animal models are an essential component of biomedical research. Over the past century, the overwhelming majority of Nobel prizes awarded in medicine and related sciences have involved significant work utilizing animal models. It is clear that information provided through the use of animal models, and in particular nonhuman primate models, will be essential for continued development of effective solutions to issues affecting the health and well-being of both human and animal populations.

Although nonhuman primates are a relatively small component of the total animal use in biomedical research, they are an extremely important resource for a wide range of investigations. Of over 1.1 million animals[1] (not including rodents, fish, or birds) used in biomedical research annually, less than 0.3% are nonhuman primates.[2] The foundation for this disproportionate significance lies in the quality of information available through nonhuman primate studies. Nonhuman primates are arguably the most applicable animal model to the human condition based on their anatomic, physiological, and genetic similarity to humans. Genetic relatedness with the chimpanzee [*Pan (Homo) troglodytes*], the most closely related nonhuman primate species, approaches 98%, suggesting a high likelihood of correlation for nonhuman primate studies[3,4] Other nonhuman primate species, while less related to humans, still demonstrate important similarities and differences, offering an incredible resource for studies spanning a broad spectrum ranging from infectious disease to evolution.

A wide variety of nonhuman primate species have been developed for biomedical studies.[2,5,6] These include several major families of primates spanning the spectrum from new to old world, apes to prosimians. Examples of commonly used species include the genera *Macaca, Pan (Homo), Papio*, *Aotus*, *Callithrix*, *Saimiri*, *Chlorocebus*, *Saquinus*, and *Cercocebus*. Again, species variations provide the basis for selective advantages between specific nonhuman primate species in certain research applications (Table 28–1).

CONSIDERATIONS FOR USE OF NONHUMAN PRIMATES

Ethical considerations associated with nonhuman primate use in biomedical research are a significant factor in model selection. Nonhuman primates occupy an exceptional position in the hierarchy of animal models due to their close relatedness to humans. As described in earlier chapters, both physical and cognitive needs must be met in order to guarantee appropriate use. Nonhuman primates are recognized as having elevated cognitive requirements in addition to standard physiological needs. Strict minimum housing space requirements as well as dietary and environmental regulations are in place through the Animal Welfare Act and subsequent amendments as well as the Guide for the Care and Use of Laboratory Animals.[7,8] These requirements are enforced through an extensive network of federal, state, and local regulations, scheduled and unscheduled site visits, and oversight by local Institutional Animal Care and Use Committees (IACUC). Use of nonhuman primates, as well as other commonly used laboratory animal species, requires prior justification and approval by the appropriate IACUC to safeguard the appropriate care and use of every animal.

As outlined in other chapters, the selection process utilized to evaluate the appropriateness of nonhuman primates as a model system involves a variety of factors. Of primary importance is the ability of the specific model system to address the biological question posed for each specific investigation. Nonhuman primates typically provide information very relevant to human application and therefore are highly recommended. Other factors, however, must also be considered in the process. If the biological question

Table 28–1
Nonhuman primate species used in biomedical studies

Species name	Common name	Applications
Macaca nemestrina	Pigtail macaque	AIDS, reproduction, growth and development, behavior, neuroscience
Macaca mulatta	Rhesus macaque	AIDS, reproduction, growth and development, behavior, neuroscience
Macaca fascicularis	Long-tailed crab-eating macaque, cynomolgus	Infectious disease (AIDS, SARS), neuroscience
Papio anubis	Olive baboon	Reproduction, neuroscience, hematology, transplantation
Papio cynocephalus	Yellow baboon	Reproduction, neuroscience, hematology, transplantation
Cercocebus atys	Sooty mangabey	AIDS, leprosy, reproduction
Chlorocebus sabaeus	African green monkey	AIDS, neuroscience
Chlorocebus aethiops	Vervet	AIDS, neuroscience
Sanquinus oedipus	Cotton-top tamarin	Enteric disease, colitis, colorectal cancer
Callithirx jacchus	Common marmoset	Reproduction, endocrinology, vision, behavior
Aotus vociferans	Owl monkey	Vaccines (dengue, malaria), behavior, endocrinology, vision
Aotus nancymaae	Night monkey	Vaccines (dengue, malaria), behavior, endocrinology, vision
Saimiri sciureus	Squirrel monkey	Malaria, neuroscience
Pan (Homo) troglodytes	Common chimpanzee	Infectious disease (AIDS, hepatitis C), behavior

can be addressed through the use of lower species or nonanimal resources, current guidelines require use of these alternative means. In addition, issues such as housing requirements, species availability, and price can be important factors for consideration in the animal model selection process.

The relevancy of information developed in nonhuman primate models is well documented (see Table 28–1). Information developed in nonhuman primate models is critical to the design of subsequent human studies and the evaluation of new medical interventions into human health care. This correlation between human and nonhuman primate studies has application for both positive and negative results. Preliminary studies in nonhuman primate models of new lentiviral vaccines have closely mirrored subsequent results developed in human clinical studies.[9–16] Similarly, results in gene transfer studies have been generally disappointing in both human and nonhuman primates, reinforcing the similarity in response.

INFECTIOUS DISEASE

Nonhuman primates have been a critical resource in biomedical investigations related to infectious disease.[2,5,6] Based on the physiological similarities between species, nonhuman primates and humans share susceptibility to a wide variety of pathogens.[2,5,6] In addition, the balance of the host–pathogen relationship is largely dependent on both innate and adaptive immune responses, both of which are founded in the expression of the genetic code. Nonhuman primates possess a striking similarity to humans, a fact that has formed the basis for development of an extensive range of nonhuman primate models focused on transmission, pathogenesis, therapeutic intervention, and vaccine development for a variety of infectious pathogens.[2,5,6,9–24]

Macaque species are the most commonly utilized nonhuman primate species with the majority of public-funded studies directed toward infectious diseases. Macaques have a history of critical contributions to health concerns including the extensive utilization of rhesus monkeys (*Macaca mulatta*) for the development of the polio vaccine.[24] More recently, extensive use of a variety of species including, but not limited to, *M. mulatta*, *Macaca fascicularis*, and *Macaca nemestrina* has been closely integrated into the research on AIDS on a national and international basis.[10–16] Haigwood *et al.* have provided an excellent overview of this effort in Chapter 58 (this volume).

Of particular note is the expected utilization of nonhuman primate species for research related to "biodefense agents."[25] These agents have been identified by the Centers for Disease Control (CDC) and National Institutes of Health (NIH) as having potential use against the public health. To mitigate this potential, significant resources have been directed toward development of effective means for diagnosis, vaccination, and therapeutic intervention. It is highly likely that macaques will play a pivotal role in the development of these capabilities. This effort will require development and refinement of new nonhuman primate models to provide the necessary information required prior to implementation of human studies. This area of research emphasizes the highly relevant application of nonhuman primates as a precursor and substitute for human clinical studies. Serious ethical considerations accompany the concept of challenging humans with potentially deadly pathogens as part of the development process. Nonhuman primates offer the opportunity to evaluate the safety and efficacy of new vaccines and therapeutic agents with a high degree of confidence, yet without endangering human subjects.

New world nonhuman primate models, although less heavily utilized on a national basis, still provide critical contributions for specific diseases. From an international perspective, malaria is one of the most prevalent and devastating diseases, with a disproportionate impact in developing areas.[26–28] Development of effective malaria vaccines and therapeutics is a high priority for the national and international health care community.[26–30] *Saimiri* and *Aotus* species have proven to be excellent models for these investigations, contributing significant information on transmission, pathogenesis, and therapeutic and vaccine interventions.[22–24] Although availability and maintenance of these species are limiting factors, they will continue to be the most effective models for future translational research.

NEUROSCIENCE

Nonhuman primates have made significant contributions to neuroscience research through utilization of a variety of models over several decades. Nonhuman primates share more anatomic and physiological similarities to humans than any other species, and these factors have been used to provide important advances into the structure and function of the human brain.[31–35]

Nonhuman primates offer several advantages over other animals models, making them a unique resource for neurological investigation. The relatively close behavioral and cognitive abilities of nonhuman primates provide the opportunity to study the anatomic and physiological basis for these characteristics, a capability absent in lower species. In addition, nonhuman primates can be trained to perform a broad range of tasks, allowing development of models designed to investigate very specific neurological activities.[36–38]

As a correlate, many human neurological and cognitive tests have been adapted to nonhuman primates, allowing investigation of developmental aspects of cognition.[39–47] Developmental testing of reflex development, visual acuity, recognition memory, motor development, object concept development, and social interactions has been adapted to evaluate macaque species. These provide exceptional investigative tools and have been effectively utilized to identify important factors affecting cognitive and motor development.

A variety of nonhuman primate models have been developed to explore a broad spectrum of neurological structure and function. Extensive developmental work over several decades has resulted in well-characterized neurophysiological recording models allowing ongoing, active electrical recording from individual neurons within specific neurological networks. Characterization of interrelated neurophysiological function between specific neuronal groups has led to greater understanding of function within both motor and visual systems.[48–51]

Advances in imaging technology have provided exceptional opportunities for investigation of neurological function in nonhuman primate models. A wide variety of imaging modalities have been applied to nonhuman primate models including computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). These noninvasive techniques offer the opportunity to perform multiple, detailed interrogations of specific neurological, anatomic, and physiological functions without physical disruption of neurological tissues. Additionally, the tractability of nonhuman primates has allowed awake models of neurological function using advanced imaging techniques including fMRI and fPET. These models provide a unique opportunity to investigate higher cognitive functions in species with a high degree of correlation to humans.

FUNCTIONAL GENOMICS

Perhaps the most important similarity between humans and nonhuman primates lies in the relative similarity of the genetic code.[52] In fact, the genomic similarities form the foundation for the physiological, morphological, and phenotypic likeness between species. Similarly, it appears that relatively small differences in either code or expression provide the basis for differences between species.[53–55] Investigation of the genetic code potentially provides an incredible resource for information across a wide spectrum of issues including evolution, cancer, basic physiology, immunology, cognition, and development.

Development of essential investigative resources for genomic studies has proceeded relatively quickly. Taking advantage of the research infrastructure established for sequencing the human genome, subsequent sequencing projects have provided initial full-length sequences for a number of nonhuman primate species including the chimpanzee, bonobo, and rhesus macaque, with sequencing projects underway for additional species.[54,55] Additionally, more limited information is available from a variety of other nonhuman primate species. Consistent with founding principles of the Genome Project, these data and others are available in public reference sites such as GenBank.[56] Microarray chips developed by both Agilent Technologies and Affymetrix have recently become available allowing directed investigation of specific genomic responses across a variety of biological studies. Advances in bioinformatics software capable of distilling complex microarray information into biologically relevant mechanistic pathways have provided a critical link between genome and phenotypic response. Taken together, these resources provide an exceptional opportunity to apply novel, highly sophisticated research methods to important biological issues.

Application of these research resources is rapidly increasing across a variety of biological fields. Evolutionary studies have derived significant benefit through comparative genomic studies of humans and nonhuman primates. Current genomic capabilities allow comparative studies of specific genetic composition and expression, providing important insights on evolutionary pressures underlying the relative divergence or convergence of primate species. In addition, genomic studies can provide significant comparative data for other investigations based on mitochondrial DNA and Y-chromosomal sequencing. Comparative genomic analyses provide the potential for extremely powerful genetic investigations defining the genetic basis for the morphological and phenotypic differences between species.

Infectious disease investigation is likely to be a primary beneficiary of genomic research capabilities. Both innate and adaptive immunity are critical components of the host–pathogen interaction. Recent microarray studies have clearly highlighted the vigorous host inflammatory response in animal models of pathogenic influenza infection.[57–59] It is clear that a more complete understanding of the host response to infection will provide critical information for the development of new generations of therapies and vaccines. As an example, the development of an effective AIDS vaccine has proven to be an exceptional challenge despite more than two decades of intense research. As yet, significant questions remain concerning the transmission events, viral pathogenesis, means of obstructing viral replication, and correlates of protection necessary for effective vaccination. Functional genomic analysis in appropriate nonhuman primate models has the potential to provide important evidence for the resolution of these issues.

In addition to functional genomics, proteomic studies in nonhuman primates have the potential to provide a key link between genome and phenotype. In that the proteome is the sum of expressed proteins based on the genetic composition of the organism and the current environment, the proteome is a critical link in achieving a more complete understanding of the ongoing relationship between genome, proteins, and function. Investigative tools are currently under development and promise to provide

information necessary to fully characterize a wide variety of physiological responses. Efforts are currently underway to provide complementary experimental designs allowing for simultaneous investigation of genomic response, protein expression, and physiological phenotype in nonhuman primate model of infectious disease.[60,61]

HOUSING CONSIDERATIONS

Maintenance of nonhuman primates in laboratory environments presents significant challenges. A considerable commitment in expertise, resources, and effort is required to ensure appropriate care and support for nonhuman primates in research facilities. While outdoors, free-ranging native housing environments might be an ideal for nonhuman primate housing, it is likely that research protocol constraints, existing facilities, and geographic location would render this a nonviable option for most research institutions. Instead, current housing environments are designed to meet the environmental, nutritional, and psychological needs of each animal, allowing individual animals to express species-specific normative behaviors, albeit in a different setting.

While many components of an effective animal care program are similar among species used in biomedical research, nonhuman primate care programs typically must provide additional emphasis on provision for the psychological health of housed animals.[62] Specific elements of a comprehensive animal care program for nonhuman primates include effective health surveillance and veterinary care, husbandry, psychological enrichment/well-being, management of breeding activities, and oversight for research activities. Appropriate documentation of animal-related activities is a critical component of effective management as well as being necessary for regulatory compliance.

In particular, nonhuman primates have special requirements for environmental enrichment, social housing, and mitigation of experimental impacts, both physiological and cognitive. The perceptive and cognitive characteristics of nonhuman primates impose additional responsibility concerning their care and use in biomedical research. Behavioral assessment of nonhuman primates is considered an essential component of health and well-being monitoring programs. Assessment of behavior is considered an indicator of the psychological state of nonhuman primates, with the implication that abnormal behavior may be an outward manifestation of physiological or psychological stress.[63] Novak and Suomi[64] have provided recommendations for the assessment of nonhuman primate well-being, although additional measures may be considered.

The need for positive social interaction between conspecifics is recognized as a critical requirement for the psychological well-being of nonhuman primates. In natural settings, nonhuman primates are generally highly social animals maintaining a range of social relationships. These social interactions are influenced by age, sex, parentage, social standing, as well as a variety of other factors. Social housing of conspecific nonhuman primates is the accepted standard housing configuration unless precluded by specific research or medical or behavioral constraints.[62]

Social housing can take many forms and spans a spectrum of housing configurations. Basic elements of social housing include access to sensory cues from other nonhuman primates including sight, smell, hearing, and touch. The standard cage design for nonhuman primates typically employs spaced bars that restrict the animals to a desired space but also allow extensive interaction with environmental visual, olfactory, and auditory cues.

The highest level of social interaction is achieved through direct physical contact between conspecifics. Group housing configurations such as harem groups, family units, single-sex cohorts, age-matched cohorts, pair housing, or limited contact housing (grooming bars)[65] have all been utilized to provide effective socialization of nonhuman primates. Although a detailed examination of each housing configuration is beyond the scope of this chapter, each has the potential to provide the opportunity for species-specific behavioral interactions, thereby increasing environmental enrichment. Care must be taken, however, in the selection process for social housing partners. Significant differences in the success of social housing configurations can occur based on age, sex, and relatedness of individual animals. While direct social housing has the potential to provide significant environmental enrichment, unsuccessful social interactions can result in significant psychological and physical trauma, including death. Additionally, social interactions can precipitously change from stable, positive physical interactions to aggressive, potentially traumatic interactions without identification of a precipitating cause. Experience and ongoing close observation of socially housed nonhuman primates is necessary to establish and maintain positive social interactions.

Maintenance of health care for nonhuman primates is central to an effective animal care program. This point is substantiated in the Animal Welfare Act through placement of independent judgment and final decision authority in the Institutional Veterinarian. An effective health care program has several components including well-defined programs for preventive health care, health surveillance, and treatment of health issues. These components must be effectively combined into a seamless program that not only meets the spontaneous health needs of each animal but actively intercedes to avoid potential health care issues. Specific components are (1) preventive health care, including quarantine, isolation, annual physical examination, dentistry, and vaccination when appropriate; (2) health surveillance, including viral testing, microbiological testing, daily observations, behavioral assessment, and weight monitoring; and (3) treatment/veterinary care, including veterinary care and surgery support, treatment of spontaneous disease, and treatment of health issues associated with research activities.

CHARACTERIZATION

As the complexity of biomedical research increases, so too does the demand for better characterization of the nonhuman primate resource. Increasing sophistication of scientific investigation requires a more thorough knowledge of potentially confounding factors, including the health status and genetic profile of the experimental animals. Most recently, AIDS-related research in nonhuman primate models has provided the overriding impetus to characterize and segregate animals based on health status and genotype.[66–69] The heavy reliance on normal immune function in AIDS-related research studies requires well-characterized research animals capable of exhibiting a normal immune response to viral infection or vaccination.

Both ethical and financial considerations provide additional motivation for characterization of individual animals prior to recruitment into research projects. Ethical guidelines require the use of minimum numbers of animals to meet experimental objec-

tives, typically the demonstration of the experimental hypothesis with statistically significant results.[70] Biostatistical consultation during the study design is a common part of the protocol development process. Additionally, nonhuman primate studies require a significant financial commitment for purchase and appropriate maintenance of experimental animals. These factors combine to produce an experimental design that includes the minimum number of animals necessary to produce statistically significant results. The dropout of individual animals due to unknown pathogen status can be the difference between experimental results that are statistically significant and results that are merely suggestive. Therefore, significant effort is directed toward the designation of specific health status prior to inclusion in research projects.

In addition to research requirements, significant motivation exists to eliminate agents with the potential for zoonotic transmission. Investigative and animal care staff typically work in close proximity to housed animals, providing the potential for transmission events between species. Several nonhuman primate pathogens have the potential to induce significant morbidity or mortality when transmitted into human hosts.[71–74] Although rare, a number of documented incidents of mortality associated with zoonotic transmission of herpes B virus from nonhuman primates to human hosts are available in the literature. Based on this potential, many facilities now require documented negative status of nonhuman primates for several infectious agents.

Genetic characterization of nonhuman primates has proven to be an important factor in studies involving immune response to infectious agents. Characterization of the MHC Class I alleles in the rhesus monkey has revealed a pattern of differential response to lentiviral infection based on the presence or absence of specific alleles.[66,67] It is therefore important to characterize the genetic composition of study animals prior to study onset either to avoid undesirable and unintended impacts to the study results or to provide important information for the interpretation of the results. As an important correlate, identification of these alleles may provide valuable information regarding relative susceptibility or resistance to disease progression following lentivirus infection.

Genetic characterization can extend beyond individual differences to important differences between subspecies. At one point in time, rhesus monkeys from either Indian or Chinese origin were largely considered interchangeable for infectious disease studies. Detailed comparative studies have revealed, however, that although both species appear to be equally susceptible to lentiviral infection, a significant difference exists in their ability to support ongoing, long-term viral replication.[75] Current recommendations therefore include segregation of subspecies for these studies, or equal distribution in experimental groups to avoid spurious distortion of experimental results.

Of note is the historically imprecise confirmation of the genetic origin of many domestic breeding colonies. Breeding records for many domestic nonhuman primate breeding colonies have depended on imprecise observational methods for documentation of parentage. Long-term productive management of breeding colonies requires accurate, ongoing genetic characterization to avoid undesirable inbreeding and to confirm the genotype for individual animals. Recent advances in genetic sequencing have provided more precise methods for confirmation of parentage and genetic origin. Techniques such as single nucleotide polymorphisms (SNPs) and microsatellite assays have made it possible to determine with relative certainty the parentage and general genetic stock and subspecies of individual animals.[76–79]

SPECIFIC PATHOGEN FREE

The characterization of the health status of nonhuman primates is critically important for a wide variety of experimental applications. In particular, studies involving exposure to infectious diseases or characterization of immunological response have the potential for unintended distortion due to coinfection with spurious pathogens. To avoid this potential confounding effect, significant efforts have been directed toward the development and characterization of nonhuman primates guaranteed negative for specific pathogens, designated specific pathogen free (SPF).

The SPF designation indicates negative status for one or more infectious agents. Although the SPF designation can technically be used for any pathogen, standard use refers to viral pathogens with a negative status for *Mycobacterium tuberculosis* assumed. Several different levels of SPF status exist; however, the most common designation refers to animals negative for simian retrovirus (SRV), simian T-lymphotrophic virus (STLV), simian immunodeficiency virus (SIV), and herpes B virus (B virus, CHV-1). This SPF designation has been established by the National Center for Research Resources (NCRR) and the Office of AIDS Research (OAR) through the development and support of SPF macaque colonies for AIDS-related research. This designation reflects the need to have animals free of indigenous retroviruses potentially capable of skewing immune function while at the same time providing protection for investigative and husbandry staffs against potential exposure to a significant zoonotic agent, herpes B virus.

Effective maintenance of SPF status requires ongoing surveillance, typically provided by strict viral testing programs. SPF status can be documented through a variety of techniques; however, several constraints exist for use of laboratory assays for colony surveillance screening. Sensitivity and specificity are key parameters for any laboratory assay and each should be quantitated and confirmed through a rigorous, ongoing quality control program. Additionally, colony surveillance assays must be relatively easy to perform and must be cost effective for efficient inclusion in a colony management program.

Under these considerations, a spectrum of screening tests has been developed to survey the viral status of macaques in breeding and research colonies. Serology is an industry standard with enzyme-linked immunosorbent assay (ELISA) and, more recently, multiplexed bead assays[80–86] utilized to identify antibody response to previous viral exposure. In addition, standard confirmatory tests such as Western blot, polymerase chain reaction (PCR), and virus isolation are utilized to confirm positive tests and resolve indeterminate results. While some institutions maintain proprietary diagnostic laboratories, commercial laboratories are available for support of viral surveillance programs.

Management of SPF colonies depends heavily on the surveillance of colony viral status. Typical management strategies include the means to test all animals with removal of individual animals testing positive or indeterminate for specified pathogens. Management protocols are available in the literature for the derivation of SPF nonhuman primate colonies from non-SPF founder stock as well as expansion of current SPF colonies.[87] These protocols rely heavily on rigorous adherence to surveillance testing schedules with exclusion of animals from the SPF colony until negative

pathogen status is confirmed. Subsequent monitoring of the colony is maintained on a continuing basis to ensure identification and isolation of individual animals demonstrating conversion to positive status.

CONCLUSIONS

Nonhuman primates are a critical resource for biomedical research. The genetic, morphological, and physiological similarity to humans provides an invaluable resource for information directly applicable to both human and nonhuman primates. Although housing and maintenance of these species are challenging, well-defined programs are in place to meet the physiological and psychological needs of animals in research settings. Ongoing efforts continue to better characterize nonhuman primate genetics and pathogen status, thereby improving the application of these models to biomedical research questions. Given the current emphasis on translational research, it is highly likely that demand for these extremely relevant animal models will continue to increase in the foreseeable future.

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