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Article

Nonhuman Primates and Translational Research: Progress, Opportunities, and Challenges

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Abstract

Nonhuman primates (NHPs) are the closest animal models to humans regarding genetics, physiology and behavior. Therefore, NHPs are usually a critical component in translational research projects aimed at developing therapeutics, vaccines, devices or other interventions aimed at preventing, curing or ameliorating human disease. NHPs are often used in conjunction with other animal models, such as rodents, and results obtained using NHPs must often be used as the final criterion for establishing the potential efficacy of a pharmaceutical or vaccine before transition to human clinical trails. In some cases, NHPs may be the only relevant animal models for a particlular translational study. This issue of the *ILAR* journal brings together, in one place, articles that discuss the use of NHP models for studying human diseases that are highly prevalent and that cause extraordinary human suffering and financial and social burdens. Topics covered in detail include: tuberculosis; viral hepatitis; HIV/AIDS; neurodegenerative disorders; Substance abuse disorders; vision and prevention of blindness; disorder associated with psychosocial processes, such as anxiety, depression and loneliness; cardiovascular disease; metabolic disease, such as obesity and metabolic syndrome; respiratory disease; and female reproduction, prenatal development and women's health. Proper husbandry of NHPs that reduces stress and maintains animal health is critical for the development of NHP models. This issue of the journal includes a review of procedures for environmental enrichment, which helps assure animal health and wellbeing. Taken together, these articles provide detailed reviews of the use of NHP models for translational investigations and discuss successes, limitations, challenges and opportunities associated with this research.

Key words: animal model; animal rights; chimpanzee; genomics; macaque; monkey; nonhuman primate; translational research

Introduction

This issue of the *ILAR Journal* provides in-depth reviews of the use and value of nonhuman primates (NHPs) for translational research. These reviews discuss the use of NHPs to facilitate preclinical research related to many of the diseases that have the highest prevalence in humans and cause extraordinary amounts of human suffering and financial and social burdens. These prevalent diseases include HIV/AIDS, tuberculosis, hepatitis, neurodegenerative disease, diabetes, cardiovascular disease, respiratory disease, and defects in vision, among others.

Over the past several years, a modest number of publications have provided high-quality reviews of the use of NHPs for translational research, including papers by Capitanio and Emborg (2008), Belmonte et al. (2015), Phillips et al. (2014), and Van Rompay (2017). In 2016, a white paper published by the

National Association for Biomedical Research briefly discussed scientific breakthroughs that required the use of NHPs (National Association for Biomedical Research 2016). The website for the US National Primate Research Centers (NPRCs) includes short discussions of the use of NHPs to study several different diseases (https://www.nprcresearch.org). In response to a request by members of the US Congress, the US National Institutes of Health (NIH) convened a workshop in December of 2016 entitled, "NIH Workshop on Ensuring the Continued Responsible Oversight of Research with Nonhuman Primates." The final report can be accessed at https://www.cnprc.ucdavis.edu/wp-content/uploads/2017/01/NIH-NHP-Workshop-Report.pdf. This report contains brief comments on some aspects of the use of NHPs for translational research and concludes: "The NIH remains confident that the oversight framework for the use of nonhuman primates in research is robust and has provided sufficient protections to date." A more detailed report published by the Scientific Committee on Health, the Environment and Emerging Risks of the European Union (The SCHEER Report) discusses the same conclusion from the European perspective, and, in addition, provides information about several aspects of the use of NHPs for translational research (SCHEER 2017).

The reviews herein provide detailed information that updates and augments these papers and websites. As issue editor, I expect that these reviews will be of use to many different researchers, including clinical investigators as well as personnel in funding agencies, as would be expected. I also hope these reviews will provide critical basic information on the use of NHPs for translational research to the very large number of stakeholders, including patients, involved with these high-profile diseases and to the general public.

The Critical Role of NHPs in Translational Research

For the papers in this issue of the journal, "translational research" is defined broadly to include both efforts aimed at developing or validating NHP models that are directly relevant to studies of human disease as well as the use of NHPs in later stages of translation that help validate drug targets; evaluate the efficacy of therapies, vaccines, or devices; and test toxicity of pharmaceuticals before testing in human clinical trials. If the three-stage model of human translational research is used as a reference (Rubio et al. 2010), NHPs are used in discovery phases that directly precede formal translational research and in the T1 phase, which transfers the results of basic research to clinical research, often called "bench to bedside."

NHPs are critical for translational research and are sometimes the only relevant animal models because of their close genetic, physiological, and behavioral similarity to humans versus other animals, such as rodents. The relatively large size of NHPs can also be important for sample collection, application of diagnostic assays, and imaging (see e.g., Havel et al. 2017). In practice, as is clear from the papers in this issue, several different animal models may be used to study a specific problem in translational science. Some basic discoveries may be obtained from studies using rodents (reviewed by Zubari and Lutz 2016) and other models, such as zebrafish (*Danio rario*), fruit flies (*Drosophila melanogaster*), and nematodes (*Caenorhabditis elegans*) (reviewed by Strange (2017)). Large animal models such as dogs and swine are also used in some fields, such as oncology and transplantation medicine. However, although not always attained in practice, NHPs must usually be the animal model that is ultimately employed in the preclinical setting for evaluating efficacy and safety of a pharmaceutical, vaccine, or device prior to human clinical trials. The literature cites many instances where NHPs have not been used and in which investigators have relied only on information from other models, particularly rodents. In many cases, preclinical investigations that have avoided use of NHPs have failed, for example for development of therapies for tuberculosis (Foreman et al. 2017). Rodents may not be relevant models, because their physiology and response to infection are not sufficiently close to those of humans. In these cases, NHPs must be the primary, and perhaps only, animal model relevant to translation. Examples include studies of several visual abnormalities (Mustari 2017), development of HIV/AIDS vaccines and therapeutics (Van Rompay 2017; Veazey and Lackner 2017), and therapies for tuberculosis (Foreman et al. 2017). An extreme example of this exclusivity is the essential use of chimpanzees (*Pan troglodytes*), relative even to macaques or other monkeys, for the development of vaccines against infection by Hepatitis A and B viruses and of therapeutics to cure Hepatitis C disease (Lanford et al. 2017).

A further feature of the process of translation is that it is often cyclical. A therapy or vaccine may, in its first application to humans, provide only partial efficacy or may raise critical questions that can only be answered by going back to animal models. A particularly timely example is development of a highly efficacious vaccine for HIV/AIDS. HIV/AIDS vaccine development is one of the most important and difficult goals of modern translational medicine. Human vaccine trials based on NHP preclinical models have not demonstrated sufficient efficacy to warrant large-scale application to human populations but have provided critical information that can be used to develop more effective vaccines (Office of AIDS Research 2017; Van Rompay 2017; Veazey and Lackner 2017, and references therein). Promising approaches for developing immunogens and adjuvants must now be reapplied to NHP models to develop more efficacious vaccines that can then be retested in humans. This is a major goal of the US National Institutes of Health (NIH) and their partners' efforts to develop a vaccine with sufficient efficacy, as discussed in the NIH Office of AIDS Research Strategic Plan for fiscal year 2018 (Office of AIDS Research 2017). Another example from the field of HIV/AIDS relates to the successful development of antiviral therapy (ART) that has turned HIV infection from one that is invariably fatal to one that can be ameliorated by the use of drugs. NHPs were critical preclinical models for development of these antiviral drugs (Van Rompay 2017; Veazey and Lackner 2017). ART, however, does not completely eliminate HIV from the body; a reservoir of the virus can be reactivated if drug therapy is stopped. A major goal is to develop better drug formulations or new approaches that can sustain viral remission, also called a functional cure without ART, or eradicate virus completely, called a sterilizing or classic cure (NIH Office of AIDS Research 2017). The preclinical research essential for meeting these goals requires use of NHP preclinical models.

Role of Large Centers for NHP-based Translational Research

Translational studies using NHPs depend on the availability and characterization of animals, high-quality animal husbandry and welfare, and the development of assays and technologies. These necessary components of NHP-based research usually require housing animals in large centers, each of which can harbor more than 1000 (often 4000–5000 or more) animals because of

the expense and complexity of husbandry. NHP centers provide animals and collaborate with diverse researchers from both academic and commercial laboratories. These centers also include the laboratories of researchers that are experts in NHP-related research. NHP centers are therefore foci for developing improvements in experimental techniques, animal welfare, and breeding in addition to their function as suppliers of animals. Large nonprofit NHP centers in the United States include the seven US National Primate Research Centers (NPRCs, https://www.nprcresearch.org) and other facilities, such as the New Iberia Research Center (http://nirc.louisiana.edu/), The Michale E. Keeling Center for Comparative Medicine and Research (https://www.mdanderson.org/research/departments-labs-institutes/programs-centers/michale-e-keeling-center-for-comparative-medicine-and-research.html), the Caribbean Primate Research Center (http://cprc.rcm.upr.edu/), and the Wake Forest University Primate Center (http://www.wakehealth.edu/ccmr/). These nonprofit centers seldom import animals. Domestic breeding colonies of animals, originally of Asian origin, such as rhesus (*Macaca mulata*), pigtail (*Macaca nemestrina*), and cynomolgus (*Macaca fascicularis*) macaques, and NHPs of African origin, baboons (*Papio* sp.) and vervets (*Chlorocebus aethiops*), are the most common animals in these colonies. Smaller colonies of the new world marmoset (*Callithrix jaccus*) are also housed at the NPRCs. For-profit centers (Contract Research Organizations (CROs)) also are a major source of animals for preclinical research. CROs may both breed and import animals, where importation is possible. Animals provided by CROs are often used by pharmaceutical companies for toxicology testing and for some preclinical research.

For many years, the United States has been the center of gravity for breeding and characterizing NHPs for preclinical research, with some breeding in Europe. The development of breeding and technology centers in China and Japan is a more recent phenomenon that is impacting translational research (Cyranoski 2014, 2016). These nations have made major efforts to increase the supply of NHPs available to researchers in their domestic institutions and to enhance infrastructure to breed animals and perform large scale investigations, including genomic analysis (see Harding 2017 for selected references) and development of new NHP disease models using Assisted Reproductive Technologies (see, e.g., Chen et al. 2016; Ke et al. 2016; Sasaki 2015; Sasaki et al. 2009). China has also increased its capacity to perform contract research that can be outsourced to pharmaceutical companies (Xia and Gautam 2015). Investigators in all parts of the world, including the United States, Europe, and Asia, would greatly benefit from reviews that summarize the capabilities of these Asian centers in more detail. In addition, the rigor of the requirements for animal protection (for example for cage sizes) and welfare has, in my opinion, not been described in sufficient detail for these Asian centers. The field of NHP-based translational research will benefit from a direct comparison of requirements for animal welfare across centers in all countries that supply NHPs for translational investigations.

Governmental and Institutional Regulation of NHP Research

Research using all vertebrate animals, including NHPs, and the characteristics of the facilities that house and breed animals are regulated in North America (Griffin and Locke 2016; Tardif et al. 2013; Vasbinder and Locke 2016), the European Union (Olsson et al. 2016; SCHEER 2017), and some Asian countries, such as China, Japan, and Korea (Ogden et al. 2016). In the United States, primary roles for regulating NHP husbandry, breeding, and use is performed by the US Department of Agriculture and the NIH Office of Laboratory Animal Welfare. Local Institutional Animal Care and Use Committees must approve any procedure at a given institution utilizing laboratory animals, including NHPs (Tardif et al. 2013). This combination of local control of specific protocols and country-wide governmental regulation provides codified, publicly available regulations, such that a given Center or Laboratory knows exactly how it must house and use NHPs for research. Similar regulations and mechanisms exist in Europe (for reviews, see Bayne and Morris 2012; Olsson et al. 2016; SCHEER, 2017).

Challenges and Opportunities

Given the complexity of translational research and the fact that any animal model cannot totally reproduce the human condition, there are many challenges and opportunities when NHPs are used for translation. As stated by Capitanio (2017), "A model is, by definition, a simplified representation of a phenomenon, and the very process of simplification means that some of the complex reality is lost." Furthermore, use of NHPs versus other models such as rodents is complicated by the many human-like characteristics of NHPs, leading to questions from researchers, stakeholders, and the public about their use for research. The articles in this issue of the *ILAR Journal* discuss many of these challenges and opportunities, some of which are noted briefly, below.

Reproducibility

In the past several years, there has been considerable discussion regarding the reproducibility of animal studies and therefore the utility of animals for translational research. Much of this discussion is centered around the use of rodents, not NHPs, and is based, in part, on two observations: First, data obtained from animal models obtained by academic laboratories and their industrial collaborators, published in highly regarded peer-reviewed journals, often cannot be reproduced by development teams within pharmaceutical companies. As reported by company scientists, estimates of failure rate range from 89% (Begley and Ellis 2012) to 75–80% (Prinz et al. 2011). Second, once drugs have been developed by the companies and their collaborators, Phase II and Phase III human clinical trials can have failure rates of 50% or more (Hay et al. 2014; other examples reviewed by Hewitt et al. 2017). Failure of Phase II and III clinical trials can reflect lack of efficacy, safety concerns, or both (Arrowsmith and Miller 2013). As discussed by Hewitt et al. (2017), lack of rigor in preclinical animal studies, including basic aspects of reproducibility such as blinding, sample sizes, and use of both males and females, contribute to lack of reproducibility. These conclusions regarding rigor appear mainly to be based on studies using rodents. I am not aware of any publication that has examined the comparative rigor with which studies using different species (for example, rodents versus NHPs or other large animals) have been performed. The point for the current review is not that problems related to reproducibility of animal studies exist, but that dealing with systemic issues such as sample size and blinding can be a major issue for translational studies using NHPs, as it is for rodents.

The rigor with which experimental animals are described in publications also influences the reproducibility of animal-based translational studies. The US National Research Council Institute for Laboratory Animal Research and the National Center for the Replacement, Refinement and Reduction of

Animals in Research in the United Kingdom have provided guidance to journal editors, authors, and reviewers regarding information that should be included in publications relating to experimental animals (National Research Council 2011; Kilkenny et al. 2010). Information includes, among others, the animals' age and sex, source of supply, genetic constitution, pathogen status, diet, housing, and others. These data are now expected for publications that describe rodent-based experiments.

In the context of NHP-based studies, it is important to note that centers such as the NPRCs maintain very detailed computerized animal records that include many of these data. Although it would be impractical to publish the entire animal record for each experimental subject, these records contain a great deal of information that could be mined by qualified investigators to aid in the design of experiments and to form the basis of retrospective studies that could include animals or tissues derived from them from several different centers and investigations.

There is increasing realization that variation in genetics or other factors such as the social interactions of individual rhesus or cynomolgus macaques can influence experimental results. The well-established difference in the response of Indian- versus Chinese-origin rhesus macaques to infection by simian immunodeficiency virus is a graphic example of this phenomenon (Harding 2017 and references therein; Van Rompay 2017). A second example is the varied stress that is experienced by macaques that are in different places in the dominance hierarchies within the colonies that are the basis of group housing of these animals. The physiological response to stress can differentially affect the animals' reaction to a given experimental intervention (Capitanio 2017). In summary, more detailed reporting of the characteristics of NHPs used in experiments can improve reproducibility and can lead to new avenues of investigation.

In response to the general problem of reproducibility of both basic and preclinical research using animals, the NIH has recently published new requirements for grant applications, which will be part of peer-review and programmatic consideration (Hewitt et al. 2017 and references therein). These new requirements will be challenging for investigators proposing NHP-based research and for peer reviewers, in part because of the expense associated with each animal in an NHP-based study and the potential difficulty of obtaining adequate numbers of females, neonates, or juveniles. These classes of animals are needed to keep breeding colonies at optimal levels to support the number and diversity of NHP-based translational studies and thus can be in short supply for investigations. It will be important for the NHP research community to have a discussion with NIH to fully understand expectations for NHP-based grant proposals.

Funding agencies, in collaboration with investigators, may need to find mechanisms to increase support for NHP-related investigations that require larger numbers of certain types of animals, such as females. Among other approaches, strategies such as pooling animals and resources from a number of centers for a single NHP-based study lead by multiple, collaborating investigators should be considered. This type of multicenter approach is often used for clinical studies by the medical community (Llovera and Liesz 2016 and references therein) and by the human genomics community for large-scale investigations of large human disease cohorts, using genome-wide association studies (Manolio 2013, 2017). The NPRCs and the other large US NHP Centers are a logical choice to champion dialog regarding approaches to multi-center studies related to NHPs and translational research.

One response by some researchers and funding agencies aimed at increasing the reproducibility and decreasing the cost of translational studies has been to develop alternatives to the use of whole animals, such as cell lines, artificial tissue arrays ("tissues on a chip"), in silico modeling, and micro-dosing of pharmaceuticals in human patients and volunteer controls. These concepts are in quite early stages of development, and their eventual contribution to translational research is uncertain (see, e.g., a discussion in the SCHEER Report, 2017). Details of these alternative approaches are outside the purview of this review. The purpose of mentioning them is to acknowledge that there are potential alternatives to NHPs and other animal models. Parallel and coordinated use of whole animals with development of alternative technologies provides an opportunity to validate or abandon alternatives and to enlarge the suite of tools that can be applied to a given translational problem. Combined and coordinated use of NHPs, other relevant animal models, and alternatives could enhance the ability to successfully develop pharmaceuticals with improved probability of success in human clinical trials.

Supply and Demand

Discussions of NHP-related research often cite shortages in the supply of some species, leading to high prices and long waiting times to obtain animals. This is, however, a highly nuanced issue. To my knowledge, there is actually very little hard data on this subject, aside from anecdote. A feature of NHP research is that the demand for some species waxes and wanes over time, for example for baboons and marmosets. In contrast, the demand for rhesus and cynomolgus macaques has been relatively constant over the past several years. There is little evidence at present for a shortage of macaques. Cynomolgus macaques are imported from Asia and provided to academic and industrial investigators by CROs. The rhesus macaque, which is the species most frequently used by academic investigators in the United States, can be obtained from the NPRCs, CROs, and other US centers, with reasonable waiting times. Supply problems for rhesus macaques for HIV/AIDS-related investigations have been largely alleviated since the early 1990s, when colonies of specific pathogen-free animals at the NPRCs and the Caribbean Primate Center were reestablished and supported by funding from the NIH.

The sudden emergence of a pathogen such as Zika or Ebola virus can cause problems related to supply and demand, since rapid response to these infections often requires use of specific types of animals in excess of plans in place at the time. For example, very shortly after Zika virus was identified as a teratogen in human populations, the demand for female NHPs, such as rhesus macaques, increased. However, despite a relative lack of supply, investigators were able to develop a macaque-based model to help understand pathogenesis (Dudley et al. 2016), using small numbers of females. This effort was accompanied by an innovative use of the internet to report the details of experiments in real time before publication, as an aid to other investigators. One solution that has been proposed to meet acute, high-demand situations is to maintain modestly sized "reserve" colonies of macaques that can be used specifically to deal with sudden increases in demand due to emerging pathogens. However, this concept has gained little traction with funding agencies.

One species for which a supply-demand problem does potentially exist at present in the United States is the marmoset, which may be particularly useful for neurobiological

studies and for development of new NHP disease models using genetic engineering and assisted reproductive technologies (Belmonte et al. 2015). This contrasts with the situation in Japan, where the marmoset has served as the basis of extensive efforts to develop transgenic animals and appears to be in relatively plentiful supply for domestic researchers (Cyranoski 2014). Supplies of marmosets can be increased rapidly relative to macaques, because of the relatively short generation time of marmosets and the fact that pregnancies usually result in twins rather than singletons. However, there are always difficulties in quickly securing additional support from funding agencies to increase breeding of any given species, since most funding is already committed to support existing breeding colonies and on-going investigations. The ability of primate centers to quickly respond to relatively sudden increases in demand remains an unsolved problem.

Transportation of Animals

The ability to transport NHPs by air has changed dramatically in the past several years. Commercial airlines that once transported NHPs no longer do so. Problems related to transportation can therefore be troublesome for researchers. There is no good solution to this problem, which is outside the control of the end users of the animals. This phenomenon, however, is also highly nuanced. Reduced ability to transport animals by air has partially inhibited the ability to move NHPs from one center to another, and for US academic researchers, has inhibited transfer of animals from one continent to another. In some cases, animals can be moved to the continental United States from offshore sites by charter companies. CROs still have the ability to move animals from Asia to the United States. Therefore, changes in transportation policies by the major airlines have been inhibitory, but not disastrous.

Within the continental United States, most species of NHPs can be moved very safely by truck. An example is the transfer of macaques and marmosets from the New England NPRC to four other NPRCs when the New England Center closed in 2015. Approximately 2000 animals were transferred, without loss of a single animal; specific pathogen-free animals remained free of viruses. Productive breeding colonies were rapidly reestablished at the recipient NPRCs, suggesting a relative lack of stress on macaques and marmosets caused by movement among centers. Chimpanzees have also been moved by truck from various centers to the Chimp Haven sanctuary within the United States, as mandated by the NIH (Reardon 2015), following a complex review process that involved, among others, the US National Academy of Medicine and the NIH Council of Councils and Director's Office with input from researchers and stakeholders. In regard to transportation, a question still remains as to whether some chimpanzees experienced stress that can affect their longevity at the Chimp Haven sanctuary.

Public Support for NHP-based Research

I will confine this discussion to the United States, although similar considerations also obtain in Europe. There is a distinct disconnect between the very high level of support of the pubic for medical research aimed at ameliorating disease and the discomfort of many with the animal-based research that is essential for development of pharmaceuticals and vaccines. Highly visible organizations, such as the Humane Society of the United States (http://www.humanesociety.org/) and the New England Anti-Vivisection Society (http://www.neavs.org/), called here, for shorthand, "animal rights organizations," actively oppose animal-based research. For examples, New England Anti-Vivisection Society states on its website that it is "dedicated to ending the use of animals in research, testing, and science education." These societies are well funded and skilled at communicating to the public. Furthermore, major users of research animals, such as pharmaceutical companies and universities, are often reticent to acknowledge use of animals and therefore are limited in their ability to defend investigations that require them. Exceptions to this are the NPRCs and other US centers that have publicly available websites that actively promote use of NHPs for translational research (https://nprcresearch.org/primate/). Furthermore, there are a few organizations, such as the Foundation for Biomedical Research (https://fbresearch.org/), that seek to inform the public about medical breakthroughs that have required the use of animals. In practice, however, this is an unequal struggle. The animal rights organizations are more successful at molding public opinion than are the research organizations that use animals.

Significantly, animal rights activity appears to be aimed at influencing members of the US Congress or the executive branch, who have direct influence over the budgets that are provided by the US government to the funding agencies. Recent examples include the influence of the animal rights organizations on the NIH decision to prohibit research using chimpanzees (Reardon 2015, see also commentary in this issue by Veazey and Lackner 2017 and Lanford et al. 2017) and the mandate by a small number of US Congress members in 2016 for the NIH to review its policies on the oversight of NHP research (Grimm 2016; see the NIH final report at https://www.cnprc.ucdavis.edu/wp-content/uploads/2017/01/NIH-NHP-Workshop-Report.pdf, which reaffirmed the importance of NHP research). As this article was being written, the US Food and Drug Administration temporarily suspended research aimed at understanding aspects of nicotine addiction using squirrel monkeys, apparently based on a direct request of the primatologist, Dr. Jane Goodall, to the FDA Commissioner (McGinley 2017). A major challenge for the research community is to find means to communicate with the public and the US Congress and executive branch more clearly and decisively regarding the importance of the animal-based investigations that are the current basis of translational research.

Technologies: Genetics and Genomics

A highly accurate and comprehensive genome sequence is important for the optimal use of any animal model for translational studies. Fairly complete draft-level genomic sequences have been published for rhesus and cynomolgus macaques, vervets, and marmosets. The baboon sequence, while not yet published, is available from public databases (reviewed in Harding 2017). After an initial sequence is obtained, usually from one or two animals, refinement of the sequence assembly is performed based on sequencing the genome and transcriptomes of many more animals. For example, Xue et al. (2016) and Bimber et al. (2017) have reported sequencing of several additional rhesus, which can be used to further refine the rhesus genome assembly and allow new inferences about rhesus genome organization and potential for identifying disease genes in captive populations.

In brief, the whole genome sequence or the exomic sequence (expressed genes) of an NHP can be used for the following (for a more detailed discussion, see Harding 2013, 2017 and references

therein; Bimber et al. 2016): (1) characterization of gene content and organization of the experimental animal species in comparison to humans, to understand the potential use of an animal model to study a specific human disease or genetic condition; (2) analysis of genetic variation in individual animals to identify potential mutant NHPs that can be developed as models of specific human diseases; (3) development of genome arrays that can be used for mapping studies that identify potential disease genes, or more generally, for identifying Quantitative Trait Loci, genomic regions that contain disease genes (Cox 2013); (4) development of arrays or other assays that can be used to stratify the population of animals to be used as experimental subjects to enhance the accuracy of preclinical studies and to reduce animal numbers needed for translational studies. Arrays have also been developed to aid in aspects of colony management, such as testing for parentage or geographic origin (Kanthaswamy et al. 2014, reviewed in Harding 2017). (5) Design of primers for assays that utilize the polymerase chain reaction (PCR) and for genetic modification using CRISPR/Cas9 or other techniques for developing genetically modifying animals. There are many examples of all of these uses of NHP genomic sequences in the literature, and I expect that the use of genomic sequences will increase markedly in the future.

Genomic and exomic sequences of NHPs can now be obtained readily, at moderate, but still significant, cost. The ability to readily sequence individual animals has prompted the champions of this technology to suggest large-scale sequencing of many more animals, particularly rhesus at the NPRCs (Bimber et al. 2016, 2017; Cornish et al. 2016). This could facilitate, for example, identification of potentially deleterious gene variants present in the heterozygous state that can be developed into homozygous animal models of specific human diseases, particularly for Mendelian (single gene) diseases. Proof of principal of this approach has been reported by Cornish et al. (2016).

There are, however, several challenges involved with the application of large-scale genomic sequencing to develop new NHP models for translational medicine. The cost of genomic sequencing is, at best, approximately $2000 per animal, not including the significant personnel costs necessary to analyze the data (Bimber et al. 2016). Cost must probably be decreased further to allow for whole genome sequencing of large numbers of animals. Alternatively, less expensive and less comprehensive approaches can be used, such as exomic sequencing or genotyping-by-sequencing (Bimber et al. 2016). These approaches, whether whole genome sequencing or alternatives, are likely to primarily identify Mendelian (single gene) mutations but are less likely to identify the great majority of genetic variants that contribute to complex diseases, such as cardiovascular conditions, which are caused by several genes (each of which contributes a low probability of risk to disease). Alternatively, NHP whole genome sequence data could potentially be used to develop tools analogous to the human HapMap (International HapMap consortium 2007), which has been used to identify human genes involved in complex diseases (reviewed by Manolio 2013, 2017). There are currently no plans to develop an NHP version of the human HapMap. Mapping, rather than sequencing per se, has been used successfully to identify Quantitative Trait Loci in baboons that contribute to complex diseases (Cox et al. 2013, 2017).

Even when a variant animal is identified, there may be no phenotypic assay for the potential disease condition in the NHP. Thus, there must be a parallel effort to accomplish more sophisticated NHP phenotyping in large numbers of animals. Efforts aimed at more extensive phenotyping of NHPs will be facilitated by the existing capability for imaging and behavioral testing already present at the NPRCs and elsewhere in the research community.

NHP sequencing efforts currently are being pursued in multiple laboratories, but, in general, are not coordinated among laboratories. The NHP translational community would benefit from a coordinated plan that shares animals and phenotyping data. There are currently some efforts within the NPRC consortium to identify cohorts of animals with specific phenotypes across centers. This type of coordination will, among other benefits, identify enough animals of high potential in regard to a specific disease, such that a relatively small number of animals, prescreened as likely candidates, can be sequenced.

In summary, advances in NHP genomics have high potential to identify and develop NHP models of use for translational research. The ability to lower costs, develop more cost-effective sequencing technologies and more sophisticated phenotyping assays, and increase coordination and cooperation among centers will likely be needed to realize this potential.

Technologies: Genetically Modified NHP Disease Models

Technologies to modify the genomes of rodents have formed the basis of developing many disease models, particularly in the laboratory mouse. CRISPR/Cas9-based approaches make it possible to rather easily modify virtually any gene, or to create inactivated or deleted genes (knockouts) or to introduce genes of interest into the genome (knockins). The short generation times of mice and their fecundity contribute to the success of these technologies. NHPs present difficulties relative to rodents in regard to genetic modification. These include: the expense of isolating oocytes or embryos from NHPs and the small numbers of these biological materials that can be obtained from any given female; the long generation time of NHPs relative to rodents (months or years rather than weeks); expense associated with maintaining colonies of genetically modified animals; and the relatively small numbers of laboratories that have command of all the technologies necessary for successful generation and propagation of mutant animals. Nevertheless, there are now several examples of creation of genetically modified monkeys that can be used for translational research. These include models for Huntington's Disease (Chan 2013), diseases caused by mitochondrial mutations (Takibana et al. 2009), autism (Liu et al. 2016), microcephaly (Ke et al. 2016), Duchenne muscular dystrophy (Chen et al. 2015), and animals with genetically encoded calcium indicators that can be used for imaging neural activity in vivo (Park et al. 2016). Development of transgenic animals that closely replicate human diseases, particularly using CRISPR/Cas9-based approaches (reviewed by Chen et al. 2016) should increase and contribute to the utility of NHPs for translational research.

Organization of the Current Issue

The articles in this issue of the *ILAR Journal* provide a rich and nuanced picture of the utility of NHP preclinical models for understanding and treating human disease. These articles are briefly described as follows.

Tuberculosis

Taylor Foreman and colleagues discuss the use of NHPs as preclinical models for development of vaccines that protect against infection by *Mycobacterium tuberculosis* (*Mtb*) and for therapies

that can ameliorate tuberculosis. They also discuss the relationship between studies that utilize rodents and those that use NHPs, as well as details regarding the relative merits of using rhesus macaques versus cynomolgus macaques. In contrast to mice, NHPs demonstrate all of the features of infection of humans by *Mtb*, particularly the formation of granulomas that have very similar physiology to those in human infection and immune responses that parallel those of humans. These authors discuss many aspects of preclinical studies using NHPs, including the necessity for having the proper containment facilities, and the ability to study the effects of co-morbidities such as infection by simian immunodeficiency virus (leading potentially to simian AIDS), long-term exposure to alcohol, and diabetes. They cite approaches to vaccine development that were first tested successfully in NHPs and have now moved to Phase II human clinical trials and also make the point that some vaccine approaches that appeared to be successful in mice, but were not tested in NHPs, failed in humans. These authors recommend that all TB vaccine trials first be tested in NHPs before being performed in humans.

HIV/AIDS

NHPs have been critical animal models for development of all current approaches aimed at halting the AIDS epidemic in humans, including developing anti-retroviral therapies, decreasing maternal-fetal transmission of HIV, testing microbicides, and designing more effective strategies for development of efficacious AIDS vaccines. In this article Ronald Veasey and the late Andrew Lackner emphasize studies in NHPs that underlay all of these advances: understanding the basic features of pathogenesis of HIV-like viruses (SIVs) using monkeys such as rhesus macaques. They also discuss early experiments using chimpanzees, which, while unsuccessful in themselves, provided information that was useful for development of the SIV-macaque animal model.

Viral Hepatitis

The development of vaccines that prevent infection of humans by Hepatitis A and B viruses and antivirals that provide curative therapies for hepatitis C virus infections is one of the clearest examples of the critical nature of an NHP preclinical model, in this case the chimpanzee (*Pan troglodytes*). Robert Lanford and colleagues provide an in-depth review of the history and continuing issues around the use of chimpanzees to ameliorate these three forms of hepatitis, which are caused by three different, unrelated viruses. Chimpanzees are the only animal model that can be used to test strategies for prevention or amelioration of Hepatis B and C and are the most useful model for Hepatitis A. Use of chimpanzees led to development of vaccines that prevent Hepatis A and B and antivirals that are curative for Hepatitis C. These authors also provide a summary and timeline of the events that lead to a cessation of the use of chimpanzees for invasive biomedical research and the decision by the NIH and US Congress to move these animals to sanctuaries, a process that is not yet complete. The authors point out that there are still important medical issues regarding Hepatitis, such as the development of curative therapies for chronic Hepatitis B, as opposed to the successful development of vaccines to thwart new infections, and the development of a vaccine for Hepatitis C, as opposed to the successful development of antivirals. These new approaches cannot be pursued in the absence of an animal model that can replace the chimpanzee.

Neurodegenerative Disorders

Marina Emborg summarizes the features of three major neurodegenerative disorders: Alzheimer's, Parkinson's, and Huntington's diseases. This article discusses the development of preclinical NHP models, tests of potential therapies, and methods for neuroprotection. As pointed out in the article, several different animal models have been used, and model development remains a very active area of preclinical research. NHPs are particularly relevant because of their highly developed cerebral cortex, cognitive functions, complex motor skills, and neuroanatomy, all of which are similar to the human. This article discusses various approaches toward therapies developed in NHPs that are in the early clinical trial stage in humans.

Substance Abuse Disorders

Matthew Banks and colleagues review many of the preclinical studies aimed at understanding the etiology of substance abuse disorders (drug addiction) and developing therapies using preclinical animal models, particularly NHPs. They emphasize advantages of NHP preclinical models relative to other animals, particularly rodents. These advantages relate, in part, to the anatomy and physiology of NHPs, which allow the same types of investigations as are performed using human subjects and to the ability to pair longitudinal drug self-administration studies with noninvasive imaging studies. The authors also point out that NHPs are well positioned as models to understand the effects of new drugs of abuse as they are introduced into societies.

Vision and Prevention of Blindness

Michael Mustari reviews the use of NHP models aimed at understanding the causes of and ameliorating conditions that lead to blindness or lack of visual acuity in humans, including problems of eye alignment (strabismus), "lazy eye" (amblyopia), unsteady gaze (nystagmus), and defective eye movements. NHPs provide particularly useful preclinical models for studying these conditions, because, like humans, optimal visual acuity depends on frontally placed eyes, retinal specializations, and binocular vision. This review also briefly discusses approaches such as gene therapy, stem cell-based technologies, neuro-prosthetics, and optogenetics that can be used to restore retinal function, leading to amelioration of retinal diseases. Vision research provides one of the most clear-cut examples of the advantages of NHP preclinical models because of the close physiological similarities between humans and NHPs.

Disorders Associated with Psychosocial Processes, Such as Anxiety, Depression, and Loneliness

John Capitanio describes research that identifies individual rhesus macaques housed at the California NPRC that can be used as models for loneliness, behavioral inhibition, and social functioning, among others. These conditions are correlated with negative health outcomes in humans, including development of autism and asthma. This article describes and discusses the details of this program (termed the Biobehavioral Assessment Program), the differences between these naturally occurring NHP models and induced models of conditions such as loneliness, and measurement of relevant physiological and genetic parameters in these animal models.

Cardiovascular Disease

Laura Cox and colleagues provide a detailed review of the use of NHPs to study several aspects of cardiovascular disease (CVD). The authors emphasize many different aspects of this topic, including effects of genetics, epigenetics, and diet on CVD; the relationship of infectious disease and CVD, as exemplified by studies of Chagas disease; and the use of stem cell-based technologies to develop NHP preclinical models. They note that NHPs are particularly useful preclinical models because of their genetic similarity to humans, size, and the relatively faster rate of development of CVD compared to humans. NHP-related studies of CVD also provide good examples of the use of multiple NHP species; the authors discuss the relative merits of different species for specific types of investigations.

Metabolic Disease

Peter Havel and colleagues review the use of NHP preclinical models for metabolic conditions, concentrating on obesity and metabolic syndrome. They point out that NHPs are more similar to humans than are rodents in a number of aspects of energy metabolism, including the major site of de novo lipogenesis (liver vs. adipose tissue), major classes of circulating lipoproteins, and the physiology of thermogenesis and insulin-mediated glucose utilization. They discuss a number of studies in which the physiological features related to endocrine and metabolic studies in nonobese animals have been translated from original findings in rodents to the more relevant NHP model. These studies include the effects of long-term energy restriction in aging NHPs. This article provides a detailed review of the use of NHP preclinical models to study several aspects of conditions associated with metabolic syndrome, including glucose resistance, diabetes, dyslipidemia, and hypertension. The authors cite several examples of the use of NHP models to validate therapies in humans.

Respiratory Disease

Lisa Miller and colleagues review the role of NHP preclinical models for understanding and treating a number of severe respiratory conditions, including damage to the airway associated with inhaled ozone and tobacco smoke, diseases associated with airway inflammation (asthma and chronic obstructive pulmonary disease), and infection with viruses such as influenza virus, respiratory syncytial virus, severe acute respiratory syndrome virus, and others. Underpinning all of these studies is the close similarity in physiology between the NHP and human respiratory systems.

Female Reproduction, Prenatal Development, and Women's Health

Richard Stouffer and Teresa Woodruff review the use of NHPs to study critical issues related to women's reproductive health. They summarize data for disorders associated with ovarian and uterine function and deleterious conditions associated with pregnancy. These common disorders of the female reproductive system include polycystic ovary syndrome, endometriosis, placental dysfunction, and preterm labor, among others. These authors also comment on emerging areas in the field of female reproduction, including infection with Zika virus and the potential use of transgenic technologies such as the CRISPR/Cas9 system, to create new disease models relevant to translational research related to women's reproductive health.

Environmental Enrichment

Kris Coleman and Melinda Novak review best practices for environmental enrichment in the husbandry of NHPs. Environmental enrichment is required by the Animal Welfare Act and is essential for maintaining the welfare of NHPs, reducing stress, and maintaining optimal health in these animals. Optimal health of animals is also a component of experimental reproducibility. These authors review the objectives of a typical enhancement plan for animals, relevant outcome measures, challenges, costs, and benefits. This paper emphasizes the fact that environmental enrichment is a dynamic process that has evolved over time and will continue to be optimized in the future.

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I dedicate this paper to the memory of Dr. Andrew Lackner: scientist, mentor, and great champion of translational research using NHPs.

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