Demands for Rhesus Monkeys in Biomedical Research: A Workshop Report[1-3]

Introduction

As the closest phylogenetic relatives to humans, nonhuman primates play an indispensable role in biomedical research. These animals are often the best—and sometimes the only—available model for studying a variety of human health issues, ranging from diseases and disorders to potential therapies and preventive strategies. National efforts to address the medical threats of bioterrorism and reduce the prevalence of AIDS depend on scientific access to nonhuman primates.

Today, however, scientists are facing a shortage of nonhuman primates available for biomedical studies. Through the mid-1970s, the rhesus macaque could be readily imported from India, and the scientific community grew dependent on access to this laboratory animal. Today, however, rhesus macaques can no longer be imported from India. Although US breeding colonies are operating at peak capacity, scientific demand for these and other nonhuman primates exceeds the available supply.

Concerned that shortage of nonhuman primates could seriously impede scientific progress, two components of the National Institutes of Health (NIH)—the Office of AIDS Research (OAR), and the National Center for Research Resources (NCRR)—have taken a lead role in addressing this issue. At the direction of US Department of Health and Human Services Secretary, Tommy G. Thompson, NIH is coordinating an effort to assess the needs for nonhuman primates in a variety of federal research agencies and is mapping out strategies to meet these needs.

To explore alternatives to rhesus macaques in biomedical research, NCRR and OAR convened a panel of investigators who utilize other nonhuman primate species in their research. The Workshop on Demands for Rhesus Monkeys in Biomedical Research, held April 19-20, 2002, at the National Academy of Sciences in Washington, DC, had three goals:

1. To identify the specific demands for rhesus macaques and to determine rhesus resource availability and limitations.

[1]Based on a workshop held April 19-20, 2002, at the National Academy of Sciences, Washington, DC.

[2]Prepared by the Research Resources Information Center, Equals Three Communications, 7910 Woodmont Avenue, Suite 200, Bethesda, MD 20814-3015.

[3]The complete Proceedings of this workshop, which were in final preparation when this issue went to press, are now available by contacting the Institute for Laboratory Animal Research (ILAR), 500 Fifth Street, NW, Washington, DC 20001 (Tel: 202-334-2590; Email: ILAR@nas.edu).

2. To identify other nonhuman primate species that can also be excellent animal models and be used for biomedical research.
3. To put forth recommendations (possibly in the form of a "White Paper" or other publication format) to alleviate the demands on rhesus macaques.

Invited speakers were each asked to address a particular topic or animal model in a 25-minute presentation. Speakers were also requested to address the following issues relative to specific animal models:

- What are the human diseases for which they are good models?
- What are the physiological and genetic similarities/dissimilarities to humans and/or rhesus macaques?
- What is their availability? Are they endangered?
- Are there importation problems?
- Are there specific problems/considerations in animal husbandry?
- Are there specific pathogen concerns?

The workshop culminated in a series of recommendations intended to alleviate scientific demands for rhesus macaques, especially those of Indian origin. This report provides highlights of Workshop presentations and summarizes the Panel's recommendations and the rationale behind each.

Summary of Recommendations

The Workshop Panel on Rhesus Monkey Demands in Biomedical Research issued a series of recommendations designed to alleviate dependence on rhesus monkeys for biomedical studies. Although the majority of their suggestions focused on characterizing and enhancing access to alternative species, the Panel also addressed broader issues that limit the nation's capacity for conducting nonhuman primate research. These larger concerns include an urgent need for adequate research infrastructure—especially facilities—and the need for stable funding and long-term planning for nonhuman primate resources. Failure to provide such critical research resources may seriously jeopardize our nation's ability to protect its citizens from bioterrorism, AIDS, and other major threats to human health.

The Panel's recommendations, and a brief rationale for each, are summarized below.

I. Encourage Broader Use of Alternative Species by the Biomedical Research Community

1. **Develop a portfolio of nonhuman primate models for a variety of human diseases and conditions.** Although rhesus macaques have become the primary laboratory primate for biomedical studies, they are in short supply in the United States, and this shortage is expected to continue for at least 5 to 10 years. Therefore, the Panel recommends that NIH support the development of alternative nonhuman primate models, including models for which the rhesus macaque is currently the species of choice.
2. **Encourage development of alternatives to Indian rhesus macaques for AIDS research.**

   - ***Develop common reagents*** for analysis of viral load and immunological parameters that can be used across species.
   - Support **cross-species comparisons** of several vaccines and challenge models.
   - ***Cynomolgus Macaques.*** Given the ready availability of cynomolgus macaques, their reasonable cost, and their susceptibility to SIV/DeltaB670 (a primary isolate of SIV), this species should be used for trials examining SIV pathogenesis and therapy. However, assays must be optimized for this species.
   - ***Pigtailed Macaques.*** Encourage broader use of these animals in microbicide studies and develop related virus stocks for this research.
   - ***Chinese Rhesus Macaques.*** Although these animals have not yet been used for preclinical AIDS research, they are less expensive and more readily available than their Indian counterparts.

3. **Enhance access to alternative species.** Before the biomedical community can reduce its reliance on the rhesus macaque, alternative nonhuman primate species must be readily accessible. Therefore, the Panel recommends the following:

   - ***Establish national resource centers devoted to selected primate species.*** Because the nation cannot realistically maintain research resources devoted to every species of nonhuman primate, priority should be given to species that have an established track record in enhancing understanding of human health and disease. The panel suggests that NCRR convene and seek the counsel of an expert panel to determine which species—such as the vervet—might merit special attention in a national resource center, perhaps similar to the NCRR-supported squirrel monkey resource at the University of South Alabama.
   - ***Support increased breeding of species with demonstrated biomedical relevance,*** especially cynomolgus macaques. Moderate expansion of baboon breeding is also recommended.
   - ***Diversify the NPRCs.*** Advise each NPRC to support and develop expertise on more than one species of nonhuman primate to help encourage use of alternative models. As respected and influential institutions within the primate research community, the NPRCs are ideally positioned to lead a shift to alternative species.

4. **Develop new reagents and test new and existing reagents in alternative species.** Resulting reagents and data will greatly enhance the utility of alternative species for studies of infectious disease, immunology, genetics, and aging.
5. **Support research that expands basic knowledge about alternative species.** Increase research on the natural biology, husbandry, and veterinary care of alternative species, particularly New World monkeys, which are less widely understood. Expertise must be developed and expanded in the husbandry and research uses of these species. This capacity-building is best supported in laboratories where expertise currently exists with each of the species targeted for support.
6. **Educate the biomedical community about available alternatives to rhesus macaques.** Possible mechanisms for disseminating information include Web sites and presentations at scientific conferences.

II. Enhance the National Capacity for Conducting Nonhuman Primate Research

7. **Develop a comprehensive National Primate Plan** to preserve and expand resources for primates that have proven to be important biomedical research models. Although the plan would focus on meeting the needs of US scientists, development and implementation of the plan must include partnerships and collaborations with scientists in source countries. The plan should include both Old World and New World monkey species and address the need for research infrastructure and facilities.
8. **Significantly expand breeding of rhesus monkeys, and enhance access to primates in source countries.** Despite a search for alternatives, the rhesus macaque is currently indispensable to many aspects of biomedical research. Partnerships with source countries would help ensure a continuing supply of nonhuman primates and enhance genetic diversity in captive breeding colonies.

    - ***Address issues related to transportation.*** Transportation of nonhuman primates is becoming increasingly difficult, both from source countries and from one US laboratory to another.

9. **Address the critical need for adequate facilities.** Insufficient infrastructure—especially facilities for housing nonhuman primates—compromises our nation's ability to address health-related emergencies. Facilities are needed for:

    - Breeding
    - Experimental use and holding space
    - Quarantine

10. **Recognize the need for long-term planning and stable funding for nonhuman primate resources.** The Panel noted that years of planning are required to establish viable primate colonies and create the infrastructure needed for nonhuman primate research. In addition, a chronic shortage of core funds for primate research centers jeopardizes the viability of current programs.

    Nonhuman primate resources must be in place if the biomedical community is to respond appropriately to any threats or challenges to the nation's health. Therefore, the Panel urges NIH to take steps now to ensure adequate primate resources for the future.

Workshop Presentations

Session 1: Rhesus Resource Needs, Availability, and Limitations

Overview of NCRR-Supported Rhesus Resources

Dr. Jerry Robinson, National Center for Research Resources

NCRR supports a network of eight National Primate Research Centers (NPRCs), as well as additional primate resources. Combined, these NCRR-supported facilities house more than 25,000 nonhuman primates, including more than 15,000 rhesus macaques (*Macaca mulatta)* and more than 4,000 baboons (*Papio sp.).*

Although the NPRCs are enhancing their breeding colonies to meet increased scientific demands for rhesus macaques, such efforts require years to reach fruition. The female rhesus does not become reproductively capable until 3.5 to 4 years of age, and the male at over 4 years of age.

SPF colonies. In collaboration with the NIH Office of AIDS Research, NCRR has awarded six grants (five of which went to NPRCs) to establish specific-pathogen-free (SPF) rhesus and pigtailed macaque colonies. However, it may take more than 5 years to step up production of these animals and increase their availability. Additional grants for SPF colonies are expected to be awarded later this year.

Survey of NIH Grantees. Dr. Robinson outlined the results of an NCRR-supported survey of NIH grantees who use nonhuman primates in their research. More than 1,000 scientists were polled, and 641 responded. According to the survey, an estimated 13,000 nonhuman primates were used in 1999 in NIH-funded investigations; an estimated 65% of those animals were rhesus macaques.

Two thirds of respondents do not typically use NPRC resources. Of scientists who requested access to NPRCs, 95% had their requests granted. The remaining 5% were denied access primarily because of lack of animals or lack of space.

Survey respondents gave the NPRCs high marks for quality of resources and services, but low marks for availability of animals and space. Of respondents who had used

NPRC resources in 1999, 70% said they had no difficulties receiving requested materials within 1 to 3 months, and 75% did not consider animal costs unreasonable.

Recommendations that arose from the survey include:

- Increase breeding capacity.
- Address limited availability of nonhuman primates.
- Make nonhuman primates available to scientists outside the NPRCs.
- Increase publicity and information about NPRC resources.
- Increase funding and/or reduce costs.

Overview of Specific Monkey Needs for AIDS Research

Dr. Bonnie Mathieson, NIH Office of AIDS Research

In the mid-1980s, researchers found that isolates of SIV derived from sooty mangabeys and African green monkeys caused AIDS in rhesus macaques of Asian origin, thereby providing researchers with a model to study AIDS pathogenesis, treatment, and vaccines. At the time, rhesus macaques (mostly of Indian origin) were commonly available in the United States because they had been bred for polio vaccine testing.

Once virus genes were identified, researchers developed candidate vaccines and began testing them in rhesus and other macaque species, including pigtailed and cynomolgus macaques. Lack of standardization in macaque species, virus stocks, and outcome measures made comparisons among models difficult. Scientists began calling for a single model system, particularly for vaccine research.

The opposite approach is to develop methodologies that can be used across monkey species, such as quantitative tests to measure viral RNA copies in plasma and immunological reagents for cell surface markers and cytokines. Dr. Keith Reimann of the New England NPRC has a Web site with information about commercially available immunological reagents that can be used across species.

As scientists began exploring new concepts for preventing AIDS, the demand for macaques grew. The new field of microbicide research, for example, began to use the same macaque models as in vaccine research because the stocks for virus challenge were already titered for vaginal and rectal use in these animals.

Recently, NIAID's Division of AIDS queried its grantees about their use of macaques in 2000 and 2001. These extramural investigators indicated using about 1,400 animals in 2000 and more than 2,000 animals in 2001. Because the NPRCs at current capacity can produce only about 1,800 macaques per year, NIH grantees were forced to acquire some macaques from commercial sources.

Macaques used for AIDS research comprise less than half of those used for all biomedical research, and the demand for macaques is expected to grow as research in fields such as transplantation and bioterrorism uses more of these animals. Possible solutions include:

- Utilization of pigtailed macaques and bonnet monkeys for microbicide research, as the menstrual cycling of both species and the vagina of bonnet monkeys are similar to those of humans. Virus stocks need to be developed for this research.
- Develop more reagents for analysis of virus load and immunological parameters that can be used across species. Compile a database of reagents that have been cross-tested.
- Compare effects of vaccines and viruses across species.
- Support breeding of various macaque species, thereby enabling investigators to explore alternative models.
- Develop international resources for breeding macaques.
- Compile a list of primary and secondary animals that can be used for research on various diseases. For each disease model, include a knowledgeable contact person.

Session 2: Alternative Macaque Models for AIDS Research

Pigtailed Macaques

Dr. Jon Warren, National Institute of Allergy and Infectious Diseases

In a study conducted by Dr. Nancy Haigwood at the Seattle Biomedical Research Institute, SIV-infected pigtailed macaques received immune globulin purified from the plasma of an SIV-infected long-term nonprogressor macaque. The animals and controls were followed up to 3 years postinfection. Although passive vaccination led to prolonged viral suppression and doubled the mean time to disease, it did not lead to viral clearance, and most vaccinated animals still developed simian AIDS (SAIDS). Because some animals developed SAIDS and died despite having had prolonged suppression of plasma virus, the researchers concluded that viral load was not an absolute predictor of survival.

A study by Dr. Janet Harouse at the Aaron Diamond AIDS Research Center in New York City demonstrates that researchers should consider the combination of virus and monkey species they choose to study, rather than just the monkey species. The researchers compared the effects of intravenously administering the CCR5-tropic virus SHIVSF162P3 in pigtailed macaques and Indian rhesus. In the pigtailed macaques, peak viral load was 10^7-10^8 vRNA/mL and the set point varied greatly. All four pigtailed macaques showed moderate $CD4^+$ cell declines in the peripheral blood, while $CD4^+$ cell levels in the gut-associated lymphoid tissue (GALT) were not reported. In the Indian rhesus, peak viral load was 10^7 vRNA/mL and the set point varied between 10^2-10^6 vRNA/mL. Both infected Indian rhesus monkeys had a gradual drop in peripheral blood $CD4^+$ cell levels and significant acute drops of

GALT $CD4^+$ cells. This GALT $CD4^+$ cell depletion, as well as the changes in viral load and the pattern of opportunistic infections, is similar to what occurs in HIV-1 infection in humans. The researchers concluded that improving the efficiency of infection in the Indian rhesus model is an important goal.

Dr. Shiu-Lok Hu of the Washington NPRC in Seattle intravenously administered SHIV KU-1 to immunized and control pigtailed macaques. Plasma viral loads peaked and then were maintained at a high level. After 2 to 3 weeks of infection, a rapid and irreversible $CD4^+$ cell depletion occurred. Some immunized animals were able to control their set point virus loads, although not their peak loads, and were able to maintain adequate $CD4^+$ cell counts. The kinetics of the disease course did not appear to parallel that of HIV-1 infection in humans.

Indian versus Chinese Rhesus Macaques

Presentation by Dr. Jon Warren, NIAID

A survey of studies published from 1999 to 2002 of live lentivirus (SIV, SHIV, HIV-1, HIV-2) challenges to vaccinated nonhuman primates indicates that most of the challenges are being done intravenously, despite the fact that lentiviruses ordinarily enter mucosally. Of the mucosal challenge studies, most have been conducted with Indian rhesus macaques and with SIV. More lentivirus challenge studies should be conducted using alternative macaque models, including Chinese rhesus, cynomolgus, and pigtailed macaques. Also, more challenge studies should be conducted with SHIV.

Because the number of HIV recombinants is growing and researchers cannot predict either their behavior or their virulence, more research should be conducted on the natural history of lentiviral infection in nonhuman primates, and the information should be stored in a shared database. NIAID's Division of AIDS is developing criteria to determine which animal models should be supported for long-term follow-up. Also, the division is considering establishment of a nonhuman primate lentiviral natural history database, which would supplement the HIV databases supported by the Los Alamos National Laboratory in New Mexico.

Chinese rhesus macaques are potentially a useful alternative to Indian rhesus macaques. While Indian rhesus are in short supply and expensive, Chinese rhesus are available and less costly. Also, Chinese rhesus are susceptible to SIVmac and certain SHIVs, and reagents that have been used with Indian rhesus can be used with Chinese rhesus, since both are members of the same species. In addition, Indian rhesus may not provide the best model for human HIV infection, since AIDS develops much more rapidly in Indian rhesus than humans and the SIV plasma viral loads in Indian rhesus are as much as 1,000 times higher than the HIV loads in untreated humans.

Researchers have compared the results of SIVmac251 intravenous challenges in Indian rhesus, Chinese rhesus, and cynomolgus macaques. Results showed that the peaks of viral load were comparable in the three species, but the set points were lower in both Chinese rhesus and cynomolgus. Also, $CD4^+$ T cell loss in the peripheral blood appeared to be less in Chinese rhesus and cynomolgus than Indian rhesus.

In a study involving intravenous challenge with SIVmac239, a molecular clone of SIVmac251, the viral load set points were again higher in Indian than Chinese rhesus. When comparing viral loads in the first 6 months of infection in both groups of SIVmac239-infected rhesus and HIV-1-infected humans, the researchers found that the virus load set points were more similar between Chinese rhesus and human patients than in the other two comparisons: Chinese versus Indian rhesus or Indian rhesus versus human patients. Also, $CD4^+$ T cell count and CD4/CD8 T cell ratios in peripheral blood were higher in Chinese than Indian rhesus and more similar to those of human patients. Similarly, the strong anti-SIV antibody responses in Chinese rhesus were more similar to the strong anti-HIV antibody responses in HIV-infected patients than the practically nonexistent antibody responses in Indian rhesus. These findings imply that Chinese rhesus may provide more relevant models of human HIV infection than Indian rhesus.

In the intestinal mucosa, SIV primarily targets $CCR5^+$ $CD4^+$ T cells. In the SIVmac239 infection study, most of the intestinal $CD4^+$ T cells died during early infection in Indian rhesus, while higher numbers of them survived in the intestinal mucosa of SIVmac239-infected Chinese rhesus. The numbers of surviving intestinal $CD4^+$ T cells were inversely correlated with viral load.

In this study, three-quarters of the Indian rhesus were rapid progressors, developing AIDS 3 to 6 months postinfection. In contrast, none of the Chinese rhesus were rapid progressors, although one monkey developed AIDS-related B-cell lymphoma at 19 months.

Indian versus Chinese Rhesus Macaques

Presentation by Dr. Marta Marthas, California NPRC

Variation *within* Indian or Chinese rhesus populations is as great as variation *between* these populations. The within-population variation can affect study results, particularly when a small number of subjects is used. This variation may partly explain differences between Dr. Marthas' study results and those cited by Dr. Warren.

Dr. Marthas' studies found no difference between Indian and Chinese rhesus in susceptibility to two different SIV viruses administered intravaginally. Few animals in either group were rapid progressors. In the animals that were not rapid progressors, antibody responses among Indian and Chinese rhesus were similar; however, titers among SIV-infected Chinese rhesus were more consistent (differing by up to 50-fold), whereas titers among infected Indian rhesus were more highly variable (in the 4,000-fold range).

While Dr. Marthas' experiments used intravaginal challenge, and those cited by Dr. Warren used the intravenous route, both sets of studies found that peak plasma SIV RNA levels were similar in Indian and Chinese rhesus, although the set points were lower in Chinese rhesus. Adding more animal subjects produced more variation—an effect also seen with human subjects.

Vaccination with an SIV vaccine provided some protection to the animals, as measured by plasma viral load and CD4/CD8 T cell ratio. Indian and Chinese rhesus did not differ in their response to the vaccine.

Vaccination studies have also been done in cynomolgus macaques, using a vaccine consisting of a Sabin polioviral vector carrying SIV genes. Cynomolgus macaques were used because they can be infected with Sabin poliovirus administered orally, while rhesus generally cannot. The vaccine provided some protection against SIVmac251 in the monkeys, as measured by viral load and changes in body weight. However, cellular immune response measurements were difficult to perform because of inverted CD4/CD8 ratios compared to rhesus, highly active and variable natural killer cells, and other factors.

Just as variation is seen in Indian and Chinese rhesus populations from different geographic locations, variation is likely in cynomolgus macaques from different locations.

Cynomolgus Macaques

Dr. Anita Trichel, University of Pittsburgh

Infection with SIV/DeltaB670, a primary isolate of SIV that consists of 12 variants, was initially studied in Indian and Chinese rhesus macaques. In Indian rhesus, virus load usually peaked at 10^6 viral copies/ml plasma within the first 2 weeks. From then on, the animals followed one of three patterns. About 20%, known as rapid progressors, did not live long enough to establish a set point but died after 2 to 3 months of infection. About 60%, known as intermediate progressors, established a set point between 10^4 and 10^5 viral copies/ml plasma and died within 1 to 3 years postinfection. A final 20%, known as slow progressors, established a set point of 10^3 viral copies/ml plasma and lived longer than 3 years postinfection.

A cohort of seven Chinese rhesus developed a peak virus load similar to Indian rhesus and established set points comparable to intermediate and slow progressors. One animal succumbed to fulminant *Pneumocystis carinii* pneumonia at 260 days, but six are still alive at greater than 570 days postinfection. Overall, Chinese rhesus survived significantly longer than Indian rhesus.

To determine whether the differential response to SIV/DeltaB670 in Indian and Chinese rhesus was due to differential amplification of one or more variants after inoculation, the researchers analyzed the variant composition in the inoculum and the monkeys. Analysis showed that the variants formed three groups. Group A variants were more virulent, Group B variants were more attenuated, and Group C variants were uncommon and probably of little consequence. The inoculum consisted of approximately 70% Group B and 30% Group A. Inoculated Indian rhesus had about equal amounts of Group A and B, while inoculated Chinese rhesus had 87% Group B and 13% Group A. These results suggest that Chinese rhesus were living longer postinfection because they had a less virulent mix of viruses. This longevity could be a problem in vaccine studies, because animals in the vaccine arm would have to survive about 1000 days or more to provide good data.

Because rhesus macaques were becoming difficult to obtain, the researchers began to use cynomolgus macaques (*Macacca fascicularus),* also known as long-tailed or crab-eating macaques. These monkeys live mostly on islands in Indonesia and Malaysia and are readily available at reasonable cost from a number of vendors. Also, they require less cage space than other monkeys because they rarely grow taller than 32 inches, and some researchers feel that they have a better disposition than rhesus.

Seven Indonesia cynomolgus macaques were obtained and a 100% infectious dose of SIV/DeltaB670 was administered intravaginally. All seven became infected.

Postinfection plasma viremia was compared in Indonesian cynomolgus macaques, Indian rhesus, and Chinese rhesus. No significant differences were found between Indonesian cynomolgus and Indian rhesus at 1, 2, and 8 weeks postinfection, although Indian rhesus had significantly higher plasma viremia than Chinese rhesus at 1 and 8 weeks postinfection.

In conclusion, cynomolgus macaques seem well-suited for studying many aspects of SIV infection, including intravaginal transmission. Some research teams have already begun to develop immunological assays for use in such studies.

Session 3: Alternative Old World Primate Models for Non-AIDS Research

Cynomolgus Macaques

Dr. Jay Kaplan, Wake Forest University

General Considerations. Cynomolgus macaques are genetically, physiologically, and behaviorally similar to rhesus macaques, although smaller in size, with adult males averaging 5 kg and females averaging 2.5 kg. Like rhesus, they routinely live more than 20 years. Even though cynomolgus macaques are from tropical regions, they survive reasonably well in laboratories located in temperate climates.

Cynomolgus macaques generally are infected with herpes B-virus but are less susceptible to tuberculosis than are rhesus macaques. Also, cynomolgus macaques absorb dietary cholesterol more easily than rhesus, vervets, baboons, and humans. Cynomolgus macaques from different geographic regions (Malaysia, Singapore, Philippine Islands, Indonesia) vary in a number of physiological measures and anatomical dimensions.

Availability and Cost. Large numbers of cynomolgus monkeys have been imported over the last few years, providing a ready resource for researchers and those wishing to stock breeding colonies. Current prices for this species range from $750 to $2,000, depending on age, sex, and origin. As with other primate species, the future availability from countries of origin is uncertain.

Research Areas/Models for Human Disease

- *General*. Cynomolgus macaques readily adapt to diets similar to those consumed by people living in highly industrialized societies. Consumption of such diets makes this species vulnerable to many of the chronic diseases affecting human populations.
- *Cancers of the reproductive tract.* Cynomolgus monkeys have been used to investigate breast and uterine cancer, particularly in relation to estrogen exposure. Oophorectomized monkeys given exogenous estrogen exhibit an increased proliferation of breast and uterine tissue, thus increasing vulnerability to reproductive tract cancer and modeling what is thought to be the human response. Importantly, progestins inhibit the estrogenic effect. The effects of phytoestrogens, which are widely consumed as a health food supplement, are under investigation using this animal model.
- *Cardiovascular disease.* Atherosclerosis (the pathobiological process underlying the development of coronary heart disease and stroke) and coronary function have been studied extensively in male and female cynomolgus macaques. These studies have elucidated the role of dietary cholesterol, stress, and reproductive hormones in the development of coronary and cerebral artery disease. Of particular interest, use of this model has enabled researchers to identify the numerous, and sometimes competing, effects of estrogen on the development of atherosclerosis as well as vascular function. Premenopausal estrogen deficiency can have adverse effects on the vasculature.
- *Diabetes.* Each year, at least 1% of cynomolgus macaques in domestic colonies develop diabetes, primarily type 2. Besides high levels of glucose, these animals also have elevated blood levels of fructosamine, glycated hemoglobin, and triglyceride. The development of type 2 diabetes is marked by elevated insulin levels in the blood and pancreas, as well as accumulation of amyloid in the pancreas. In the final stages of the disease, insulin levels drop.
- *Drug abuse*. Cynomolgus monkeys have been used extensively to study both cocaine and alcohol abuse, particularly in relation to social factors. One recent study demonstrated that subordinate animals found cocaine to be more reinforcing than did their dominant counterparts. Using positron emission tomography, the researchers determined that social housing increased the amount or availability of dopamine D2 receptors in the dominant monkeys but had no effect on these receptors in subordinate monkeys.
- *Osteoporosis.* Cynomolgus macaques reach peak bone mass at about 9 years of age. Bone mass and density are easily measured with procedures similar to those applied to women. As in women, estrogen deficiency (e.g., through surgical menopause) causes rapid bone loss that persists for at least 18 months and that can be completely prevented with estrogen treatment. Current evidence suggests that conditions related to premenopausal estrogen deficiency can accelerate bone loss in affected individuals.
- *Reproductive function.* Cynomolgus monkeys, like rhesus macaques, have a menstrual cycle and reproductive hormone profile similar to that of women. Unlike rhesus monkeys, the cynomolgus macaque is not a seasonal breeder, which allows reproductive function to be studied year-round. One of the most consistent observations in this species is that when housed in small social groups, subordinates become relatively estrogen-deficient. This fully reversible condition closely resembles functional hypothalamic amenorrhea (also called "psychogenic" amenorrhea), an outcome manifested both clinically and subclinically in women.
- *Other diseases:* Cynomolgus macaques have also been used to model depression and various infectious diseases, including tuberculosis and AIDS.

Pigtailed Macaques

Dr. William Morton, Washington National Primate Research Center

Most pigtailed macaques in this country originated from southern Sumatra, while others came from Kalimantan, or Borneo. Pigtails also live in Malaysia, Burma, Thailand, and Vietnam. They are omnivorous and weigh about the same as rhesus. Behaviorally, pigtailed macaques are somewhat more tractable than rhesus.

Pigtails in breeding colonies and in the wild have a high seroprevalence of herpes B-virus, but the Washington NPRC has produced several herpes B-negative colonies at its facilities at the Tulane NPRC. As with other macaque species, tuberculosis is also of concern with pigtailed macaques.

Availability. The Washington NPRC has the nation's largest supply of pigtailed macaques; the only other facility with significant numbers is the Yerkes NPRC. Only small colonies of pigtails exist elsewhere, and few are imported. If pigtails are to be used more in biomedical research, breeding colonies will need to be expanded.

Research Areas/Models of Human Disease

- *Infectious Diseases.* Pigtailed macaques are useful for studying infectious diseases, including AIDS. The most consistent AIDS model in the pigtail is infection with HIV-2_{287}. This model always leads to AIDS, has a realistic time frame, and is good for testing antiviral drugs. Pigtails are remarkably susceptible to SHIVs and can even be infected with HIV-1, but the persistence of infection and the level of viral load are not sufficient to evaluate vaccines.

- *Reproductive physiology.* The female's pronounced perineal tumescence and detumescence at different stages of the menstrual cycle allow researchers to easily spot ovulation times. As with humans, pigtail menstrual cycles last about 28 days and occur throughout the year rather than in particular seasons. The pigtail vagina is also human-like in its epithelial thickness, pH, and flora. Because pigtails are susceptible to varieties of *Chlamydia trachomatis* that also infect humans, the animals provide a good model for studying potential microbicides.

Baboons

Dr. John VandeBerg, Southwest National Primate Research Center

The baboon is an appropriate alternative to rhesus macaques for certain kinds of non-AIDS research, and it may be a superior laboratory animal for certain types of studies. Macaques and baboons became evolutionarily separated from each other about 7 million years ago, which is the same duration of time that chimpanzees and humans have been evolving independently. The DNA sequences of macaques and baboons are about 98 to 99% identical; likewise, chimp and human DNA sequences are 98 to 99% identical. In addition, the karyotypes of macaque and baboons are identical; no chromosome rearrangements have occurred during the 7 million years the animals have independently evolved. Therefore, there is no innate genetic reason for choosing a macaque over a baboon for biomedical research.

Five subspecies of baboons are currently used in biomedical research in the United States: olive baboon, native to Tanzania and Kenya; red baboon, native to west Africa; yellow baboon from Tanzania; chacma baboon from South Africa; and hamadryas baboon, native to Ethiopia, Somalia, and the Arabian Peninsula. Because each subspecies has unique physiological characteristics, scientists must be aware of the type of baboon under study.

The olive baboon is the predominant subspecies at the Southwest NPRC, which also houses large numbers of yellow baboons. These two subspecies interbreed naturally in the wild, and they are closely related genetically.

Availability. Baboons are readily available from the Southwest NPRC and other sources. The Southwest center maintains about 3,800 baboons in a steady state; most were born at the center. In the last few years, the NPRC has been able to supply baboons to all US scientists who have requested the animals.

Baboon production could be enhanced with relative ease, if scientific demand should increase. It may also be possible to import additional animals from Africa, although air transportation is a primary obstacle. Additional funds would also be needed to house a larger number of baboons, construct necessary breeding facilities, and support any influx of animals.

Quick Facts

- *Fecundity.* About 0.8 to 0.85 live births annually per female, compared with an estimated rate of 0.6 to 0.8 in the rhesus macaque.
- *Breeding.* A single male can serve as an effective stud for 20 to 40 females. Single-sire breeding groups can be kept in large outdoor pens.
- *Size.* Baboon females can weigh 10 to 20 kilograms, or about twice the size of a female rhesus macaque. Male baboons can weigh 20 to 40 kilograms, which is up to 3 or 4 times larger than a male rhesus.

Advantages of Baboons in Biomedical Research

- *Hardy.* Can live in a variety of weather conditions, including temperatures ranging from about less than 10 to more than with to rhesus, baboons can be more easily arranged in different social groups, and they exhibit little intragroup hostility.
- *Genetic studies.* Although gene maps are now being developed for the rhesus macaque and the vervet, the baboon gene map is farther along and expanding at an accelerating pace. In addition, it is easy to rapidly expand sire families to large numbers (e.g., to propagate a particular gene in a colony) by housing a male baboon with up to 40 females at a time—in contrast to the male rhesus macaque, which is generally housed with only 10 to 12 females.
- *Size.* Large size can be helpful for experimental procedures that require delicate surgical manipulations, especially for studies of prenatal and neonatal baboons. The baboon's larger blood volume is also an advantage for studies that require extensive blood drawing.
- *Pathogens.* Baboons do not carry herpes B-virus, and they are more resistant to TB than are rhesus macaques.
- *Cost.* To buy adult baboons reared at the Southwest NPRC, investigators pay between $3,500 and $5,000. For comparison, the estimated cost of a US-born, Indian-derived rhesus macaque is about $5,400, and about $7,600 or more for a specific-pathogen-free macaque.

Disadvantages of Baboons in Biomedical Research

- *Size.* Can be a disadvantage, since larger animals require larger cages and larger doses of medications in pharmaceutical studies.

Research Areas/Models for Human Disease

- *Aging.* The Southwest NPRC houses a colony of about 200 to 300 geriatric baboons, over 16 years of age. It is the world's largest colony of old primates. Most of these animals are pedigreed and genotyped, with progeny, grand progeny, and great-grand progeny in the colony, which provide an opportunity to study aging through several generations of a family.
- *Alcoholic liver disease.* Studies of the baboon proved that this disease arises from alcohol, rather than the poor nutrition that is often associated with alcohol consumption in humans.
- *Cardiovascular.* Like humans, baboons have a relatively low glycemic response to dietary fat and cholesterol, whereas rhesus and cynomolgus macaques easily

absorb dietary cholesterol and develop very high blood cholesterol. Baboons, therefore, provide a more human-like model for studying the genetics of blood cholesterol control and atherosclerosis. Baboons may also be more appropriate than the rhesus macaque for studying the mechanisms of cholesterol metabolism.

- *Developmental biology.* Baboons are widely used in studies of prenatal and neonatal development, including embryology and teratology, dental development, bronchopulmonary dysplasia, infant nutrition, and chronic lung diseases of infancy.
- *Diabetes.* Type 2 diabetes can develop spontaneously in the baboon.
- *Genetic studies.* The Southwest baboon colony has been extensively pedigreed, beginning in the 1970s, making it an incomparable primate resource for genetic research. The pedigrees include thousands of baboons. Baboon chromosomes are remarkably similar to human chromosomes in their genetic arrangement. With a few rearrangements, the baboon gene map can be aligned, chromosome-for-chromosome, with the human gene map.
- *Hypertension.* High blood pressure can develop naturally in baboons and can be induced by renal artery stenosis.
- *Infectious disease.* Some baboons have become naturally infected with *Trypanosoma cruzi,* which causes Chagas disease and is endemic to the southern United States. Baboons also develop cardiac pathologies similar to humans with the disorder. Baboons can also develop schistosomiasis, as well as periodontal disease and dental caries. Scientists are studying baboons to examine the harmful effects that maternal periodontal disease may have on fetal development.
- *Neonatology.* Reboons—a cross between a baboon female and a male rhesus—tend to be born prematurely. A neonatal intensive care unit was developed at the Southwest Foundation to care for these fragile newborns. This has become an excellent model for studying premature births, prenatal biology, neonatal lung and heart development, hyaline membrane disease, and bronchopulmonary dysplasia.
- *Neurobiology.* Baboons are used in studies of drug abuse, epilepsy, and other brain disorders.
- *Obesity.* Baboons can develop spontaneous obesity.
- *Osteoporosis.* Elderly female baboons develop bone pathology similar to humans with osteoporosis. Because some baboons appear to be resistant to osteoporosis, scientists are also studying the underlying genetics of the condition.
- *Reproduction.* As with the pigtailed macaque, the baboon has a visually obvious change to the perineum when females are in estrus. This characteristic enables easy tracking of timed pregnancies, since it is apparent when the female is ovulating. Baboons also enable research on pregnancy, endometriosis, and contraception.
- *Transplantation.* Because of its large size, the baboon is a suitable model for xenotransplantation studies of pig organs into primates.

Other research uses of baboons include immunogenicity testing, pharmacokinetics, somatic cell gene therapy, bone implants, disc replacement testing, and metabolic research.

African Green Monkeys (Vervets)

Dr. Lynn Fairbanks, University of California, Los Angeles

The African green monkey, also known as the vervet, is found throughout sub-Saharan Africa, with populations in the Caribbean on the islands of St. Kitts, Nevis, and Barbados accidentally introduced during the slave trade in the 1600s.

Vervets are in the same subfamily as the macaques and baboons. They are similar to rhesus in anatomy, physiology, hematology, blood chemistry, and social organization. Female vervets reach puberty at 2 to 3 years of age, and adult size at 4. Males mature between 3 and 4 years of age and reach adult size at age 5. As adults, vervets are slightly smaller than rhesus and considerably smaller than baboons.

Female vervets have a slightly higher fecundity rate than rhesus. They typically produce one infant per year, although they are capable of producing three infants in 2 years. Females can begin producing infants at 3 years of age, but at that age they generally have pregnancy problems and a higher infant mortality rate compared with mothers 4 years of age and older. Gestation takes 165 days, and the mother-infant relationships are similar to rhesus and baboons.

Vervets of African origin carry natural pathogens, including the African green monkey variant of SIV (SIV-agm), Cercopithicine herpesvirus 2 (SA-8), and Marburg virus, which has killed laboratory workers in Europe. They are not carriers of herpes B-virus. In contrast, vervets of Caribbean origin are free of significant disease, including tuberculosis and hepatitis, and remain healthy in captivity.

Avaliability. Vervets are readily available for import from the Caribbean, where they are abundant and considered an agricultural pest. Research facilities that house and export vervets include the St. Kitts Biomedical Research Foundation, Caribbean Primate Research Laboratories, and Barbados Primate Research Center. Approximately 1,000 animals per year are available for export from Barbados, with the potential for similar numbers from St. Kitts.

In the United States, large colonies are located at the New Iberia Research Center (1,200) and at University of California, Los Angeles/Veterans Administration (UCLA/VA) Vervet Research Colony (550), while smaller numbers are housed at Wake Forest University and the University of Texas at Austin.

Advantages of Vervets as an Alternative to Rhesus

- *Cost.* Vervets are available for prices between $1,000 and $2,000.
- *Pathogens.* Vervets do not carry herpes B-virus, and animals from the Caribbean are relatively disease-free compared with Asian macaques.

- *Caging.* Vervets can be housed in the same cages as rhesus and handled using the similar procedures.
- *Uniformity.* Because the Caribbean vervets are descendants of a small founder population, they are relatively homogeneous genetically.

Research Areas/Models for Human Disease

- *Cell biology.* Green monkey kidney cell lines are widely used as an in vitro system for research in cell biology and physiology.
- *AIDS.* Although they have not been used extensively for AIDS research in the United States, vervets are being used for this purpose in Germany.
- *Behavior.* Vervets have been used to study maternal behavior, behavioral development, communication, cognition, aggression, impulsivity, and socioecology.
- *Cardiovascular/pulmonary disorders.* Vervets have been used to study atherosclerosis, heart disease, and chronic obstructive pulmonary disease.
- *Diabetes.* About 4% of vervets have higher than normal blood glucose levels, and some vervets develop type 2 diabetes.
- *Genetics.* Researchers increasingly use vervets to study the genetics of individual differences. The St. Kitts Biomedical Research Foundation, operated by McGill University, has been screening large numbers of vervets for traits related to alcohol preference and anxiety. These researchers also have developed more than 400 microsatellite markers. Plans are being made to use these markers and the pedigree developed at UCLA/VA Vervet Research Colony to produce a map of the vervet genome. A bacterial artificial chromosome library of vervet genes also is being developed.
- *Neurobiology.* Vervets have become one of the primary models for studying neurobiological systems and disorders. The Caribbean Primate Research Laboratories, operated by Yale University, and the UCLA/VA Vervet Research Colony use vervets as their primary model for Parkinson's disease. These two centers also study neural development and regeneration and the role of serotonin and dopamine in behavior.

Session 4: Alternative New World Primate Models for Non-AIDS Research

Overview of New World Primates

Christian R. Abee, University of South Alabama

Indigenous to Central and South America, New World Monkeys are not as closely related genetically to humans as are Old World monkeys. However, their genetic divergence creates biological characteristics that make New World monkeys uniquely suitable for studying certain human conditions, including infectious diseases like malaria.

Workshop participants focused on four types of New World monkeys that have been important to biomedical research: the squirrel monkey and owl monkey (family Cebidae), and the tamarin and marmoset (family Callitrichidae).

Availability. Prior to the mid-1980s, biomedical scientists in the United States could readily import New World monkeys, which were widely used to study malaria, behavior, and virus-induced cancers. However, most South American countries have since banned the export of nonhuman primates, and many of these animals have become nearly impossible to obtain in the United States. A few US breeding colonies produce New World monkeys—including squirrel monkeys and marmosets—but their availability to biomedical researchers remains very limited.

In general, workshop participants agreed that lack of availability has muted the demand for New World species in US biomedical research. However, increased supply of these animals could renew their study and enhance their usefulness as biomedical models, perhaps ultimately helping to alleviate demands for rhesus monkeys.

Care and Handling. New World monkeys require different care and husbandry than Old World monkeys, including an emphasis on routine and regular handling. The animals are easily stressed by environmental changes, leaving them vulnerable to disease. Improper handling has given New World monkeys a reputation as frail creatures in captivity; however, they are hardy if habituated to procedures and housed in suitable settings.

Advantages of New World Monkeys. Squirrel monkeys, owl monkeys, tamarins, and marmosets all share the following characteristics, which make them suitable for biomedical research:

- *Small size.* Because these animals are generally smaller than the Old World primates, they can be more economical to feed and house, requiring smaller cages than the larger primates.
- *Pathogens.* Unlike Old World monkeys, New World monkeys do not carry herpes B-virus, which can be deadly to human handlers.

Disadvantages of New World Monkeys. Among the potential drawbacks of using New World primates in biomedical research are:

- *Limited availability.* Many countries have banned the export of their native nonhuman primates, and existing animal colonies in the United States have only limited numbers of New World primates. Marmosets, however, are bred commercially in the United States and Europe, and their domestic production could be ramped up to meet scientific demand.
- *Small size.* Although their diminutive size offers advantages, it also makes New World monkeys inappropriate for some studies that require extensive blood work or delicate surgical procedures.

Squirrel Monkeys and Owl Monkeys (Family Cebidae)

Christian R. Abee, University of South Alabama

Squirrel Monkeys

Dr. Abee heads the NCRR-supported Squirrel Monkey Breeding and Research Resource, established in 1980 at the University of South Alabama in Mobile. The resource currently houses about 450 squirrel monkeys, making it the largest breeding colony of these animals in the United States.

Of the species of squirrel monkey used in biomedical studies, the Bolivian subspecies (*Saimiri boliviensis boliviensis)* is considered the best biomedical research model, and it is the type most prevalent at the Alabama resource. Only one species of squirrel monkey—S*aimiri oerstedii*—is considered to be endangered.

Before their exportation from South America was banned, squirrel monkeys were used extensively for biomedical research; about 3,000 animals per year were imported to the United States in the early 1980s. Today the primary US source for Bolivian squirrel monkeys is the Alabama resource.

Scientific Demand. In the last 3 years, about 1,400 papers published in peer-reviewed biomedical journals have cited use of the squirrel monkey. Dr. Abee suspects this number would increase dramatically if the animals were more readily available to scientists.

In the past 3 years, the Alabama resource has seen a doubling of formal requests for squirrel monkeys. Dr. Abee estimates that about 300 animals could easily be placed in biomedical studies each year, and demand would increase even more if the animals were more readily available. The squirrel monkey's relatively small body size makes it a practical model for drug studies. Pharmaceutical companies have long depended on squirrel monkeys for psychopharmacology and other research.

Availability. The Squirrel Monkey Breeding and Research Resource in Alabama records about 115 live births per year. But to maintain a self-sustained breeding colony, only about 60 to 70 of these animals can be harvested each year for biomedical studies.

Although the animals cannot be imported from South America, Dr. Abee has been working with the government of Bolivia to reach a collaborative arrangement that might overcome the ban on exportation. The primary obstacle, however, is funding limitations—both for development of a breeding colony in Bolivia and for expansion of US facilities to handle an influx of new animals.

Quick Facts

- *Seasonally polyestrus*. Female squirrel monkeys have a cluster of ovulatory cycles for about 3 months each year. The animals are in an anestrus state for the remainder of the year.
- *Gestation.* About 150 days.
- *Live-birth rate.* Mature females at the Alabama resource produce about 0.65 live offspring per year.
- *Sexual maturity.* Females begin breeding at about 2.5 to 3 years of age.
- *Social groups.* In captivity, squirrel monkeys can be housed in harem groups, with one or two males per group (or more, in larger living spaces). Group size can range from a single pair up to 35-50 animals, depending on available housing.
- *Pathogens.* Squirrel monkeys carry two natural viruses: herpesvirus saimiri 1 and herpesvirus saimiri 2 (previously known as herpesvirus tamarinis). Neither appears to infect humans. Herpesvirus saimiri was the first oncogenic virus found in primates. Although it produces no disease in squirrel monkeys, it produces a lymphoma leukemia in other New World primates.

Research Areas/Models for Human Disease

- *Aging.* The National Institute on Aging's studies of calorie-restricted animals include a small colony of squirrel monkeys, as well as a larger colony of rhesus monkeys. Participating squirrel monkeys are now more than 16 years old.
- *Central Nervous System*. The brain/body weight ratio is similar to that of humans.
- *Creutzfeldt-Jakob disease (CJD).* Squirrel monkeys are susceptible to all of the spongiform encephalopathies, including Creutzfeldt-Jakob disease (known as mad-cow disease in infected livestock). The squirrel monkey predictably develops clinical signs of CJD between 20 and 22 months after inoculation, which is a shorter incubation period than with the rhesus monkey.
- *Genetic characterization.* Satellite markers are available. Maternal pedigrees have been developed for the colony in Alabama, and Dr. Abee expects the entire colony will be pedigreed within the next 5 to 6 years.
- *Leishmaniasis and Chagas diseases.* Squirrel monkeys are susceptible to the infectious agents that cause both of these tropical diseases. About 10% of imported Bolivian squirrel monkeys tested were found to be naturally infected with *Trypanosoma cruzi,* the causative organism of human Chagas disease. Squirrel monkeys also develop the fibrous cardiomyopathy characteristic of the human disease.
- *HTLV1.* Recent papers have shown that squirrel monkeys infected with HTLV1 can develop a persistent viremic state, making it a possible model for vaccine development.
- *Malaria.* Each year an estimated 1 million to 2 million people worldwide die from malaria, and about 300 million to 500 million develop the disease. In Africa, half the deaths of children under 5 years of age are caused by malaria.

Both the squirrel monkey and the owl monkey are unique animal models for malaria, since they are among the few animals that can be infected with human malarial parasites—including *Plasmodium falciparum* (which causes the most severe human malaria) and *P. vivax* (the most prevalent human malaria). In contrast, most other animals, including Old World primates, can only be infected with nonhuman malarial parasites that are specific to their species. Both squirrel and owl monkeys are therefore in great demand for malaria vaccine research. Some immuno-

logical reagents have been developed for such studies (e.g., markers for peripheral T-cells, assays), but they are less extensive and well-developed than those available for the rhesus macaque.

- *Parkinson's disease.* The original research that identified MPTP as a neurotoxin that causes Parkinson's disease was done in the squirrel monkey. MPTP induces a parkinsonian state in the animals that has enabled study of the underlying mechanisms and potential therapies for Parkinson's disease.
- *Periodontal disease.* Periodontal disease is prevalent in captive-housed squirrel monkeys, a much-studied animal model.
- *Pelvic Organ Prolapse.* As in humans, the incidence of POP in squirrel monkeys increases with age, parity, and infant weights.

Owl Monkeys

The genus *Aotus* is found throughout the Amazon basin and the southern part of Central America. The most commonly used species in biomedical research is the Peruvian red-necked owl monkey (*Aotis nancymae),* about 300 of which are housed at the University of South Alabama primate center. It is one of the nation's largest colonies of owl monkeys.

Monogamous pairing of owl monkeys necessitates relatively large breeding colonies compared with other primate species. However, this drawback may be offset by the ability of female owl monkeys to get pregnant more than once per year.

Availability. Because export of these animals has been banned, owl monkeys are in extremely limited supply for biomedical research.

Quick Facts

- Nocturnal. Unique as the only nocturnal simian primate.
- *Nonseasonal breeding.* Breeds throughout the year.
- *Gestation.* About 150 days.
- *Sexual maturity.* Females usually begin to cycle at about 2 to 2.5 years of age.
- *Birth.* Usually gives birth to single offspring, at intervals of about 13 months.
- *Social groups.* Live as monogamous pairs with offspring (also true of marmosets and tamarins).

Research Areas/Models of Human Disease

- *Malaria.* Some types of owl monkeys are considered the best animal models for human malaria—especially *P. falciparum.*

Overview of Tamarins and Marmosets (Family Callithrichidae)

Suzette Tardiff, Southwest National Primate Research Center

Tamarins and marmosets are exceptionally small primates, ranging from the pygmy marmoset (*Cebuella pygmaea:* about 120-130 grams) to the lion tamarin (*Leontopithecus rosalia:* about 700 grams). They are the only simian primates that routinely produce more than one young at a time. Fetuses within a litter share a blood supply, making them hematopoietic chimeras—a characteristic that has proven useful in some areas of research. Recent research suggests that marmosets may be chimeric for somatic tissues beyond hematopoietic tissues. Such a finding has even more application for studying immunological tolerance and autoimmune diseases, such as multiple sclerosis, ulcerative colitis, and type 1 diabetes.

Quick Facts. *Social groups.* Marmosets and tamarins have a cooperative breeding system (older offspring and subordinate adults carry and care for the young of the dominant pair), so male-female pairs, families, and mixed-sex groups provide optimal captive groupings.

Advantages of Tamarins and Marmosets. Besides the advantages they share with other New World primates—including small size and lack of susceptibility to herpes B-viruses, the Callithrichidae family also offers the following benefits to biomedical research:

- *Fertility.* They are unusually fecund in captivity, relative to other primates. In a healthy colony, tamarins and marmosets produce offspring with an efficiency rate of 1.5 to 2.8, compared with 0.65 to 0.85 efficiency rates in most other primate species.

Research Areas/Models for Human Disease

- *Aging.* Because their lifespan is relatively short, both tamarins and marmosets are suitable for aging research.
- *Transplantation/immunology.* The hematopoietic chimerism of twins has made these animals useful in studies of transplantation and associated immunology.

Tamarins

Suzette Tardif, Southwest National Primate Research Center

Tamarins are indigenous to South America, ranging from southern Amazonia to Panama. Tamarin usually refers to two genera—*Saguinus* and *Leontopithecus* (lion tamarin), although the latter is endangered and plays no role in biomedical research. *Saguinus* includes more than a dozen species, but only four have been important to US biomedical research:

Cotton-top tamarin (*Saguinus oedipus),* which is geographically and phylogenetically distinct from the three species below. Native only to Columbia, the cotton-top tamarin is unusual because it lives in northern deciduous forest.

Saddleback tamarin *(Saguinus fusicolis),* which is the smallest tamarin. *S. fusicolis* is native to the Amazon, as are the other two species below.

Moustache tamarin *(Saguinus mystax).*

White-lipped tamarin *(Saguinus labiatus).*

Availability. There is one remaining large colony of cotton-top tamarins in the United States, located at the NCRR-supported New England NPRC. Because this species is not available from other sources, Dr. Tardif suggests that it is important to continue to maintain this captive breeding colony. Although it may never again be widely used by biomedical researchers, studies of the cotton-top tamarin may offer unique insights into hepatitis C, colitis, and colon cancer.

Research Areas/Models for Human Disease. *Saguinus* was an important research primate from the 1960s to the early 1990s in the United States. Today, however, they are used primarily in only two research areas:

- *Colitis and colon cancer.* Ongoing studies of these disorders in the cotton-top tamarin are defining the natural history and pathology, examining the role of environmental factors, and searching for relevant infectious agents. Scientists estimate that about 20 to 50% of tamarin deaths in US research colonies in the 1980s and 1990s were caused by colon cancer. Recent published papers have fingered *E. coli* and a new helicobacter species as playing a role in colitis and possibly contributing to the development of colon cancer in these animals.
- *Hepatitis and viral oncology.* A virus known as GBV, primarily studied using *S. labiatus* and *S. mustax,* is able to infect cotton-top tamarins and serves as an excellent model for human hepatitis C. This characteristic is important, because there are no other good animal models (besides the chimpanzee) for this disease. The cotton-top model has been used to test potential antiviral compounds and may enable development of rapid screening tests for hepatitis C virus.

Comparison with Marmosets. Although the tamarin has a distinctive niche in biomedical research, Dr. Tardif suggests that the common marmoset is actually a more suitable animal model for biomedical studies. Common marmosets have better reproductive performance, they are better able to withstand variations in management and handling, they are more readily available, and they are not endangered.

Marmosets

David Abbott, Wisconsin National Primate Research Center

Marmosets are indigenous to Brazil. Marmoset usually refers to one genus, *Callithrix,* which includes nine species segregated into two groups:

Atlantic coastal forest species, subdivided into the "Aurita" group (*C. aurita and C. flaviceps*) and "Jacchus" group or "true marmosets" (*C. penicillata, C, jacchus, C. kuhli, and C. geoffroyi*).

Amazonian species or "Argentata" group (*C. argentata, C. humeralifer, C. emiliae*).

Only one species, however, has been important to US biomedical research: the common marmoset (*Callithrix jacchus).* This species is native to the Atlantic coastal forests and scrub of northeastern Brazil.

The common marmoset has been widely used in Europe for biomedical research since the 1960s. Today, it is the primate of choice in European biomedical studies, in part because it is more widely available there and is less expensive to care for than the macaque. Although marmosets are not suitable for AIDS research and could not entirely replace the macaque, marmosets have characteristics that make them uniquely suited for particular areas of biomedical research.

Availability. Europe has several commercial suppliers, and several marmoset breeding facilities have recently been established in the United States.

Quick Facts

- *Social groups.* Marmosets live in families with a high degree of infant parental care by adult males and females, which is why they cannot be singly housed. These animals form bonds that must be preserved in order to provide quality care and ensure infant survival.
- *Gestation.* About 145 days for the common marmoset.
- *Multiple offspring.* Breeding females typically produce dizygotic twins every 6 months. There is no reproductive seasonality in this species.
- *Sexual maturity.* Both sexes mature within 1.5 years.
- *Lifespan.* About 12 to 14 years.

Advantages of Marmosets

- *Reasonable housing space*. Compared with larger primates, marmosets can be housed in cages with much less floor space; they can also be housed socially. Because marmosets are tree-dwellers, they prefer vertical cages. Therefore, the Wisconsin NPRC has obtained a waiver from the USDA regarding the requirements for cage floor space.
- *Easy handling.* If handled routinely, marmosets are very tractable and responsive.
- *Economical.* Marmosets require less food and use of materials.
- *Reproductive capacity.* Of all the primates discussed at the Workshop, marmosets have the greatest reproductive capacity by far, which could enable rapid increase in animal numbers.

Disadvantages of Marmosets

- Historical data are lacking to judge toxicological outcomes of drug studies effectively. However, pharmaceutical companies in Europe are now building up databases to demonstrate the appropriateness of drug testing in marmosets.
- *Gastrointestinal problems.* Inflammatory bowel disease and other gastrointestinal disorders plague marmoset colonies.

Research Areas/Models of Human Disease

- *Adrenal dysfunction.* Since the adrenal cortex of the marmoset has a fetal zone at birth, but a poorly developed inner zone (zona reticularis) in adulthood, it is

providing new insight into the molecular regulation of adrenal hormones key to the stress response.

- *Aging.* Marmosets maintain bone mass during age-associated estrogen depletion. They also apparently lack reproductive senescence.
- *Behavior.* Marmosets have been used to study the neural basis of parental neglect and abuse of offspring. Unlike rhesus monkeys, if marmosets fail to rear younger offspring, even though they're socialized with their parents, they can become neglectful and abusive parents themselves.
- *Infectious disease.* Marmosets are extremely susceptible to human herpes simplex virus and other common human pathogens.
- *Multiple sclerosis.* Marmosets can be immunized against human myelin and thereby develop an autoimmune response similar to human multiple sclerosis.
- *Neurobiology.* Although the marmoset brain is small, it is still 2% of body weight and is typical of the relatively large size of primate brains compared with that of rodents (0.5% of body weight). However, the smooth cerebrum of the marmoset lacks the sophisticated folding of the macaque and other primate brains, which limits the type of research that can be performed (e.g., marmosets cannot complete the two-step puzzle task, which a rhesus monkey can easily master).

Yet the marmoset offers a unique advantage for noninvasive imaging of primate brain function (e.g., functional MRI [fMRI] and micro-PET) because marmosets can be trained to sit still in the scanner without sedation. The small, enclosed space of a brain scanner resembles the marmoset's nest box, where they often fall asleep, and has allowed scientists to take functional brain images, using a 9.4 tesla fMRI scanner, on conscious male marmosets.

- *Obesity.* Marmosets provide a model for viral-induced adiposity, a problem in about 10% of humans. This condition cannot be studied in the rhesus macaque since most rhesus are already infected with human viruses that predispose them to adiposity.
- *Parkinson's disease.* The MPTP model is also used in the marmoset. Recent studies are examining the effects of embryonic cell transplantation in treating Parkinson's disease and stroke.
- *Reproduction.* Studies include development of transgenic marmosets, male contraception, and social regulation of female fertility and fecundity.

Appendix 1. WORKSHOP SPEAKERS AND ATTENDEES

Workshop on Demands for Rhesus Monkey in Biomedical Research
April 19-20, 2002
Washington, DC

Speakers

David H. Abbott, Ph.D.
Professor
Department of OB/GYN
Wisconsin National Primate Research Center
University of Wisconsin
Madison, WI

Christian R. Abee, D.V.M.
Professor and Chair
Department of Comparative Medicine
University of South Alabama
Mobile, AL

Lynn A. Fairbanks, Ph.D.
Professor
Neuropsychiatric Institute
University of California
Los Angeles, CA

Jay R. Kaplan, Ph.D.
Professor of Pathology and Anthropology
Department of Comparative Medicine
Wake Forest University School of Medicine
Winston-Salem, NC

Martha L. Marthas, Ph.D.
Associate Professor
California National Primate Research Center
Davis, CA

Bonnie Mathieson, Ph.D.
Office of AIDS Research
National Institutes of Health
Bethesda, MD

William Morton, V.M.D.
Director
National Primate Research Center
University of Washington
Seattle, WA

Jerry A. Robinson, Ph.D.
Director
National Primate Research Centers Program
National Center for Research Resources
National Institutes of Health
Bethesda, MD

Suzette D. Tardif, Ph.D.
Associate Director
Southwest National Primate Research Center
San Antonio, TX

Anita M. Trichel, D.V.M., Ph.D.
Research Assistant Professor
Department of Molecular Genetics and Biochemistry
University of Pittsburgh
Pittsburgh, PA

John L. VandeBerg, Ph.D.
Scientific Director
Southwest National Primate Research Center
San Antonio, TX

Jon Warren, Ph.D.
Director
Innovation Grants Program
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda, MD

Attendees

Gary Baskin
Tulane National Primate Research Center

J. Gregory Beattie
Sierra Biomedical, Charles River Laboratories

Jack Bley
Pharmacia Kalamazoo, Michigan

Tom DeMarcus
Centers for Disease Control and Prevention

Richard Eberle
Oklahoma State University College of Veterinary Medicine

Gale Galland
Centers for Disease Control and Prevention

Gary Heckman
Oregon National Primate Research Center

Edmundo Kraiselburd
Caribbean Primate Research Center

Marta Leon-Monzon
Office of AIDS Research, NIH

Keith Mansfield
New England National Primate Research Center

Michael Murphy-Corb
University of Pittsburgh

Jason M. Mwenda
I.P.R., Kenya

Nancy Nadon
National Institute on Aging, NIH

Ray O'Neill
National Center for Research Resources, NIH

Barbara Perrone
National Center for Research Resources, NIH

Jeffrey Roberts
California National Primate Research Center

Pete Schultheiss
US Army, Department of Defense

Gary L. White
Oklahoma University Health Science Center

Sarah Williams-Blangero
Southwest Foundation for Biomedical Research

Appendix 2. Workshop Agenda

Workshop on Demands for Rhesus Monkeys in Biomedical Research

Friday, April 19, 2002

1:00 – 1:15 p.m. Welcome and Charges to Participants

1:15 – 2:00 Session 1: Rhesus Resource Needs, Availability, and Limitations

A. Overview of NCRR-supported Rhesus Resources, Dr. Jerry Robinson, NCRR US Rhesus Resources:
   1) US Rhesus Resources
   2) NIH Grantee NHP Utilization (NCRR Survey)
   3) Current NCRR Activities To Address Macaque Shortages

B. Overview of Specific Monkey Needs for AIDS Research, Dr. Bonnie Mathieson, OAR
   1) AIDS Vaccine Development
   2) AIDS Pathogenesis

2:00 – 3:00 Session 2: Alternative Nonhuman Primate Animal Models for AIDS Research

1) NIAID Workshop Summary, Dr. Jon Warren, NIAID
2) Indian versus Chinese Rhesus, Dr. Marta Martas, California NPRC
3) Cynomolgus Macaques, Dr. Anita Trichel, University of Pittsburgh

3:00 – 3:15 Coffee Break

3:15 – 5:15 Session 3: Alternative Old World Primate Models for Non-AIDS Research

1) Cynomolgus macaques, Dr. Jay Kaplan, Wake Forest University
2) Pigtailed macaques, Dr. William Morton, Washington NPRC
3) Baboons, Dr. John VandeBerg, Southwest NPRC
4) African Green Monkeys (Vervets), Dr. Lynn Fairbanks, UCLA

Saturday, April 20, 2002

7:30 – 9:00 a.m. Session 4: Alternative New World Primate Models for Non-AIDS Research

1) Squirrel and Owl Monkeys, Dr. Chris Abee, University of South Alabama
2) Tamarins, Dr. Suzette Tardif, Southwest NPRC
3) Marmosets, Dr. David Abbott, Wisconsin NPRC

9:00 – 9:10 Charges to Working Groups

9:15 – 11:15 Working Group Sessions

11:15 – 12:15 Reports with recommendations from the Working Groups

12:30 Adjourn