Cynomolgus Macaques (*Macaca fascicularis*) in Biomedical Research

Overview

Cynomolgus macaques are also known as crab-eating macaques or long-tailed macaques. They are one of the most widely used nonhuman primate species in research. Cynomolgus are slightly smaller than rhesus macaques and have significantly longer tails (1). Their coats range in color from light red-brown to gray and vary depending on the subspecies (2, 3). Male cynomolgus macaques may be distinguished from females by their facial hair. Males have mustaches and cheek whiskers; females have cheek whiskers and beards (3).

Cynomolgus macaques are the most widely used macaque contributing to pharmaceutical discoveries and toxicology research. They have also enhanced our understanding of reproductive biology, behavior, oncology, diabetes, cardiovascular disease, and infectious diseases (3).

Natural History

Cynomolgus macaques are native to Southeast Asia. They can be found in Bangladesh, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Singapore, the Philippines, Indonesia, and Timor-Leste. Introduced populations are present in Mauritius, Palau, and Papua New Guinea (4). Cynomolgus macaques live in a wide variety of habitats including mangrove, rainforest, swamp, shrubland, coastal, deciduous, and evergreen forests. The availability of significant food resources attracts cynomolgus macaques to human settlements (5). Approximately two-thirds of their diet consists of fruits (6). Other food items include leaves, flowers, insects, mollusks, and crustaceans (6, 7). Cynomolgus macaques live in large social groups containing around 3-20 females, their offspring, and 1-14 males (8). The females have a clear dominance hierarchy (8). In contrast to rhesus macaques, they are nonseasonal breeders (12).

There are ten different subspecies of cynomolgus macaques (3, 4). The geographic origin of cynomolgus macaques is an important consideration for study design (3). There are four major genetic populations of cynomolgus macaques: the Indonesian-Malaysian, Indochinese, Philippine, and Mauritian (9). Different populations display different genetic features. Ideally, researchers should select animals from the same geographic origin to avoid confounding variables. The Mauritian cynomolgus macaque population has particularly unique genetic features since they are descended from a very small founder population that was introduced to the island about 500 years ago (10). Compared to all other populations of cynomolgus macaques, they show very minimal variation in a particular class of genes called the “major histocompatibility complex” (10, 11). This

genetic feature is especially helpful for researchers studying cellular immune responses and organ transplantation (11, 12).

Research Contributions

The whole genome for cynomolgus macaques was first sequenced in 2012 (13). This discovery has helped us understand the genetic differences between cynomolgus macaques, rhesus macaques, and humans and has laid the foundation for understanding genetic influences on disease processes and drug metabolism.

Reproductive studies*:*

Nonhuman primates are ideal for studying reproductive diseases, given their similarities to women in anatomy and physiology. Macaques have a true menstrual cycle, unlike rodents (14). Since cynomolgus macaques are non-seasonal breeders, it is easier to study reproduction and developmental biology in a cynomolgus model compared to rhesus macaques. Cynomolgus macaques have been used to establish safety and efficacy of contraceptives (15). Uterine transplantation has also been performed in cynomolgus macaques, and recently the first successful delivery with a transplanted uterus has been documented (16,17).

Pharmaceutical studies*:*

Cynomolgus macaques offer unique advantages in ensuring that new drugs are safe. Their similarities in reproductive physiology to humans allow us to ensure that any new therapeutics will not have adverse effects on the developing fetus.

Cynomolgus macaques have been particularly helpful in the discovery of numerous monoclonal antibody drugs (18). Monoclonal antibodies are designed to target very specific portions of chemical markers within the body. They have been developed as treatments for multiple cancer types, asthma, and rheumatoid arthritis, as well as for the prevention of organ transplant rejection (18). Cynomolgus macaques are ideal for studies of these drugs' safety and efficacy because many of their target molecules are remarkably similar to the targets in humans (18).

***Infectious Disease*:**

Cynomolgus macaques have improved our understanding of numerous infectious diseases. Research with cynomolgus macaques has helped us understand the cells targeted in Ebola virus infection and has shown that monoclonal antibodies can be an effective treatment (19, 20). Cynomolgus macaques have been the best model to replicate the different disease states of tuberculosis seen in human infection (21, 22, 23).

Cynomolgus macaques helped prove that Severe Acute Respiratory Syndrome (SARS) is caused by the SARS-CoV virus (24). SARS is a respiratory disease that emerged in 2003, producing symptoms ranging in severity from flu-like signs to pneumonia, with a case fatality rate of 11% (25). More recently, cynomolgus macaques have contributed to

our understanding of the disease processes occurring in COVID-19, caused by SARS-CoV-2 virus (26, 27).

***Cardiovascular studies**:*

Cynomolgus macaques have contributed to decades of research on arterial atherosclerosis. Atherosclerosis is a disease in which cholesterol, lipids, and other substances build up in the walls of arteries, causing them to harden and narrow. Cynomolgus macaques develop coronary artery atherosclerosis analogous to the disease process in humans when they are fed diets containing large amounts of fat and cholesterol (14, 28, 29). Specifically, the cynomolgus atherosclerosis model has been helpful for understanding sex differences in the progression of this disease (14, 28-32). Studies with cynomolgus macaques have demonstrated that presence of estrogen plays a key role in preventing the development of coronary artery atherosclerosis (14, 29).

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