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Nonhuman Primates in Translational Research

Alice F. Tarantal[1,2], **Stephen C. Noctor**[3], **Dennis J. Hartigan-O'Connor**[2,4]

[1]Departments of Pediatrics and Cell Biology and Human Anatomy, University of California, Davis, California, USA;

[2]California National Primate Research Center, University of California, Davis, California, USA

[3]Department of Psychiatry and Behavioral Sciences, University of California, Davis, California, USA;

[4]Medical Microbiology and Immunology, School of Medicine, University of California, Davis, California, USA;

Abstract

Nonhuman primates are critically important animal models in which to study complex human diseases, understand biological functions, and address the safety of new diagnostics and therapies proposed for human use. They have genetic, physiologic, immunologic, and developmental similarities when compared to humans and therefore provide important preclinical models of human health and disease. This review highlights select research areas that demonstrate the importance of nonhuman primates in translational research. These include pregnancy and developmental disorders, infectious diseases, gene therapy, somatic cell genome editing, and applications of in vivo imaging. The power of the immune system and our increasing understanding of the role it plays in acute and chronic illnesses are being leveraged to produce new treatments for a range of medical conditions. Given the importance of the human immune system in health and disease, detailed study of the immune system of nonhuman primates is essential to advance preclinical translational research. The need for nonhuman primates continues to remain a high priority, which has been acutely evident during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) global pandemic. Nonhuman primates will continue to address key questions and provide predictive models to identify the safety and efficiency of new diagnostics and therapies for human use across the lifespan.

Keywords

preclinical models; immune system; developmental disorders; infectious diseases; gene therapy; in vivo imaging

1. INTRODUCTION

Animal models are essential to study complex human diseases, understand biological functions, and address the safety and efficiency of new treatments proposed for human

aftarantal@ucdavis.edu .

use. Nonhuman primates are uniquely similar to humans from genetic, physiologic, immunologic, reproductive, and developmental perspectives and thus provide important models of human health and disease. Although other animal models can provide mechanistic insights, nonhuman primates, particularly Old World species, more closely simulate the human condition. Nonhuman primates and humans share many characteristic features because of their close phylogenetic relationship, which aids in overcoming the roadblocks to clinical translation.

The objective of this review is to highlight select topics that demonstrate the importance of nonhuman primates for translational research. Areas of focus include pregnancy and developmental disorders, infectious diseases, gene therapy, somatic cell genome editing, and in vivo imaging applications. The power of the immune system and our increasing understanding of it are being leveraged to produce new treatments for medical conditions including cancer, infectious diseases, metabolic disorders, and those related to the maternal–placental–fetal interface. Groundbreaking therapeutic approaches, such as gene therapy and somatic cell genome editing, depend on a nuanced understanding of the immune system and often on evasion of immune responses to therapeutic proteins. Given the importance of the human immune system in health and disease, detailed study of the immune system of nonhuman primates is needed to advance translational research.

This article is not intended to provide an exhaustive historical account or an all-inclusive review of research conducted with nonhuman primates. The most frequently used Old World species such as macaques (e.g., rhesus, *Macaca mulatta*; cynomolgus, *Macaca fascicularis*) and baboons (*Papio* spp.) are highlighted. New World species such as the common marmoset (*Callithrix jacchus*) are included as models in biomedical research. The rhesus monkey has remained the predominant species used and has a multitude of reagents available (https://www.nhpreagents.org). The authors acknowledge that many outstanding publications could not be included; readers are referred to the literature for additional information.

2. IMPORTANCE OF NONHUMAN PRIMATES

2.1. Reproduction

Old World species have a menstrual cycle similar to that of humans. Rhesus monkeys are seasonal breeders with an ~30-day menstrual cycle, and menopause occurs in several primate species; thus, they are excellent models for studies focused on female reproduction and women's health across the lifespan. Readers are referred to excellent reviews on this topic (e.g., 1). Male reproduction, particularly restoration of functional sperm production using spermatogonial stem cell transplantation, has been an active area of investigation (2).

Reproductive senescence has been studied in rhesus, although it has been challenging to distinguish seasonal acyclicity and amenorrhea from reproductive senescence (3). Shideler et al. (4) addressed the interaction of seasonality and aging with a focus on endocrine parameters. Some older females begin to exhibit abnormal ovarian function at 18 years of age, but others were shown to continue to have normal menstrual cycles until 25 years of age. Baboons breed throughout the year, with an average menstrual cycle length of ~33 days. Based on menstrual cycles, increased variation begins at ~19 years of age, whereas total

cessation of cyclicity generally occurs at ~26 years of age. The common marmoset has an approximate 28-day ovarian cycle and does not experience menopause (5).

Trimesters in rhesus monkeys are 55 days in length, with the first trimester representing 0–55 days gestation, the second trimester 56–110 days gestation, and the third trimester 111–165 days (term 165 ± 10 days). Cynomolgus are similar in length of gestation, whereas the baboon has an ~180-day gestational length and the common marmoset has a gestational length of ~150 days. The rhesus monkey placenta is chorioallantoic, discoid, villous, deciduate, and hemochorial. In humans and rhesus monkeys, trophoblasts erode through the maternal endothelium and are in direct contact with maternal blood, which results in the hemochorial categorization (6). In humans, the embryo is completely embedded within the uterine stroma, whereas in the monkey, the blastocyst remains superficially attached and adheres to the side opposite the initial attachment, forming the location of the future secondary placental disk (~80% bidiscoid placenta). The baboon embryo also implants superficially, with typically a monodiscoid, villous hemochorial placenta (7). Marmoset placentas differ from the placentas of humans and macaques in the organization of structural elements, particularly at the maternal–fetal interface (8).

2.2. Fetal and Maternal Microchimerism

Studies in humans have shown that during pregnancy fetal cells can enter the maternal circulation and persist for many years (fetal microchimerism) (9). The presence of cell-free fetal DNA during pregnancy in rhesus monkeys has been shown with gestational and postnatal dynamics that parallel findings in humans (10, 11). Similar to findings in humans, trafficking and persistence of maternal cells in the rhesus monkey fetus (maternal microchimerism) was demonstrated using a panel of validated major histocompatibility complex (MHC) sequence-specific quantitative polymerase chain reaction (PCR) assays; maternal microchimerism was also shown in postnatal tissues. Thus, the rhesus provides an excellent model for the study of fetal and maternal microchimerism, as well as the relationship to health and disease.

2.3. Immune Ontogeny

The primate immune system leverages a vast array of effector mechanisms that distinguish self from nonself and respond when necessary to eliminate pathogens, as well as deviant cells such as tumor cells. In addition, the system occasionally malfunctions to produce autoimmune conditions that include many prevalent, chronic diseases. Immunologic studies in mice are in many cases not a useful substitute because of the idiosyncrasies of mouse physiology and the inbred strains that are widely used, whereas nonhuman primates are susceptible to many of the same infectious and metabolic diseases (12). Although humanized mouse models are used in human immunodeficiency virus (HIV) and other research, these models suffer from many anatomic and histologic differences, as well as interspecies differences in homing receptors and immune modulators, which collectively restrict the relevance of mouse models to humans (13). Although the highly variable adaptive-immune loci (e.g., MHC) differ between macaques and humans, this difference is far smaller than that between mice and humans (14).

Fetal and pediatric research are particularly constrained in humans; thus, nonhuman primate models are invaluable for such studies. Several descriptive investigations have been undertaken to provide dynamic immunologic information to better understand ontogeny (15). By the second trimester, the rhesus monkey fetus has a complete complement of immune cells that are properly positioned within the developing lymphoid organ compartments. Even at this early stage, the cells appear functionally competent, that is, capable of secreting cytokines and/or immunoglobulins (Igs), as appropriate to the lineage. These findings are consistent with studies showing that human infants can respond to many test antigens or vaccines in a manner similar to adults (16–18). Fetal baboons have shown specific, de novo antibody responses following in utero immunization (19).

To assess the possible protective role that transplacentally transferred anti-rhesus cytomegalovirus (RhCMV) IgG might play in limiting fetal disease, maternal and fetal antibody titers to RhCMV antigens have been analyzed by obtaining paired maternal/fetal blood samples during gestation, at birth, and at one month postnatal age (20). In fetuses from RhCMV seropositive dams, antiviral IgG titers were first detected in the fetal circulation in the early second trimester (~1–2% of maternal titers). Mean titers in the fetus increased to 12.5% of maternal titers by the late second trimester and increased further during gestation to ~50% of maternal titers in the third trimester and at birth, reflecting increased transplacental transfer of maternal IgG. Analyses were also performed in fetuses that were directly inoculated with RhCMV and showed higher mean RhCMV IgG titers at one month postnatal age (30% versus 12% of maternal titers). These results demonstrated that fetuses are immunologically competent for de novo IgG production and that the fetal primate develops some degree of effector function at an early stage, although the contribution of this effector function to protection against pathogens remains to be determined.

CD3 is the earliest lymphoid marker shown to be expressed and was found abundantly in the first-trimester thymus and less abundantly in spleen (15). The myeloid marker and low-density lipoprotein (LDL) receptor CD68 was found on cells near the blood islands in the liver in the late first trimester. Cells expressing the dendritic-cell marker CD205 were noted in the spleen, thymus, and axillary and mesenteric lymph nodes in the mid-second trimester but not until the third trimester in the gastrointestinal tract. B cell markers were not observed until midway through the second trimester, when they appeared in liver, spleen, thymus, lymph nodes, bone marrow spaces, and occasionally gut-associated lymphoid tissue. Expression of CD3 and other T cell markers was maintained during this time in liver, spleen, and thymus as well as in Peyer’s patches of the jejunum, ileum, and colon; by the early second trimester, early lymphocyte aggregate formation was evident. Of note, the T cell markers that were examined were not found in the bone marrow medullary spaces at any gestational age, first appearing at three months postnatal age. Ig- and cytokine-secreting cells (i.e., potentially functional cells) were first detected in the early second trimester. Thus, by the second trimester, the lymphoid tissues of the rhesus monkey fetus have a reasonably complete repertoire of properly organized B cells, T cells, and antigen-presenting cells, which are at least partly functional. Expression of CCR5 was of interest due to its role as a coreceptor for HIV and the potential for in utero transmission of the virus. In both the

second and third trimesters, CCR5 expression was abundant in the submucosa and lamina propria of the gut and in Peyer's patches.

These many similarities to human immune ontogeny have been leveraged to provide insights into fetal/maternal infectious diseases and preterm birth, particularly when the latter is triggered by chorioamnionitis or intrauterine inflammation. In the Zika virus field, the fetal monkey model has proven essential for studying neuroinflammation and compromise of the blood–brain barrier (21, 22). Fetal monkeys also represent the most relevant model of preterm birth, where they have been used, for example, to model and study the contribution of interleukin-1 signaling (23) and the role of hyaluronidase in promoting bacterial invasion and preterm labor (24).

2.4. Immune Senescence

Aging is associated with changes in immune function that encompass defective effector function, inflammation, and engagement of suppressor pathways. Part of the increased inflammation may be due to increased permeability of mucosal barriers, leading to translocation of bacteria and/or bacteria-associated molecular patterns that engage the immune system (e.g., lipopolysaccharide, LPS). Adaptive immunity undergoes even more profound change with aging. Both humans and macaques experience thymic involution (or atrophy) in which thymic epithelial tissue is replaced with adipose tissue, resulting in decreasing T cell export. In humans, this process is thought to begin as early as one year after birth (25). The result is exponentially declining T cell production over time, with the rate of production having an apparent half-life in humans of ~15.7 years. Aging is also associated with progressive accumulation of memory T cells, particularly highly differentiated effector-memory $CD8^+$ T cells, which consequently leads to an inverted CD4/CD8 ratio (26–28). Moreover, aging is accompanied by a significant reduction in T and B cell repertoire diversities, partly related to expansion of adaptive cells specific for lifelong persistent viral infections such as CMV or Epstein-Barr virus (28, 29). The foregoing features of adaptive immune aging, defined as the immune risk profile, have been shown to occur in aging Chinese rhesus macaques (30). It would seem apparent that a long-lived species with genetic and physiological similarity to humans, such as the rhesus, offers distinct advantages for immunosenescence research (31). Indeed, existing data have supported the idea that rhesus monkeys offer a robust translational model of age-related changes in immunity. In aged macaques, as in humans, the representation of naïve T cells declines and the profile shifts toward a $CD95^+$ memory phenotype, as described in humans (32, 33). Although the effects of aging on macaque B cells have been less well described, a profound decrease in $CD20^+$ B cells with age has been shown (34).

The mutually reinforcing impacts of aging and CMV infection on immunity have also been investigated in nonhuman primates (35). CMV-specific T cell immunity is maintained in aged rhesus monkeys, including a dominant effector-memory phenotype; identical patterns of IFN-γ, TNF-α, and IL-2 production and cytotoxic degranulation; and comparable functional avidities (33).

2.5. Complex Nature of the MHC Loci

The MHC encodes highly polymorphic, membrane-bound glycoproteins that contribute to adaptive immunity by presenting linear peptides to T cells, among other functions (36). MHC class I antigens interact with the CD8 molecule found on cytotoxic T cells, whereas class II antigens interact with the CD4 molecule that is associated with helper T cell function. In addition, class I proteins (particularly class Ib proteins such as HLA-E/Mamu-E) can interact with receptors found on the surface of natural killer (NK) cells to regulate their cytotoxicity and/or cytokine production. Most importantly, HLA-E/Mamu-E presents self-peptides derived from class Ia molecules, attesting to proper expression of these needed molecules in the cell and inhibiting NK cell activation. Class II molecule expression is restricted predominantly to lymphoid cells, including B cells, monocytes, macrophages, endothelial cells, dendritic cells, and activated T cells.

Both the class Ia and class II genes are often highly polymorphic, presumably to allow collective resistance of the species to a collection of ever-varying pathogens (37). Nonclassical class I and class II antigens (such as HLA-E/Mamu-E), in contrast, are far less polymorphic and may serve distinctly different functions to those of the classical alleles (38). Presentation of HLA leader peptides by HLA-A/Mamu-E is one such distinct function. As one would expect, human MHC gene sequences are more similar to those of macaques than those of mice, but the class I genes are paralogous within each of the three species (39). The abundance of paralogs means that any given human MHC class I allele does not have an exact nonhuman primate counterpart—and the immune responses mediated by that allele are also not exactly replicated by nonhuman primates. In contrast, the MHC class II genes are orthologous between the species.

The Mamu genomic region has 22 class I and 11 class II genes, whereas the human MHC complex carries only 6 class I and 13 class II genes. The Mamu class I region is thus substantially more complex. Interestingly, the orthologs of the human HLA-C gene have not been identified so far in rhesus monkeys or any other Old World species, although some Mamu-B genes appear more closely related to HLA-C than to HLA-B.

2.6. Immunoglobulin Loci

The Ig-encoding genomic loci are the fundamental basis of all antibody responses; thus, the characteristics of these loci in nonhuman primates are important to all vaccine research that is based on antibody generation. As with MHC class I alleles, the macaque Ig loci are more diverse than those in humans, comprising both greater sequence diversity and copy-number variation between individuals. Humans typically carry 38 to 46 different, functional Ig heavy-chain V genes (IGHV); macaques carry a similar number of genes, but the mean pairwise distance between sequences is greater, indicating greater diversity (40, 41). In addition, a large number of distinct macaque alleles were identified in a small study of 10 animals (41), compared to all the human alleles ever identified. Comparing IGHV sequences between species, 5 of 7 sequence families are phylogenetically interdigitated, indicating intrafamily divergence before speciation, whereas 2 families segregate by species, suggesting postspeciation homogenization.

Understanding of species-specific differences is particularly important in influenza and HIV vaccine research, in which certain germline alleles are more likely to give rise to broadly neutralizing antibodies (BNAbs). For example, human IGHV1–69 and IGHV1–2 alleles appear more likely to produce BNAbs against influenza and HIV-1, respectively (42, 43). Most human germline Ig sequences do not have exact orthologs in macaques, unfortunately, although most share 90% or greater identity with one or more genes in the other species. Successful immunization in macaques may therefore proceed via recruitment of B cells carrying similar germline antibody genes to those in humans—or such immunization may proceed by a different pathway, depending on the immunogen.

3. DEVELOPMENTAL DISORDERS

3.1. Developmental Origins of Disease and the Maternal–Placental–Fetal Interface

The developmental origins of health and disease hypothesis, previously known as the Barker hypothesis, proposes that organ systems are shaped prenatally in ways that set the stage for a lifetime of health or disease (44). Although studied primarily in the context of cardiovascular and metabolic disease, the continued rise in allergic and autoimmune conditions highlights the susceptibility of developing immune pathways, with inflammation a common theme for many chronic illnesses (45, 46). It has been shown that obesity promotes a chronic inflammatory state, which in turn may lead to many downstream consequences, such as insulin resistance. Further, a range of factors has been shown to alter epigenetic programming and gene expression in utero, which can have profound implications for the developing phenotype and predisposition to future diseases (47, 48). For example, exposure to a high-fat diet in nonhuman primate species has been shown to result in offspring that are more susceptible to type-2 diabetes, altered leptin sensitivity, nonalcoholic fatty liver disease, hypertension, and obesity (49).

The maternal–placental–fetal interface provides close contact between the uterine mucosa, placenta, and fetal membranes. Inflammation and inflammatory cytokines play a role in placental function; a wide range of cytokines can be synthesized and released by cytotrophoblasts, syncytiotrophoblasts, and resident placental macrophages (50). Immune cells in the decidua (e.g., uterine NK cells) and Toll-like receptors (TLR) expressed by syncytiotrophoblasts stimulate production of interferons in response to viruses and maintain placental function (51). Despite this potential immunologic barrier, microorganisms can sometimes bypass the host adaptive and innate immune system and lead to congenital infection, which has been studied extensively in nonhuman primates.

3.2. Hematopoietic Ontogeny

Comparable to humans, the fetal rhesus monkey liver is the primary site of hematopoiesis in the first trimester (comparable to human ontogeny ~5–6 weeks), with a peak in hematopoiesis thereafter (early second trimester; 3–4 months in humans) until bone marrow hematopoiesis is established in the mid-second trimester. Early signs of bone marrow hematopoiesis begin in the fetal monkey in the early second trimester, with a significant decrease in blood islands in fetal liver. This decline continues into the early third trimester, when bone marrow takes on the primary hematopoietic role (~7 months in humans) (52,

53). The frequency of $CD34^+$ hematopoietic stem/progenitor cells (HSCs) is high in the second trimester, whereas spleen and marrow begin to dominate by the beginning of the third trimester. Similar to findings in human fetuses, there is a gradual decline of $CD34^+$ HSCs in fetal blood in the late second and third trimesters, whereas the frequency of $CD3^+$ T cells remains relatively unchanged later in gestation. Understanding hematopoietic ontogeny has been critical in designing studies focused on hematopoietic stem cell transplantation. Early-gestation in utero stem cell transplantation can avoid immune responses and target early hematopoietic organs such as the liver to treat congenital illnesses such as sickle cell disease, β-thalassemia, and immunodeficiencies. Many of these strategies have used nonhuman primates to address critical questions, such as the quantity of cells for transplant, the timing of delivery, and the route of administration (52, 54).

3.3. Ontogeny of the Kidney and Lung

Both the developing kidney and lung are highly vascularized, with complex branching networks of many specialized cells. These tissues have similar structure in humans and macaques, supporting the rhesus monkey as an excellent model to study normal development, directed differentiation of human and nonhuman primate pluripotent stem cells, and treatment of congenital diseases. Nephrogenesis begins in both species in the first trimester and continues throughout the mid-third trimester, whereas in other species, such as the mouse, nephrogenesis begins in mid-gestation and concludes postnatally. The temporal and spatial expression of key renal developmental markers from early gestation to postnatal life and into adulthood have been shown, and changes in protein and RNA expression patterns identified, indicating strong similarities when compared to humans (55, 56).

To better understand the pathogenesis of kidney disease during development, a rhesus monkey model of unilateral ureteric obstruction was developed (57). In humans, congenital obstruction of the urinary tract during active nephrogenesis results in a well-described pattern of histopathological changes, with architectural disorganization and the development of immature glomeruli, primitive tubules surrounded by fibromuscular collars, interstitial fibrosis and mesenchymal expansion, and cystic transformation of tubules with injury to the developing collecting duct epithelium (58). These well-characterized histopathological features in the human fetus were fully recapitulated in the monkey model and suggest that altered proliferation, differentiation, and/or survival of progenitor cells are involved in pathogenesis, including glomerular injury and decreased nephron endowment, expansion of the interstitial mesenchymal space and fibrosis, tubular atrophy, and apoptosis.

As is true of the kidney, the monkey lung passes through the same developmental stages at similar gestational time points as in humans, and during prenatal and postnatal life human and monkey lungs are still developing (59). At birth, both species are at comparable stages of development; the most peripheral conducting airways are absent, and only a small fraction of the adult population of alveoli is present. Humans and nonhuman primates share a mixture of cell phenotypes that are not found in other species, and the overall pattern of tracheobronchial epithelial differentiation is strikingly similar between the species (59). Rhesus monkeys also have similar segmental arrangement, branching patterns, mucosal

surfaces, and arterial structure when compared to humans, and they have been crucial for the study of airway disease, including asthma, chronic obstructive pulmonary disease, and the impact of wildfire and tobacco smoke (60). Baboons have been used extensively for the study of respiratory diseases, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; see Section 4.2, below), pneumococcal pneumonia, and bronchopulmonary dysplasia (61–64). Although New World species have served as models for inflammatory lung diseases, they have not proven to be as useful as Old World species for modeling SARS-CoV-2.

3.4. Brain Development and Neurodevelopmental Disorders

Nonhuman primates have addressed the development and function of the central nervous system (CNS). Research with rhesus monkeys has led to important insights into the development of the human cerebral cortex (65) and the etiology of neurologic and psychiatric diseases (45, 66). Neurodevelopmentally, the production of cortical neurons in fetal rhesus monkeys follows the same inside-out sequence as in the human brain, and the proliferative zones of the fetal monkey cerebral cortex possess the same classes of neural precursor cells (NPCs) that exhibit similar distribution, morphology, protein, and transcription factor expression as the human brain (67). In both the rhesus monkey and human fetal brain, the distribution of the NPC pool is more complex, and the number of NPCs is greatly expanded when compared to small animal models (68). Studies with fetal rhesus monkeys were instrumental for the development of models focused on how cortical lamination and areal differences in the human brain may arise from a proto-map of localized clusters of NPCs in cortical proliferative zones (65).

Brain size and structural organization vary considerably across nonhuman primates. For example, the lissencephalic brain of New World monkeys such as the common marmoset is considerably smaller, whereas the larger gyrencephalic brain of Old World species such as the rhesus more closely recapitulates the human brain. Research in marmosets has expanded considerably in the past decade. The more complex social behaviors of marmosets compared with rodents, combined with their small size and relatively fast growth and maturation, have made the common marmoset an attractive nonhuman primate model for studying CNS development and human disease (69). Comparative studies of the developing brain across nonhuman primates, yet within more closely related species such as simiiformes (e.g., marmosets versus rhesus monkeys), can shed light on genetic and molecular signaling programs that guide histogenesis, expansion, and ultimate size of complex human CNS structures such as the cerebral cortex.

It has become increasingly clear in the past decade that the immune system plays key functional roles in early brain development (70). The innate immune cells of the CNS, microglial cells, contribute to an array of developmental programs, including cortical layer formation, axon pathfinding, synapse development, synapse maintenance (70), and regulation of the NPC pool (71, 72). Although much of this work has been conducted in rodents, it has been reported that microglia colonize key structures in the CNS, including the telencephalon, in larger numbers and at earlier stages of fetal development in nonhuman primates than in common rodent species (71), and that microglia in the human brain differ

from those in rodents (73). The comparatively early arrival of microglia in proliferative zones of the fetal primate telencephalon, particularly during cortical neuron production, provides opportunity for microglia to play a more prominent role in cellular genesis in primates and supports the concept that microglial cells contribute to telencephalon formation from early stages of development. However, this developmental benefit may come with potential risks. Fetal microglia respond rapidly to changes in their local environment, injury, and extrinsic factors introduced through maternal exposure to viral pathogens (74), which has been shown in both the baboon and rhesus monkey (75, 76). For example, fetal exposure to Zika virus results in profound changes in these key components. At three weeks after direct Zika virus inoculation, microglial distribution in the fetal rhesus monkey was shown to be altered, with microglia collected in large heterotopic clusters throughout cortical proliferative zones. The microglial clusters were associated with disturbed distribution of NPCs, enlarged blood vessels, and a thinner cortical plate, and these altered parameters persisted at three months postexposure (76). The data indicate that microglia may be one conduit between pathogen exposure and atypical outcomes in primate neurodevelopment.

The link between fetal immune activation and psychiatric disease remains an active area of study (77). A nonhuman primate model of exposure to the viral mimic polyinosinic:polycytidylic acid during pregnancy has shown elevated striatal dopamine, a molecular hallmark of human psychosis (78), providing an unprecedented opportunity to study the underlying molecular correlates between maternal inflammation during pregnancy and disease in offspring. RNA sequencing across relevant brain regions (e.g., prefrontal cortex, anterior cingulate, hippocampus) and primary visual cortex (timing comparable to mid-adolescence in humans) showed region-specific alterations in synaptic signaling and oligodendrocytes.

3.5. Neurodegeneration

Inflammation has been correlated with the onset and appearance of neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease. Chronic systemic administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in the rhesus monkey has been shown to reproduce key features of Parkinson’s disease progression, including neuropathology of the nigrostriatal system, motor and cognitive symptoms, inflammation, and impairments of other neurochemical systems (79). Nonhuman primates also mirror the progression of human brain aging and show similar cognitive decline. A recently developed Alzheimer’s disease model induced by an adeno-associated virus (AAV) expressing a double tau mutation (80) was shown to manifest the neuropathology associated with the disease. An advantage of nonhuman primate models of Alzheimer’s disease includes the formation of amyloid plaques; amyloid precursor protein differs in the mouse and does not aggregate, and heterogeneity of the size and density of amyloid plaques is seen in humans but not in murine models.

The similarity of neuroanatomical structure and function in nonhuman primates and molecular advances have yielded nonhuman primate models of Huntington’s disease. Delivery of fragments of the mutant human *HTT* containing CAG repeats into the putamen via viral vectors produces dyskinesias that mirror human symptoms, as well as

neuropathological findings that include formation of inclusions, loss of NeuN-expressing neurons, and astrogliosis in the putamen (81). The common marmoset has also been used to model multiple sclerosis through experimental autoimmune encephalomyelitis that produces inflammation, demyelination, and axonal injury that closely mirror human findings (82). These studies highlight the importance of translational nonhuman primate models to study human neurodegenerative conditions and to test novel therapeutics.

4. INFECTIOUS DISEASES

4.1. Simian Immunodeficiency Virus, Cytomegalovirus, and Zika Virus

Nonhuman primates are indispensable models for HIV infection. Indeed, several different HIV models are available (83–86), which differ according to the version of simian immunodeficiency virus (SIV) used for infection and the target species. Different models provide excellent tools for addressing various phases and aspects of infection, with some resulting routinely in rapid disease progression and others in chronic, indolent infection. Nonhuman primate models have provided a wealth of information about pathogenesis and treatment that is not possible to catalog here (87).

Several species have provided valuable information about early transmission and the post-transmission phase of virus replication in gut tissue. HIV targets during early infection are activated, $CCR5^+$ cells in the gut, which are critical targets of infectious, transmissible HIV—a fact first appreciated in the nonhuman primate model (88). In early infection, patients heterozygous for the CCR5 delta 32 allele exhibit significantly lower cell-associated HIV DNA loads than those homozygous for the normal allele, suggesting delayed establishment of the virus in reservoirs (89). Furthermore, most HIV-infected but untreated people maintain a predominantly CCR5-tropic viral population for extended periods (8 years), with potent antiretroviral therapy (ART) creating the conditions only in some patients for emergence of X4 variants in cellular reservoirs after 30–60 months of treatment (90). These virologic and cellular dynamics are well captured in macaques. To further define the importance of CCR5 in chronic infection, strategies for depletion of $CCR5^+$ cells were developed, including use of immunotoxins and bispecific antibodies (91). These agents proved capable of both very effective depletion and long-term suppression of viremia, presumably related to depletion of $CCR5^+$ reservoir cells harboring SIV.

SIV-infected nonhuman primates have more recently become excellent models of chronic, treated HIV infection, the most common situation for human patients. Testing of ART in nonhuman primates provided critical data necessary to their later lifesaving success (92). Nonetheless, for many years, the lack of efficacy of non-nucleoside reverse transcriptase inhibitors against SIV made study of chronic, treated disease in the nonhuman primate model very difficult. With new drug classes, the viremia can be suppressed to below the level of detection, as achieved routinely for human subjects. These ART-suppressed animals can then serve as valuable models for testing HIV cure or therapeutic vaccine strategies (93). For example, using SHIV-SF_{162}P3-infected rhesus monkeys in which ART was initiated on day seven of infection, Borducchi et al. (94) demonstrated that NAb (PGT121) and a TLR7 agonist (GS-9620) delayed viral rebound following ART withdrawal. Okoye et al. (95) found that 0/6 animals started on ART on day four or five showed post-ART viral rebound

during nine months of follow-up; indeed, at tissue collection, these macaques showed no evidence of replication-competent SIV. Such studies provide insight into the durability of the viral reservoir and its vulnerability to immunologic intervention.

Outbreaks of both Zika virus and SARS-CoV-2 have demonstrated the indispensability of nonhuman primates for research into emerging diseases. Prenatal exposure to Zika virus and resulting congenital Zika syndrome in Latin America and United States territories showed Zika virus to be a global public health concern. Zika virus is distributed across Africa, the Americas, Asia, and the Pacific, with virus detected in blood, semen, saliva, and urine. Known modes of transmission include mosquito, vertical, and sexual transmission, and potentially through the blood supply. Zika virus has been identified as a teratogen similar to other neurotropic viruses, notably CMV and rubella (96).

As demonstrated in studies with RhCMV, the rhesus monkey model provides strategic advantages for investigating teratogenic agents. Many developmental features are similar between the species, as noted above. These overlapping features, as well as immunologic similarities, make the macaque an excellent model for studying prenatal infections. For example, in Zika virus infection, maternal T cell activation and maturation were assessed by following expression of CCR5, HLA-DR, and memory/effector subset markers (76). Maternal CCR5 expression by $CD4^+$ T cells followed a pattern of early upregulation followed by later decline that was reminiscent of maternal viral loads. Indeed, maximum maternal viral loads and CCR5 expression appeared to be associated, and increased expression of CCR5 followed the early spike in maternal viral loads by 10–30 days. Furthermore, significant morphologic changes were noted in the fetal cerebral cortex at three weeks post-Zika virus inoculation as described above in Section 3.4.

Zika virus vaccine research in nonhuman primates was in some ways a successful forerunner to the SARS-CoV-2 vaccine research that would follow beginning in 2020. Both viruses are far more tractable for vaccine development than HIV, lending themselves more readily to elicitation of NAbs, which are indeed protective against infection and/or its most undesirable sequelae. In the case of Zika virus, effective vaccine platforms tested in monkeys include inactivated virus, plasmid DNA, messenger RNA (mRNA), and adenovectors (97, 98). For both Zika virus and SARS-CoV-2, there is concern for the possibility of vaccine-mediated enhancement of disease, which may occur in relatively uncommon circumstances and is therefore difficult to model in an animal species. This concern is particularly acute in the case of Zika virus given its similarity to Dengue virus, another flavivirus for which the risk of severe disease was found to increase among dengue-naïve children following vaccination (99). Nonetheless, Zika vaccine research has led to several candidate vaccines that are currently in human clinical trials (100, 101).

4.2. SARS-CoV-2

As of this writing, SARS-CoV-2 infection has resulted in more than 330 million infections and more than 5.5 million deaths globally (https://coronavirus.jhu.edu/map.html). Extremely rapid progress in understanding disease pathogenesis and correlates of immune-mediated protection would not have been possible without intensive use of nonhuman primates (102, 103). Experiments in macaques have offered a window into the mechanistic basis

of SARS-CoV-2-associated vascular disease and thrombosis, for example, including to coordinate production of thrombotic and inflammatory mediators, demonstrating critical interactions between the two pathways (104). Additionally, it is fair to say that much baseline knowledge about coronavirus biology, known before the SARS-CoV-2 outbreak, relied on the nonhuman primate model.

Early important results from nonhuman primates demonstrated that vaccine-mediated protection against SARS-CoV-2 was possible and was correlated with the NAb titer achieved. As such results were increasingly refined, they began to define a lower limiting titer at which protection could be achieved, thus critically informing interpretation of early phase-I clinical trial results. Working early in the pandemic with DNA vaccines, for example, Barouch and colleagues (105) were able to define an apparent NAb threshold separating completely protected from partially protected macaques (~1:100). Such work remains important today as we explore the decrement in NAb titer that is associated with variant spike proteins, and the likelihood that booster vaccines are needed to protect against them (106–108). Nonhuman primates also provided a crucial relevant species in which to perform detailed mechanistic studies. In the first experimental tests of the importance of antibodies versus $CD8^+$ T cells, McMahan et al. (109) showed that adoptive transfer of purified IgG from convalescent macaques protected naïve recipient rhesus monkeys against SARS-CoV-2 challenge, whereas depletion of $CD8^+$ T cells from convalescent animals degraded but did not eliminate the protection afforded by previous infection. The authors concluded that relatively low titers of NAb are protective against SARS-CoV-2 infection and that $CD8^+$ T cells make an important contribution only when titers fall below the completely protective level. Working with the Moderna mRNA vaccine, investigators from the National Institutes of Health (NIH) Vaccine Research Center similarly found that passive transfer of vaccine-induced IgG from macaques to naïve hamsters was sufficient to mediate protection. They concluded that humoral immune responses are a mechanistic correlate of protection against SARS-CoV-2 (110).

It is now apparent that the most effective vaccines induce NAbs that are almost exclusively directed against the viral spike protein. Within that target, NAbs have been identified that target the receptor binding domain, the N-terminal domain, or the S1 base. Understanding the immunological mechanisms characteristic of SARS-CoV-2 infection and vaccination, specifically immune responses associated with NAb production, was foundational in the selection of a vaccine capable of abating the pandemic and to evaluation of candidate monoclonal-antibody therapies (111).

Attention has turned to the durability of antibody responses and the immunologic mechanisms that support long-term responses. Use of long-lived macaques will allow investigation of such questions. One prevalent hypothesis being tested in macaques is that durable antibody responses, including long-lived plasma cells, hinge on $CD4^+$ T follicular helper (Tfh) cells. Generation of peripheral Tfh cells predicts antibody durability after HIV or influenza vaccination (112). SARS-CoV-2-specific $CD4^+$ T cells responding to spike proteins were observed in the peripheral blood of recovered patients, as well as vaccinated macaques or humans (113). Similar findings were previously observed with the 2002 SARS-CoV (114), and studies in mouse models demonstrated a critical role for $CD4^+$ T cells in

viral clearance (115). Together, these data illustrate the importance of the nonhuman primate model for addressing follow-up questions about the longitudinal relationship between Tfh cells and antibody responses that might provide protection over the span of years rather than months. Early immune responses, particularly in lymphoid and respiratory tissues, are challenging to study in humans; the correspondence between such early responses and durable immunity is more challenging still.

Using the rhesus macaque model, we reported that SARS-CoV-2 infection triggered acute changes in circulating innate myeloid cells (116). In particular, two days after viral exposure, a substantial rise in proinflammatory monocytes and decline in plasmacytoid dendritic cells in the peripheral blood were observed. These changes began to subside on day four postinoculation, apparently in conjunction with rapid resolution of systemic inflammation and only mild clinical signs. Of greatest importance to durable protection, infection also elicited robust germinal centers with SARS-CoV-2-reactive Tfh cells within the mediastinal lymph nodes. Additionally, SARS-CoV-2-specific Tfh cells were observed in peripheral blood following infection. Chandrashekar et al. (117) showed experimentally in rhesus monkeys that such convalescent responses do protect against rechallenge, proving the efficacy that had been inferred in human studies.

The extraordinary importance of rhesus monkeys in studying SARS-CoV-2 has led to outsized demand for the animals, which was most intense earlier in the pandemic but remains high particularly with the need to study the long-term impact of infection and post-acute sequelae. The NIH has suggested that a viable alternative to rhesus may be needed because of the difficulty in ensuring an adequate supply to sustain all NIH-supported research (102). The baboon was suggested as an alternative with the similarity of antibody subclasses in this species when compared to humans. Baboons also experience higher viral titers, longer viremia, and more pronounced lung pathology than macaques, making them a suitable model for more severe infection as observed in humans (63).

5. PRECISION MEDICINE

5.1. Stem Cell Transplantation, Gene Therapy, and Somatic Cell Genome Editing

Nonhuman primates have been used widely as preclinical models for stem/progenitor cell transplantation and gene therapy and have shown substantial advantages when compared to other species. A few studies are highlighted, with additional findings on this subject noted below in Section 6.

5.1.1. Stem cell transplantation.—A range of studies have been performed in Old World species (rhesus, baboons) to address collection of $CD34^+$ HSCs for transplantation, as well as conditioning regimens. For example, Radtke et al. (118) observed a population of early-engrafting cells displaying HSC-like behavior, which persisted long term in vivo in an autologous myeloablative transplant model in rhesus and pig-tailed macaques. The phenotype and function of defined nonhuman primate hematopoietic stem and progenitor cell (HSPC) subsets were obtained and compared to human HSPCs. These studies demonstrated that the $CD34^+CD45RA^-CD90^+$ cell phenotype is highly enriched for HSCs and fully supported rapid short-term recovery and robust multilineage hematopoiesis in

the transplant model. The frequency of cells with this phenotype quantitatively predicted transplant success and time to neutrophil and platelet recovery.

Studies in young rhesus monkeys have used SIV-based lentiviral vectors to transduce rhesus monkey $CD34^+$ HSCs for autologous transplantation, and with non-myeloablative conditioning regimens (e.g., busulfan, fludarabine) (119). SIV-based vectors overcome the posttranslational block specific to the rhesus hematopoietic system. Bone marrow $CD34^+$ HSCs were transduced in two equal fractions using SIV-based lentiviral vectors carrying a nonexpressed DNA sequence tag and the green fluorescent protein (GFP) reporter gene. Post-transplant, there was no evidence of elimination of cells containing the potentially immunogenic GFP gene. Antibodies and cellular immune responses to GFP developed in recipients with the highest levels of GFP-marked cells, although these cells were not eliminated. Studies established a clinically relevant pediatric primate model to assess the effects of conditioning regimens on the engraftment of transduced HSCs and the immune responses to cells expressing a foreign gene product.

Uchida et al. (120) evaluated whether additional immunosuppression beyond busulfan is required for efficient engraftment of gene-modified cells using an adult rhesus HSC lentiviral genetherapy model. After autologous transplantation of two transduced cell populations following myeloablative busulfan conditioning, immunological rejection of GFP-transduced cells and stable engraftment of γ-globin-transduced cells were observed. After the addition of abatacept and sirolimus to busulfan conditioning, engraftment of both GFP- and γ-globin-transduced cells were observed, demonstrating that additional immunosuppression allowed for engraftment of gene-modified cells expressing immunogenic proteins.

5.1.2. Gene therapy.—Tremendous progress has been made in the gene therapy field, with lentiviral vectors and recombinant AAV vectors at the forefront of current gene therapy clinical trials (e.g., Glybera® for lipoprotein lipase deficiency, Luxturna® for Leber congenital amaurosis, Zolgensma® for spinal muscular atrophy) (121). However, there have been some setbacks (122), and the potential for inflammation and immune responses requires further studies on safety and efficiency (123, 124).

Gruntman et al. (125) assessed two methods, peripheral venous limb perfusion and an intra-arterial push and dwell using rAAV1 and rAAV8, in a rhesus monkey study. The rhesus AAT transgene was used with a c-myc tag to enable quantification of transgene expression in five cohorts treated with a dose of 6×10^{12} vector genomes (vg)/kg. Both methods were well tolerated, with limb perfusion demonstrating higher potency per vg injected and a greater total vector retention within the muscle (compared to intramuscular administration), while enabling a much greater total dose to be delivered with equivalent safety.

Therapeutic exon skipping as a treatment for Duchenne muscular dystrophy (DMD) has focused primarily on the delivery of antisense oligomers to treat out-of-frame exon deletions. Gushchina et al. (126) reported on the preclinical development of an AAV vector containing four copies of the noncoding U7 small nuclear RNA, each targeted to either the splice donor or the splice acceptor sites of *DMD* exon 2. A dose escalation study with

cynomolgus monkeys with two doses (3×10^{13} and 8×10^{13} vg/kg) showed no evidence of toxicity, providing evidence for safety that led to initiation of a first-in-human clinical trial.

A series of studies have been instrumental in achieving the overall goal of using AAV expression of human acid alpha-glucosidase in Pompe disease patients. Investigational new drug (IND)-enabling preclinical studies for a phase I/II clinical trial in adult Pompe patients was designed to evaluate the toxicology, biodistribution, and potential for readministration of rAAV9-DES-hGAA injected intramuscularly into the tibialis anterior muscle using an immune modulation strategy in rhesus monkeys (127). In preparation for a phase I clinical trial of AAV8 gene therapy to treat homozygous familial hypercholesterolemia, Greig et al. (128) evaluated the safety of the clinical candidate vector, AAV8.TBG.hLDLR, in wild-type rhesus monkeys and those heterozygous for a nonsense mutation in the LDL receptor gene ($LDLR^{+/-}$). Intravenous (IV) infusion of 1.25×10^{13} genome copies/kg was well tolerated and associated with only mild histopathology that was restricted to the liver, with sporadic, low-level transient elevations in transaminases. Overall, this study supported the safety of AAV8.TBG.hLDLR for evaluation in a human clinical trial.

Galvan et al. (129) conducted a study focused on delivery of AAV9-PHP.B, a capsid mutant, into the lateral ventricle of rhesus monkeys. To enhance the expression of the transgene, which represented the tag protein emerald green fluorescent protein (EmGFP), a gene promoter was used to confer high neuron-specific expression of the transgene, the human synapsin 1 promoter. EmGFP was observed in neuronal cell bodies within the cerebral cortex and in the cerebellum, as well as in the striatum and hippocampus. Results demonstrated that a single injection in the lateral ventricle resulted in widespread transgene expression, avoiding the need for intraparenchymal injections.

Hordeaux et al. (130) aggregated data from 33 studies in 256 nonhuman primates and performed a meta-analysis of the severity of dorsal root ganglia (DRG) pathology that has been observed by comparing routes of administration, dose, time course, study conduct, age, sex, capsid, promoter, capsid purification method, and transgene. DRG pathology was observed in 83% of animals administered AAV via the cerebrospinal fluid (CSF) and in 32% that received an IV injection. These data from 5 different capsids, 5 different promoters, and 20 different transgenes suggest that DRG pathology can occur after AAV gene therapy in studies with nonhuman primates. None of the animals receiving a therapeutic transgene displayed any clinical signs.

Kiss et al. (131) evaluated the long-term safety of vascular endothelial growth factor (VEGF) suppression with aflibercept expression after a single intravitreal injection of ADVM-022 (AAV2.7m8.C11.CO.aflibercept) in African green monkeys. The expressed protein is an anti-VEGF immunoadhesin comprising portions of human VEGF receptors and the Fc portion of human IgG1. Sustained expression was noted and mild to moderate inflammatory responses were observed, which trended toward spontaneous resolution without treatment. No abnormalities in retinal structure or function were observed. Rodriguez-Bocanegra et al. (132) assessed different doses of rAAV8 vector (10^{11}–10^{12} vg) when injected subretinally into the left eye of cynomolgus monkeys. Animals in the high-dose group showed more hyper-reflective foci than in the low- and medium-dose

groups at 90 days postadministration. The presence of infiltrating B and T cells and microglia activation were detected in rAAV8-treated eyes. To better understand the immune and structural consequences of subretinal rAAV readministration to the same eye, Weed et al. (133) administered bilateral subretinal injections of rAAV2-*hRPE65v2* to nonhuman primates (rhesus and cynomolgus) and repeated the injections in the same eyes two months later. The repeat injections were well tolerated immunologically and structurally, even in the setting of pre-existing serum NAbs. Localized structural abnormalities confined to the outer retina and retinal pigmented epithelium after readministration did not differ from those observed after single or contralateral administration of an AAV vector carrying a nontherapeutic transgene.

5.1.3. Somatic cell genome editing.—Gene editing, through the use of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases, or clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nucleases, has revolutionized the ability to correct genetic information in living cells for the study and treatment of human disease. A significant challenge is the safe delivery of sufficient nuclease into the cells to be edited. Nowhere is this challenge more acute than in the safe delivery of nucleases into the disease-related cells of patients, which represents one of the greatest barriers for clinical use. A range of viral (e.g., AAV) and nonviral (e.g., nanoparticles) vectors are under study in nonhuman primates.

Wang et al. (134) showed the long-term durability and safety of Proprotein convertase subtilisin/kexin type 9 (PCSK9) knockdown in rhesus monkey liver by AAV-delivered meganuclease. PCSK9, an antagonist of the LDL receptor, is a reasonable target for nuclease-mediated gene disruption as an approach to treat hypercholesterolemia (135). Monkeys administered AAV-meganuclease in combination with corticosteroid treatment or an alternative AAV serotype were monitored for a period of up to three years and showed a sustained reduction in circulating PCSK9 and LDL cholesterol through the course of the study concomitant with stable gene editing of the PCSK9 locus. Low-frequency off-target editing remained stable, with no adverse findings in the liver.

Musunuru et al. (136) demonstrated that CRISPR base editors delivered with lipid nanoparticles can efficiently and precisely modify disease-related genes in cynomolgus monkeys. A near-complete knockdown of PCSK9 in the liver was shown after a single infusion of lipid nanoparticles, with concurrent reductions in PCSK9 and LDL cholesterol of ~90% and ~60%, respectively (8 months duration). Similarly, Rothgangl et al. (137) investigated the efficacy and safety of adenine base editors (ABEs) in the liver of cynomolgus monkeys for the reduction of blood LDL levels. Lipid nanoparticle-based delivery of mRNA encoding an ABE and a single-guide RNA targeting *PCSK9* induced up to 34% editing (average 26%). Plasma PCSK9 and LDL levels were stably reduced by 32% and 14%, respectively. ABE mRNA was cleared rapidly, and no off-target mutations in genomic DNA were found. Redosing in macaques did not increase editing, possibly related to the detected humoral immune response to ABEs.

Uchida et al. (138) developed high-efficiency, viral vector–free, non-footprint gene correction in sickle cell disease $CD34^+$ cells by using electroporation to deliver mutation-

targeting guide RNA, Cas9 endonuclease, and 100-mer single-strand donor DNA encoding the intact β-globin sequence, achieving therapeutic-level gene correction at DNA (~30%) and protein (~80%) levels. A rhesus β-to-βs-globin gene-conversion strategy was developed to model HSC-targeted genome editing for sickle cell disease and demonstrated the engraftment of gene-edited $CD34^+$ cells 10–12 months post-transplant in rhesus monkeys. Humbert et al. (139) evaluated the therapeutic potential of HSPCs edited with the CRISPR-Cas9 nuclease platform to recapitulate naturally occurring mutations identified in individuals who express increased levels of fetal hemoglobin (HbF). CRISPR-Cas9 treatment and transplantation of HSPCs purified on the basis of surface expression of the CD34 receptor in a nonhuman primate autologous transplantation model resulted in up to 30% engraftment of gene-edited cells for >1 year. Edited cells effectively and stably reactivated HbF, as evidenced by up to 18% HbF-expressing erythrocytes in peripheral blood. Similar results were obtained by editing highly enriched HSCs, defined by the markers $CD34^+CD90^+CD45RA^-$, allowing for a tenfold reduction in the number of transplanted target cells, thus considerably reducing the need for editing reagents. The frequency of engrafted, gene-edited cells persisting in vivo using this approach may be sufficient to ameliorate the phenotype for several genetic diseases.

Peterson et al. (140) evaluated whether the disruption of the CCR5 locus in pig-tailed macaque HSPCs by ZFNs was feasible. Macaque-specific CCR5 ZFNs efficiently induced CCR5 disruption at levels of up to 64% ex vivo, 40% in vivo early post-transplant, and 3% to 5% in long-term repopulating cells over 6 months following HSPC transplantation. These genome-edited HSPCs supported multilineage engraftment and generated progeny capable of trafficking to secondary tissues, including the gut. Together, data demonstrated that genome-edited HSPCs engraft and contribute to multilineage repopulation after autologous transplantation in nonhuman primates.

The NIH Common Fund's Somatic Cell Genome Editing Consortium was established to accelerate the development of solutions to many genome editing challenges (https://commonfund.nih.gov/editing). The consortium includes 72 principal investigators from 38 institutions addressing 45 projects on biological systems, new editing platforms and delivery systems, and small animal (mice) and large animal (pig, nonhuman primates) testing centers in which to validate and assess the safety and efficiency of these new approaches (141). The overarching goal is to accelerate the translation of genome editing technology for a wide range of tissues and diseases. Beyond the need for new editing capabilities, other areas of focus to advance the field include assessment of off-target effects and safe and efficient delivery strategies. The Consortium will also develop standards and a Toolkit, which will be shared with the greater research community.

6. TRANSLATIONAL APPLICATIONS OF IN VIVO IMAGING

Significant advancements have been made in the use of noninvasive imaging techniques, including those that make use of endogenous or exogenous contrast mechanisms to create images that can reflect anatomy, physiology, metabolism, interactions with proteins, or gene expression. The two most sensitive in vivo imaging technologies for detection of gene and protein expression are optical techniques based on bioluminescence or

fluorescence and nuclear imaging techniques such as positron emission tomography (PET) that use radioactively tagged tracers. A range of imaging applications from ultrasound to bioluminescence and fluorescence to PET/computed tomography (CT) and magnetic resonance imaging (MRI) are used with nonhuman primate models.

6.1. Pregnancy and Fetal Development

Ultrasound has been instrumental in providing information on best practices for nonhuman primate colony management and for a wide range of research applications (142). Other imaging applications during pregnancy to assess the fetus and placenta include CT, PET/CT, and MRI. For example, the assessment of placental intervillous perfusion in gravid rhesus monkeys has been performed using arterial spin labeling flow-sensitive alternating inversion recovery and ferumoxytol dynamic contrast enhanced (DCE) MRI with and without the administration of interleukin-1β to induce inflammation and disrupt perfusion (143). Ferumoxytol is a superparamagnetic iron oxide nanoparticle used off-label as an intravascular MRI contrast agent. Four-dimensional (4D) flow imaging was also studied and shown to be feasible in the primary uteroplacental vessels (144), supporting 4D flow MRI as a valuable tool in assessing uteroplacental health in humans. Additionally, ferumoxytol uptake by macrophages was shown to facilitate detection of inflammatory sites.

From a fetal perspective, exploring synaptic density changes during brain development is crucial to understanding ontogeny under normal and aberrant developmental conditions. A synaptic vesicle glycoprotein 2A (SV2A) PET radioligand was explored in gravid rhesus monkeys with total body PET and showed that fetal SV2A concentrations were greater in subcortical regions than in cortical regions in the second and third trimesters. Near-term, significant differences were observed in the motor and visual regions, providing unique in vivo insights (145).

6.2. Infectious Diseases

The impact of HIV on immune activation and systemic inflammation has led to an interest in molecular imaging techniques to better understand infection (146). PET has been used to measure metabolic activity with ^{18}F-fluoro-2-deoxy-d-glucose (^{18}F-FDG) and to quantify changes in the setting of ART. Santangelo et al. (147) used copper-64 (^{64}Cu)-radiolabeled SIV gp120-antibody to address biodistribution after infection and ART administration in rhesus monkeys. A follow-up study of anti-α4β7 treatment in SIV-infected rhesus monkeys demonstrated a reduction in SIV protein expression in the lung, spleen, and lymph nodes (148).

Quantitative MR-relaxometry and ^{18}F-FDG PET/CT was used to assess brain involvement with Ebola virus in acutely infected monkeys, including blood–brain barrier dysfunction and metabolic changes (149). In addition, ^{18}F-FDG has been used in African green monkeys infected (via aerosol or mucosal administration) with SARS-CoV-2, which showed pulmonary lesions at four days postinfection, and resolved over time (150). CT and PET/CT imaging was also used in cynomolgus monkeys infected using an intratracheal administration approach with SARS-CoV-2 (151). Here, serial CT scans demonstrated lung abnormalities characteristic of subclinical or mild-to-moderate infection

(e.g., ground-glass opacities with or without reticulation, paving, or alveolar consolidation, peri-bronchial thickening, linear opacities). PET demonstrated increased ^{18}F-FDG uptake 2 days postexposure, which resolved by 6 to 12 days postinfection.

To better understand how cell populations and metabolic activity change in granulomas over the course of *Mycobacterium tuberculosis* infection, ^{64}Cu-LLP2A, a PET probe for VLA-4, was tested and validated in cynomolgus monkeys infected with an intrabronchial approach. When used in conjunction with ^{18}F-FDG, ^{64}Cu-LLP2A was shown to be a useful tool for understanding granuloma biology (152). Epithelioid macrophages and T cells were associated most strongly with ^{64}Cu-LLP2A activity, with the greatest variation occurring in lung and lymph node granulomas during early infection, suggesting a period of intense metabolic activity followed by recruitment or differentiation of integrin α4β1-expressing cells. These studies combining two PET probes with different mechanisms of action yielded novel insights into granuloma biology that benefited basic and translational *M. tuberculosis* research.

PET/CT and near-infrared imaging have also been used to address the biodistribution of a mRNA vaccine after immunization in cynomolgus monkeys (153). PET imaging demonstrated that following administration, mRNA reached draining lymph nodes quickly and accumulated for at least 28 h after injection.

6.3. Regenerative Medicine and Gene Therapy

Bioluminescence imaging (BLI) provides a method for visualizing a variety of biological processes such as gene expression and cell trafficking in vivo. Mammalian tissues have no intrinsic bioluminescence, and thus imaging results in high signal-to-noise ratios when luciferase is expressed in the presence of its substrate, luciferin, in vivo. The safety and efficiency of lentiviral vector-mediated in utero gene transfer and the feasibility of using firefly luciferase to monitor transgene expression postnatally for up to 15 years and in parallel with PET has been reported (154, 155). Studies further investigated the safety, efficiency, and long-term expression of luciferase after intrathoracic or intramyocardial gene transfer in early-gestation fetal rhesus monkeys (156). AAV5, AAV9, and AAV10 were all shown to be highly efficient in the delivery and long-term expression of firefly luciferase, with transgene expression approximately 100-fold higher than when using a dual-fusion HIV-1-derived lentiviral vector after intraperitoneal administration (155). These studies further supported the use of BLI to monitor transgene expression in young rhesus monkeys, to assess different vector constructs simultaneously and over time, and to provide unique insights that may not be detectable by biopsy or tissue collection. Despite some limitations, BLI provides an efficient and noninvasive method to monitor the expression of reporter genes in vivo with virtually no background noise.

Other studies have demonstrated effective techniques for radiolabeling stem/progenitor cells with radioactive copper (^{64}Cu-PTSM) for PET imaging (157). Cell radiolabeling provides the best contrast-to-noise environment for cell detection because there is essentially no background when the majority of detected signal comes from the cells. These and related investigations have indicated that each cell type has a different radiolabeling efficiency and must be tested to optimize delivery. Studies have also shown improved contrast-to-noise

imaging for cell trafficking when zirconium (^{89}Zr) radioimmunoconjugates were used to identify engrafted CD34^{+} HSC post-transplantation (158). Other investigations have focused on organ-targeted delivery, such as the use of renal precursors differentiated from human pluripotent stem cells transplanted into fetal kidneys in utero (159). Imaging confirmed that the transplanted cells remained within the kidney and did not migrate to other anatomical sites.

Using iodine (^{124}I) radiolabeling, Ballon et al. (160) studied a method for quantitative analysis of the biodistribution of AAV9 and AAVrh10. The AAV vectors were administered to adult African Green monkeys either IV or using an intracisternal approach to compare and contrast outcomes. The average AAV dose of ~5×10^{12} genome copies was included and assessed with PET over four successive days. IV administration was shown to be relatively similar between serotypes, with distribution primarily to the liver and heart and some evidence at other anatomical sites (e.g., parotids, spleen, long bones). When using the intracisternal route of administration, the outcomes of the two vectors were also shown to be similar with detection in the spinal canal/CSF and liver.

Intramarrow injection of HSPC obtained from umbilical cord blood has been proposed as a potential strategy to improve transplant engraftment and prevent graft failure. Stringaris et al. (161) assessed engraftment of CD34^{+} cells transplanted in a myeloablative rhesus monkey model comparing intramarrow to IV delivery. Daily blood collection for assessments by flow cytometry quantified the proportion of engrafted cells from each source. Marrow retention was evaluated with ^{89}Zr-oxine-labeled CD34^{+} cells and PET/CT, which showed that cells injected within the long bones were retained and engrafted in all animals. However, cells transplanted directly into the marrow did not engraft faster than those delivered IV and overall contributed significantly less to hematopoiesis than IV-delivered cells at all time points. Given the potential risks of intramarrow administration, these data did not support such transplants as a strategy to improve hematopoietic engraftment. Overall, these and other published studies emphasize the critical importance of in vivo imaging to monitor safety, biodistribution, and long-term expression for cell and gene therapy.

6.4. Immunotherapy and Radiolabeled Antibodies

Adoptive transfer of allogeneic and autologous NK cells is being investigated clinically for the treatment of malignancies. Although regression of malignancies in humans with advanced cancers has been observed, the overall efficacy of NK-cell-based immunotherapy remains marginal. These findings may be related to the inability of NK cells to efficiently traffic to bone marrow. A ^{89}Zr-oxine complex was used as a cell-labeling agent to monitor the tracking of cells with PET (162). Using the rhesus monkey model, tissue distribution was shown and accurately quantitated NK-cell trafficking with low radioexposure to organs, suggesting this method could be translated safely to humans.

Studies of therapeutic antibody biodistribution have been addressed in support of regulatory filings for therapeutic candidates with the Food and Drug Administration. For example, a study with the primate total-body PET EXPLORER demonstrated ^{89}Zr antibody biodistribution out to 30 days postinjection, providing a better understanding of antibody

behavior than observed previously with the limits of short-term analysis (e.g., 9–10 days). The study also aided in the identification of chemically stable chelator moieties appropriate for use out to these unprecedented imaging time points (163). Because the ligands typically under testing have limited cross-species reactivity, preclinical imaging is feasible only in nonhuman primates.

6.5. Aging and Neuroinflammation

Both Old and New World species can address age-related pathology similar to findings in humans, including arthritis, cardiovascular disease, and neurological decline, providing excellent models for increasing our understanding of the mechanisms associated with aging (164, 165). Similar to other species, marmosets accumulate β-amyloid with age, which occurs at 7–15 years, compared to 22–31 years in Old World species. With a compressed lifespan in comparison to rhesus, marmosets are mature by approximately 2 years of age and considered aged by 8 years of age. Imaging biomarkers for Alzheimer's, Parkinson's, and Huntington's disease in these species include a range of markers beyond glucose metabolism and are focused on tracking progression and the effectiveness of new treatment strategies using both PET and MRI.

Longitudinal changes in the dopaminergic and serotonergic systems in the MPTP cynomolgus monkey Parkinson's model were assessed with PET, including changes in dopamine synthesis (^{18}F-DOPA), dopamine D_2/D_3 receptors (^{11}C-raclopride), and serotonin transporter DASB (*N,N*-dimethyl-2-2-amino-4-cyanophenylthiobenzylamine) and serotonin 1A receptor (^{18}F-MPPF) levels at baseline, early symptomatic, full symptomatic, and recovered stages (166). Degeneration of dopamine neurons in the midbrain underlies the pathogenesis of Parkinson's disease. Supplement of dopamine via L-DOPA alleviates motor symptoms but does not prevent the progressive loss of these neurons. Tao et al. (167) showed that over a two-year period without immunosuppression, monkeys receiving autologous transplantation of induced pluripotent stem cells exhibited recovery from motor and depressive signs. These behavioral improvements were accompanied by robust grafts with extensive dopamine neuron axon growth, as well as strong dopamine activity by PET. Mathematical modeling revealed correlations between the number of surviving neurons with PET signal intensity and behavior recovery.

Neuroinflammation is a complex process that occurs with neuronal degeneration. Although PET imaging has largely used ^{18}F-FDG or the translocator protein TSPO expressed in activated microglia and reactive astrocytes, both have some limitations (168). The cyclooxygenase (COX) isozymes COX-1 and COX-2 catalyze the rate-limiting step in the synthesis of several inflammatory prostanoids. COX-2 is the product of an immediate-early gene that is quickly and dramatically upregulated by inflammation and can reflect rapid changes in the inflammatory response, particularly when compared to TSPO. ^{11}C-MC1 for COX-2 was shown to effectively image and quantify COX-2 upregulation in the rhesus monkey brain after LPS-induced neuroinflammation and in human peripheral tissue with inflammation.

7. CONCLUSIONS

The need for nonhuman primates for translational research continues to remain a high priority. This need was acutely evident during the SARS-COV-2 global pandemic, when the demand for nonhuman primates was exceedingly high (169, 170). Nonhuman primate species will continue to address key questions, and to provide predictive models to identify the safety and efficiency of new diagnostics and therapies across the lifespan. Studies of infectious diseases, including Zika virus and SARS-CoV-2, support the importance of nonhuman primates, particularly for the development of safe and effective vaccines. The path to human clinical trials is challenging, and nonhuman primates provide the essential preclinical and IND-enabling studies to ensure safety and proof-of-concept data prior to human use.

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LITERATURE CITED

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