



HHS Public Access

Author manuscript

Biol Psychiatry Cogn Neurosci Neuroimaging. Author manuscript; available in PMC 2022 October 18.

Published in final edited form as:

Biol Psychiatry Cogn Neurosci Neuroimaging. 2022 May ; 7(5): 491–497. doi:10.1016/j.bpsc.2022.03.001.

Translational Utility of the Nonhuman Primate Model

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Abstract

Nonhuman primates are essential for the study of human disease and to explore the safety of new diagnostics and therapies proposed for human use. They share similar genetic, physiologic, immunologic, reproductive, and developmental features with humans and thus have proven crucial for the study of embryonic/fetal development, organ system ontogeny, and the role of the maternal-placental-fetal interface in health and disease. The fetus may be exposed to a variety of inflammatory stimuli including infectious microbes as well as maternal inflammation, which can result from infections, obesity, or environmental exposures. Growing evidence supports that inflammation is a mediator of fetal programming and that the maternal immune system is tightly integrated with fetal-placental immune responses that may set a postnatal path for future health or disease. This review addresses some of the unique features of the nonhuman primate model system, specifically the rhesus monkey (*Macaca mulatta*), and importance of the species for studies focused on organ system ontogeny and the impact of viral teratogens in relation to development and congenital disorders.

Nonhuman primates, such as the rhesus monkey (*Macaca mulatta*), are an important animal model for the study of human development and disease. Humans and rhesus monkeys share many characteristic features because of their close phylogenetic relationship, which includes similarities in genetic, physiologic, immunologic, reproductive, and developmental features (1–16). For example, the rhesus monkey placenta is discoid and hemochorial, similar to the human placenta (12). 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. In humans, the embryo is completely embedded within the uterine stroma, whereas in the rhesus monkey, the blastocyst remains superficially attached

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DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

and adheres to the side opposite the initial attachment, forming the location of the future secondary placental disk (approximately 80% with bidiscoid placenta). Trimesters in rhesus monkeys encompass 55 days with the first trimester representing 0 to 55 days of gestation; the second trimester, 56 to 110 days of gestation; and the third trimester, 111 to 165 days of gestation (term 165 ± 10 days in this species) (15). Developmental similarities include the period of organogenesis (17,18), growth trajectory, and organ ontogeny, including the hematopoietic and immune systems (2–4,9).

Comparable to humans, the fetal rhesus monkey liver is the primary site of hematopoiesis in the first trimester (human fetus approximately 5–6 weeks), with a peak in hematopoiesis thereafter (early second trimester; 3–4 months in the human fetus) 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 (approximately 7 months in the human fetus) (9,19).

Given the importance of the human immune system in health and disease, studies that focus on the immune system of nonhuman primates further advance translational research. Immune ontogeny in the rhesus monkey is similar in temporal and anatomical sequence to human development (3) and shows significant differences compared to rodents (20). These differences are well documented and highlight crucial disparities that may influence outcomes, particularly for the developing fetus and infant, and in relation to, for example, the trafficking of cells between the mother and fetus (21–25). Similar trafficking in rhesus monkeys and humans is related to the comparable placental structure. The fetus may be exposed to a variety of potential inflammatory stimuli including infectious microbes and allergenic proteins, as well as maternal inflammation that may result from infections, obesity, or environmental influences (26–30). Growing evidence supports that inflammation is a mediator of fetal programming and that the maternal immune system is tightly integrated with fetal-placental immune responses that can shape postnatal immunity and set a path for future health or disease (26,30–37). Development of the immune system begins early in gestation and continues through the postnatal period, similar to the developing brain (38). It is during this time that the fetus, neonate, and infant are most susceptible to pathogenic agents and rely primarily on innate immune responses for protection (39,40).

It has been shown that by the second trimester, the lymphoid tissues of the fetal rhesus monkey have a reasonably complete repertoire of appropriately organized B cells, T cells, and antigen-presenting cells, which are at least partly functional (3). These and other similarities in immune ontogeny when compared to humans have been leveraged to provide key insights into fetal-maternal infectious diseases and the role of the placenta. The placenta can serve as a modulator with postnatal consequences (36). A wide variety of cytokines are synthesized and released by cytotrophoblasts, syncytiotrophoblasts, and resident placental macrophages (35,41,42). Immune cells in the decidua (e.g., uterine natural killer [NK] cells) and toll-like receptors expressed by syncytiotrophoblasts can stimulate production of interferons in response to viruses and maintain placental function (43,44). It has been proposed that the interleukin 10 and uterine NK cell balance controls excessive

inflammation (45). A placental viral infection may activate both the maternal and the fetal immune systems and could promote a fetal inflammatory response resulting in high concentrations of inflammatory cytokines that can have a significant effect on the developing central nervous system (CNS).

NEURODEVELOPMENT

Nonhuman primates have proven essential to address the development and function of the CNS and have led to important insights into the formation of the human cerebral cortex. Production of cortical neurons in fetal rhesus monkeys follows an “inside out” sequence, 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 shown in small animal models (46,47). However, in the brain of both fetal rhesus monkeys and humans, the distribution of the NPC pool is more complex, and the number of NPCs is greatly expanded compared to commonly used small animal species (48). Furthermore, the elaborate folding of sulci and gyri in the mature human cerebral cortex is modeled by findings in the rhesus monkey. Consequently, studies with fetal rhesus monkeys have been instrumental for developing models that explain how cortical lamination and areal differences in the human brain may arise, for example, from a proto-map of localized clusters of NPCs in cortical proliferative zones (49).

Recent work has provided evidence of the interplay between key components of the developing CNS and immune system during gestation. The foundation for this insight was first established through decades of painstaking work that identified and characterized the form and function of NPCs in the fetal cerebral cortex. Development of the cerebral cortex begins early in gestation with proliferation of NPCs lining the ventricular lumen at the anterior end of the neural tube. Two main classes of NPCs, primary and secondary, have been identified based on expression of cell-specific molecular markers. Primary NPCs express the nuclear transcription factor Pax6 (47,50) and initially undergo self-replicating divisions. At the onset of neurogenesis, the Pax6⁺ primary NPCs undergo asymmetric divisions that generate the secondary population of NPCs (47,51–53). The secondary NPCs establish a proliferative zone that is called the subventricular zone (54), express the cell-specific nuclear transcription factor Tbr2 (47), and play a key role in generating telencephalic neurons (52,55–59). Studies in nonhuman primates leveraged previous work in small animal models to better predict human CNS development. Proliferative zones in the fetal rhesus monkey cerebral cortex are larger and more complex when compared to small animal species (60), but the timing and spatial distribution of the Pax6- and Tbr2-expressing NPCs are maintained in the rhesus monkey (61) and, importantly, model the pattern that has been described in the human fetal brain (46,48).

Primary NPCs in both fetal human and rhesus monkey cortex are distinguished from NPCs in smaller animal models by precocious expression of the cytostructural intermediate filament glial fibrillary acidic protein (GFAP) (62), which is maintained throughout the entire neurogenic period (62–64). Studies performed in fetal rhesus monkeys were instrumental in the discovery that newborn cortical neurons migrate from the proliferative zones to the developing cortical gray matter by attaching to GFAP⁺ pial processes and

using the pial process as a migratory guide during the journey (65), which also occurs in the human fetus (64,66). More recent studies have shown that intercellular communications between migrating neurons, the pial process of primary NPCs, and secondary NPCs are crucial for regulating the proper functions of these cells (67,68)—further highlighting the importance of intercellular relationships in the fetal brain that were revealed in nonhuman primate studies.

Cortical neurons are generated in a temporal sequence during gestation, with these neurons destined for deep cortical layers generated earliest, followed by neurons destined for more superficial cortical layers, as demonstrated nearly 50 years ago in the fetal rhesus monkey (69). Experimental studies have not yet determined the precise timing of cortical neurogenesis in the human cerebral cortex, but examination of fetal tissue supports that development in the rhesus monkey closely models the timing of developmental sequences in the fetal human brain (64,66,70). The neurogenic period in rhesus monkey cortex is approximately 2 months long, with neurons destined for each layer of the cerebral cortex generated over a period of at least a week (69). Similarly, in the human fetus, cortical neurogenesis occurs over a protracted period of time (71,72). The length of neurogenesis in the human and nonhuman primate contrasts significantly with the shorter 1-week-long neurogenic period in the mouse or rat (73). Critically, the months-long stages of cortical cell production in nonhuman primates allow for a thorough, temporal study of brain structural development (60), molecular mechanisms regulating growth (65,74,75), and the intersection of critical windows during development of CNS structures with exposure to pathogens.

The neurogenesis of deep brain structures in nonhuman primates has not been as thoroughly studied as in rodents. However, available data show that the neurogenic phase of these structures extends far longer in nonhuman primates such as the rhesus monkey than in small rodent models. For example, neurons destined for the rhesus monkey neostriatum are generated over a period of 45 days (76), which is double the entire length of gestation for the rat and mouse. As with the longer period of cortical formation, the extended production of striatal neurons in human and nonhuman primates may provide greater opportunity for interference through pathogen exposure.

MICROGLIA: IMMUNE CELLS OF THE CNS

It has become increasingly clear that the immune system plays a key functional role in development of the brain (77). The innate immune cells of the CNS, microglial cells, contribute to an array of developmental programs, including axon pathfinding, synapse development and maintenance, and cortical layer formation (77–81). Microglia in the fetal human and rhesus monkey brain begin to colonize the cerebral cortex at the onset of cortical neurogenesis and initially populate the neural proliferative zones (78,82–86). On arriving in the fetal cortex, microglia establish connections with NPCs and developing vasculature (78,87,88). In particular, the population of microglia located close to the ventricle establish numerous contacts with NPCs, envelop mitotic NPCs in a cell cycle-dependent manner (87,88), and phagocytose NPCs, which slows production of cortical neurons and glia (78).

Microglia colonize key structures, such as the telencephalon, in significantly larger numbers and at earlier stages of fetal development in nonhuman primates than in small animal species (87), and evidence indicates that microglia in the primate brain differ from those in rodents (89). Together, these findings highlight the importance of nonhuman primates in modeling the intersection between the developing CNS and immune system. Indeed, the comparatively early arrival of microglia in proliferative zones of the fetal primate telencephalon, particularly during the months-long period of cortical neuron production, provides an opportunity for microglia to play a more prominent role in cellular genesis and maturation in primates and supports the concept that microglial cells contribute to formation of the telencephalon from early stages of development. However, this potential developmental benefit may come with risks. Fetal microglia rapidly respond to changes in the local environment, injury, and extrinsic factors introduced through maternal exposure to viral pathogens (90), which has been demonstrated in the baboon and rhesus monkey (91,92). For example, fetal exposure to Zika virus results in profound changes in key components of the fetal cerebral cortex. At 3 weeks after fetal 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 (see below). The microglial clusters were associated with disturbed distribution of NPCs, enlarged blood vessels, and a thinner cortical plate that persisted 3 months after Zika virus exposure (92).

Further, we have found that microglia in the typically developing fetal brain exhibit multiple phenotypes that are revealed through differential expression of markers such as CD68. This includes analysis of morphological features such as an ameboid versus ramified cellular phenotype, and extension of phagocytic cups that contact precursor cells and differentiating cells in the germinal zones. These analyses have captured distinct states of microglial activation at the molecular and morphological levels and the relative proportion of these cellular phenotypes during development (78,87). Environmental stimuli that induce a maternal immune response are therefore capable of engaging immune cells in fetal tissues, including the brain, and potentially altering the normal developmental trajectory. These data support the concept that microglia may be one conduit between pathogen exposure and atypical outcomes in primate neurodevelopment. Further studies on intercellular communications in the prenatal proliferative zones will aid in defining the functional roles of microglial cells in the developing brain under typical developmental conditions and with respect to neurodevelopmental disorders. These data exemplify why rhesus monkeys are an essential model for understanding CNS development and the role of viral and other teratogens.

VIRAL TERATOGENS

As noted above, the rhesus monkey closely mirrors human development, including length of gestation, growth characteristics, and organ ontogeny. The importance of these features is clear in the historical accounting of the thalidomide tragedy (93) and, more recently, viral teratogens such as the TORCH (*Toxoplasma gondii*, other, rubella virus, cytomegalovirus [CMV], and herpes simplex virus) agents, Zika virus, and CMV (94–96). The maternal-placental-fetal interface provides close contact between the uterine mucosa, placenta, and fetal membranes. Despite this potential physical and immunologic barrier, microorganisms

can sometimes bypass the host adaptive and innate immune system and lead to congenital infection.

Microglia and Zika Virus

Although Zika virus was discovered in 1952 in Uganda (97), it remained little studied until the South American outbreak in 2015 (98). Studies in nonhuman primates soon followed, which demonstrated features consistent with typical human infections (99,100). Most often, infected adult macaques manifest only transient viremia and subclinical disease; the infection may be transmitted from dams to their fetuses and can cause adverse fetal outcomes. In humans, the congenital sequelae to in utero infection (congenital Zika syndrome) develop in approximately 1 in 7 infants born to infected mothers (101).

Our studies have addressed the impact of Zika virus on fetal development more directly, by inoculating early-gestation fetal rhesus monkeys using an ultrasound-guided approach (intraperitoneal or intracranial) (92). Transient maternal viremia was observed, and sustained maternal immune activation was detected by flow cytometry. Maternal T cell activation and maturation was assessed by following expression of CCR5, HLA-DR, and memory/effector subset markers. Maternal CCR5 expression by CD4⁺ T cells followed a pattern of early upregulation with a later decline, in a pattern similar to maternal viral load; maximum maternal viral load and CCR5 expression appeared to be associated. The increased expression of CCR5 followed the early spike in maternal viral load by 10 to 30 days. In some cases, maternal CCR5 expression was maintained despite resolution of viremia. As noted above, significant morphologic changes were observed in the fetal cerebral cortex at 3 weeks after Zika virus inoculation, including massive alterations in the distribution, density, number, and morphology of microglial cells in proliferative regions of the fetal cerebral cortex; an altered distribution of Tbr2⁺ NPCs; increased diameter and volume of blood vessels in the cortical proliferative zones; and a thinner cortical plate. At 3 months after inoculation, alterations in morphology, distribution, and microglial cell density were also observed with an increase in blood vessel volume and a thinner cortical plate.

One question under study is whether adverse fetal outcomes require direct infection of fetal tissues or if placental infection and resulting dysfunction are important contributors. Studies in macaques are particularly important in resolving this question because of similarities in placental structure as noted above. Placental Zika virus infection in humans and rhesus monkeys has been shown to result in placental inflammation and vasculitis (31,32,96,99,102,103), providing evidence to support that more studies are needed to understand the role of the placenta and the maternal-placental-fetal interface in the range of outcomes associated with infection.

CMV and Fetal Immunity

CMV is the most common congenital viral infection in the United States, with approximately 1% of newborns infected (103). A primary infection in the first trimester has a 40% risk of transmission with 25% resulting in birth defects. Fetal and/or postnatal CMV infections can have a profound, permanent impact on immune function and can result in postnatal hearing loss and neurodevelopmental delays. CMV infection influences

nearly 60% of all immune phenotypes and functional responses, including a major impact on the memory T cell pool, with about 10% of the memory T cell repertoire (both CD4⁺ and CD8⁺) being CMV specific (104). Human CMV infection is associated with expansion of and adaptive changes in subsets of NK cells, e.g., g⁻NK cells, a class of cells with memory function that do not rely on germline-encoded, antigen-specific receptors (105). Some investigators have argued that g⁻NK cells are essentially specific to CMV infection, as CMV-seronegative individuals with prevalent g⁻NK cells are frequently shown to possess CMV-specific T cells. CMV infection is also associated with expansion of adaptive immune cells with innate features, e.g., NK-like cytotoxic T lymphocytes, which are CD8⁺ cytotoxic T lymphocytes with NK-like surface markers and functional activity. We recently demonstrated that these cells are equivalent to cells previously identified variously as innate memory cells, virtual memory cells, or antimicrobial cytotoxic T lymphocytes, and that their expansion is driven by host interleukin 15 production (106). Innate memory cells express HLA class I–specific inhibitory receptors most often associated with NK cells (e.g., NKG2A) and have the capacity to kill class Ia–deficient targets, such as HIV-infected cells and many tumor cells (107).

To assess the potential protective role that transplacentally transferred anti-rhesus CMV (RhCMV) immunoglobulin G (IgG) might play in limiting fetal disease, maternal and fetal antibodies to RhCMV were analyzed in rhesus monkeys by obtaining paired maternal/fetal blood samples during gestation, at birth, and postnatally (108). In uninfected control fetuses, antiviral IgG titers were first detected in the fetal circulation in the early second trimester (approximately 1%–2% of maternal titers), which corresponded to transplacentally transferred IgG. Mean titers in the fetus increased to 12.5% of maternal titers by the late second trimester and increased further during gestation to approximately 50% of maternal titers in the third trimester and at birth. Because maternal RhCMV titers remained unchanged during gestation, relative increases in fetal IgG titers reflect increased transplacental transfer of maternal IgG. Analyses were then performed in fetuses that were directly inoculated intraperitoneally with RhCMV in the late first trimester or early second trimester. For fetuses inoculated in the first trimester, titers paralleled those from control fetuses during gestation. A distinct pattern was observed in fetuses inoculated in the early second trimester when compared to uninoculated: significantly higher RhCMV IgG responses were noted in the third trimester, at term, and at 1-month postnatal age. Since maternal RhCMV titers remained constant across gestation, the increased titers reflected IgG of fetal and neonatal origin. These results demonstrate 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.

CONCLUSIONS

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 health or disease across the lifespan (109). The continued rise in allergic and autoimmune diseases highlights the susceptibility of developing immune pathways, with inflammation a common theme for many chronic illnesses (28,110,111). The need for

nonhuman primates in translational research, particularly related to pregnancy, fetal/neonatal development including the CNS, and the role of the immune system, continues to remain a high priority (99,112–116). Ongoing studies that explore the role of the maternal-placental-fetal interface are needed to address new approaches to protect against congenital disease and the potential for neurodevelopmental disorders (117–119).

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (Grant Nos. OD023716 [to AFT, SCN], NS103658 [to AFT, SCN, DJH-O], NS109379 [to SCN, AFT], NS111313 [to SCN, AFT], AI133548 [to AFT], AI084109 [to DJHO, AFT], and AI090677 [to DJH-O]) and National Institutes of Health Primate Center base operating grant (Grant No. OD011107). In vivo imaging was performed with instrumentation funded by the National Institutes of Health S10 High-End Instrumentation Grant Program (Grant Nos. OD016261 and RR025063 [to AFT]).

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