Review

Biological Psychiatry: CNNI

Translational Utility of the Nonhuman Primate Model

Alice F. Tarantal, Dennis J. Hartigan-O'Connor, and Stephen C. Noctor

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.

https://doi.org/10.1016/j.bpsc.2022.03.001

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 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).

ISSN: 2451-9022

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 AND DISCLOSURES

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 DJH-O, 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]).

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

ARTICLE INFORMATION

From the Departments of Pediatrics (AFT), Cell Biology and Human Anatomy (AFT), Medical Microbiology and Immunology (DJH-O), and Psychiatry and Behavioral Sciences (SCN), School of Medicine, California National Primate Research Center (AFT, DJH-O), and MIND Institute (SCN), University of California Davis, Davis, California.

Address correspondence to Alice F. Tarantal, Ph.D., at aftarantal@ucdavis.edu.

Received Sep 13, 2021; revised Feb 25, 2022; accepted Mar 1, 2022.

REFERENCES

1. Abbott DH, Rogers J, Dumesic DA, Levine JE (2019): Naturally occurring and experimentally induced rhesus macaque models for polycystic ovary syndrome: Translational gateways to clinical application. Med Sci (Basel) 7:107.
2. Batchelder CA, Keyser JL, Lee CCI, Tarantal AF (2013): Characterization of growth, glomerular number, and tubular proteins in the developing rhesus monkey kidney. Anat Record 296:1747–1757.
3. Batchelder CA, Lee CCI, Duru N, Baker C, Swainson L, McCune JM, *et al.* (2014): Myeloid-lymphoid ontogeny in the rhesus monkey (*Macaca mulatta*). Anat Rec 297:1392–1406.
4. Batchelder CA, Lee CCI, Martinez ML, Tarantal AF (2010): Ontogeny of the kidney and renal developmental markers in the rhesus monkey (*Macaca mulatta*). Anat Rec (Hoboken) 293:1971–1983.
5. Cardoso-Moreira M, Sarropoulos I, Velten B, Mort M, Cooper DN, Huber W, *et al.* (2020): Developmental gene expression differences between humans and mammalian models. Cell Rep 33:108308.
6. Cox LA, Olivier M, Spradling-Reeves K, Karere GM, Comuzzie AG, VandeBerg JL (2017): Nonhuman primates and translational research—cardiovascular disease. ILAR J 58:235–250.
7. Estes JD, Wong SW, Brenchley JM (2018): Nonhuman primate models of human viral infections. Nat Rev Immunol 18:390–404.
8. Huber HF, Jenkins SL, Li C, Nathanielsz PW (2020): Strength of nonhuman primate studies of developmental programming: Review of sample sizes, challenges, and steps for future work. J Dev Orig Health Dis 11:297–306.
9. Lee CI, Fletcher MD, Tarantal AF (2005): Effects of age on the frequency, cell cycle, and lineage maturation of rhesus monkey (*Macaca mulatta*) $CD34^+$ and hematopoietic progenitor cells. Pediatr Res 58:315–322.
10. Messaoudi I, Estep R, Robinson B, Wong SW (2011): Nonhuman primate models of human immunology. Antiox Redox Sign 14:261–273.
11. Plopper CG, Hyde DM (1992): Epithelial cells of bronchioles. In: Parent RA, editor. Comparative Biology of the Normal Lung. Boca Raton, FL: CRC Press, 85–92.
12. Ramsey EM, Houston ML, Harris JW (1976): Interactions of the trophoblast and maternal tissues in three closely related primate species. Am J Obstet Gynecol 124:647–652.
13. Saravanan C, Flandre T, Hodo CL, Lewis AD, Mecklenburg L, Romeike A, *et al.* (2020): Research relevant conditions and pathology in nonhuman primates. ILAR J 61:139-166.
14. Stouffer RL, Woodruff TK (2017): Nonhuman primates: A vital model for basic and applied research on female reproduction, prenatal development, and women's health. ILAR J 58:281–294.
15. Tarantal AF (2005): Ultrasound imaging in rhesus and long-tailed macaques: Reproductive and research applications. In: Wolfe-Coote S, editor. The Laboratory Primate. London: Elsevier, 317–351.
16. Warren WC, Harris RA, Haukness M, Fiddes IT, Murali SC, Fernandes J, *et al.* (2020): Sequence diversity analyses of an improved rhesus macaque genome enhance its biomedical utility. Science 370:eabc6617.
17. Gribnau AAM, Geijsberts LGM (1985): Morphogenesis of the brain in staged rhesus monkey embryos. Adv Anat Embryol Cell Biol 91:1–69.
18. Heuser CH, Streeter GL (1941): Development of the macaque embryo. Contrib Embryol 29:15–55.
19. Cowan MJ, Chou SH, Tarantal AF (2001): Tolerance induction post in utero stem cell transplantation. In: Holzgreve W, Lessl M, editors. Stem Cells From Cord Blood, In Utero Stem Cell Development, and Transplantation-Inclusive Gene Therapy. New York: Springer-Verlag, 145–196.
20. Mestas J, Hughes CC (2004): Of mice and not men: Differences between mouse and human immunology. J Immunol 172:2731–2738.
21. Bakkour S, Baker C, Tarantal AF, Wen L, Busch MP, Lee TH, *et al.* (2014): Analysis of maternal microchimerism in rhesus monkeys (*Macaca mulatta*) using real-time quantitative PCR amplification of MHC polymorphisms. Chimerism 5:6–15.
22. Bianchi DW, Khosrotehrani K, Way SS, MacKenzie TC, Bajema I, O'Donoghue K (2021): Forever connected: The lifelong biological consequences of fetomaternal and maternofetal microchimerism. Clin Chem 67:351–362.
23. Jimenez DF, Leapley AC, Lee CI, Tarantal AF (2005): Fetal $CD34^+$ cells in the maternal circulation and long-term microchimerism in rhesus macaques. Transplantation 79:142–146.
24. Jimenez DF, Tarantal AF (2003): Quantitative analysis of male fetal DNA in maternal serum of gravid rhesus monkeys (*Macaca mulatta*). Pediatr Res 53:18–23.
25. Mold JE, McCune JM (2012): Immunological tolerance during fetal development: From mouse to man. Adv Immunol 115:73–111.
26. Apostol AC, Jensen KDC, Beaudin AE (2020): Training the fetal immune system through maternal inflammation—a layered hygiene hypothesis. Front Immunol 11:123.
27. Arora N, Sadovsky Y, Dermody TS, Coyne CB (2017): Microbial vertical transmission during human pregnancy. Cell Host Microbe 21:561–567.
28. Duan Y, Zeng L, Zheng C, Song B, Li F, Kong X, *et al.* (2018): Inflammatory links between high fat diets and diseases. Front Immunol 9:2649.
29. Sureshchandra S, Marshall NE, Messaoudi I (2019): Impact of pregravid obesity on maternal and fetal immunity: Fertile grounds for reprogramming. J Leukoc Biol 106:1035–1050.
30. Tarantal AF, Berglund L (2014): Obesity and lifespan health—importance of the fetal environment. Nutrients 6:1725–1736.
31. Chen Q, Gouilly J, Ferrat YJ, Espino A, Glaziou Q, Cartron G, *et al.* (2020): Metabolic reprogramming by Zika virus provokes inflammation in human placenta. Nature Commun 11:2967.
32. Crooks CM, Weiler AM, Rybarczyk SL, Bliss M, Jaeger AS, Murphy ME, *et al.* (2021): African-lineage Zika virus replication dynamics and maternal-fetal interface infection in pregnant rhesus macaques. J Virol 95:e0222020.
33. Heerema-McKinney A (2018): Defense and infection of the human placenta. APMIS 126:570–588.
34. Jennewein MF, Abu-Raya B, Jiang Y, Alter G, Marchant A (2017): Transfer of maternal immunity and programming of the newborn immune system. Semin Immunopathol 39:605–613.
35. Kim CJ, Romero R, Chaemsaithong P, Kim JS (2015): Chronic inflammation of the placenta: Definition, classification, pathogenesis, and clinical significance. Am J Obstet Gynecol 213:S53–S69.
36. Kreis NN, Ritter A, Louwen F, Yuan J (2020): A message from the human placenta: Structural and immunomodulatory defense against SARS-CoV-2. Cells 9:1777.
37. Ozen M, Novak C, Burd I (2018): Placenta immune infiltrates and perinatal outcomes. Am J Reprod Immunol 79:e12850.
38. Lu-Culligan A, Iwasaki A (2020): The role of immune factors in shaping fetal neurodevelopment. Annu Rev Cell Dev Biol 36:441–468.
39. Basha S, Surendran N, Pichichero M (2014): Immune responses in neonates. Expert Rev Clin Immunol 10:1171–1184.
40. Levy O (2007): Innate immunity of the newborn: Basic mechanisms and clinical correlates. Nat Rev Immunol 7:379–390.
41. Bayer A, Lennemann NJ, Puyang Y, Bramley JC, Morpsky S, De Azeved Marques ET, *et al.* (2016): Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. Cell Host Microbe 19:705–712.
42. Silasi M, Cardenas I, Kwon JY, Racicot K, Aldo P, Mor G (2015): Viral infections during pregnancy. Am J Reprod Immunol 73:199–213.
43. Koga K, Mor G (2010): Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy disorders. Am J Reprod Immunol 63:587–600.
44. Mor G (2016): Placental inflammatory response to Zika virus may affect fetal brain development. Am J Reprod Immunol 75:421–422.
45. Racicot K, Kwon JY, Aldo P, Silasi M, Mor G (2014): Understanding the complexity of the immune system during pregnancy. Am J Reprod Immunol 72:107–116.
46. Clowry G, Molnar Z, Rakic P (2010): Renewed focus on the developing human neocortex. J Anat 217:276–288.

47. Englund C, Fink A, Lau C, Pham D, Daza RA, Bulfone A, *et al.* (2005): Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 25:247–251.
48. Hansen DV, Lui JH, Parker PR, Kriegstein AR (2010): Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464:554–561.
49. Rakic P (1988): Specification of cerebral cortical areas. Science 241:170–176.
50. Gotz M, Stoykova A, Gruss P (1998): Pax6 controls radial glia differentiation in the cerebral cortex. Neuron 21:1031–1044.
51. Cai L, Hayes NL, Takahashi T, Caviness VS Jr, Nowakowski RS (2002): Size distribution of retrovirally marked lineages matches prediction from population measurements of cell cycle behavior. J Neurosci Res 69:731–744.
52. Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR (2004): Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 7:136–144.
53. Takahashi T, Nowakowski RS, Caviness V Jr (1995): Early ontogeny of the secondary proliferative population of the embryonic murine cerebral wall. J Neurosci 15:6058–6068.
54. Angevine JB, Bodian D, Coulombre AJ, Edds MV, Hamburger V, Jacobson M, *et al.* (1970): Embryonic vertebrate central nervous system: Revised terminology. Anat Rec 166:257–261.
55. Haubensak W, Attardo A, Denk W, Huttner WB (2004): Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: A major site of neurogenesis. Proc Natl Acad Sci U S A 101:3196–3201.
56. Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, Hodge R, *et al.* (2009): Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. Cereb Cortex 19:2439–2450.
57. Miyata T, Kawaguchi A, Saito K, Kawano M, Muto T, Ogawa M (2004): Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. Development 131:3133–3145.
58. Sessa A, Mao CA, Colasante G, Nini A, Klein WH, Broccoli V (2010): Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. Genes Dev 24:1816–1826.
59. Sessa A, Mao CA, Hadjantonakis AK, Klein WH, Broccoli V (2008): Tbr2 directs conversion of radial glia into basal precursors and guides neuronal amplification by indirect neurogenesis in the developing neocortex. Neuron 60:56–69.
60. Smart IH, Dehay C, Giroud P, Berland M, Kennedy H (2002): Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. Cereb Cortex 12:37–53.
61. Martinez-Cerdeño V, Cunningham CL, Camacho J, Antczak JL, Prakash AN, Cziep ME, *et al.* (2012): Comparative analysis of the subventricular zone in rat, ferret and macaque: Evidence for an outer subventricular zone in rodents. PLoS One 7:e30178.
62. Levitt P, Rakic P (1980): Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing rhesus monkey brain. J Comp Neurol 193:815–840.
63. Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013): Diversity of neural precursor cell types in the prenatal macaque cerebral cortex exists largely within the astroglial cell lineage. PLoS One 8:e63848.
64. de Azevedo LC, Fallet C, Moura-Neto V, Daumas-Duport C, Hedin-Pereira C, Lent R (2003): Cortical radial glial cells in human fetuses: Depth-correlated transformation into astrocytes. J Neurobiol 55:288–298.
65. Rakic P (1972): Mode of cell migration to the superficial layers of fetal monkey neocortex. J Comp Neurol 145:61–83.
66. Sidman RL, Rakic P (1973): Neuronal migration, with special reference to developing human brain: A review. Brain Res 62:1–35.
67. Rash BG, Ackman JB, Rakic P (2016): Bidirectional radial Ca(2+) activity regulates neurogenesis and migration during early cortical column formation. Sci Adv 2:e1501733.
68. Yoon KJ, Koo BK, Im SK, Jeong HW, Ghim J, Kwon MC, *et al.* (2008): Mind bomb 1-expressing intermediate progenitors generate notch signaling to maintain radial glial cells. Neuron 58:519–531.
69. Rakic P (1974): Neurons in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. Science 183:425–427.
70. Kostovic I, Rakic P (1980): Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. J Neurocytol 9:219–242.
71. Arshad A, Vose LR, Vinukonda G, Hu F, Yoshikawa K, Csiszar A, *et al.* (2016): Extended production of cortical interneurons into the third trimester of human gestation. Cereb Cortex 26:2242–2256.
72. Malik S, Vinukonda G, Vose LR, Diamond D, Bhimavarapu BB, Hu F, *et al.* (2013): Neurogenesis continues in the third trimester of pregnancy and is suppressed by premature birth. J Neurosci 33:411–423.
73. Bayer SA, Altman J (1991): Neocortical Development. New York: Raven Press.
74. Dominguez MH, Ayoub AE, Rakic P (2013): POU-III transcription factors (Brn1, Brn2, and Oct6) influence neurogenesis, molecular identity, and migratory destination of upper-layer cells of the cerebral cortex. Cereb Cortex 23:2632–2643.
75. Meinecke DL, Rakic P (1992): Expression of GABA and GABAA receptors by neurons of the subplate zone in developing primate occipital cortex: Evidence for transient local circuits. J Comp Neurol 317:91–101.
76. Brand S, Rakic P (1979): Genesis of the primate neostriatum: [3H] thymidine autoradiographic analysis of the time of neuron origin in the rhesus monkey. Neuroscience 4:767–778.
77. Salter MW, Stevens B (2017): Microglia emerge as central players in brain disease. Nat Med 23:1018–1027.
78. Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013): Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216–4233.
79. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, *et al.* (2011): Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456–1458.
80. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, *et al.* (2012): Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705.
81. Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, *et al.* (2014): Microglia modulate wiring of the embryonic forebrain. Cell Rep 8:1271–1279.
82. Andjelkovic AV, Nikolic B, Pachter JS, Zecevic N (1998): Macrophages/microglial cells in human central nervous system during development: An immunohistochemical study. Brain Res 814:13–25.
83. Arno B, Grassivaro F, Rossi C, Bergamaschi A, Castiglioni V, Furlan R, *et al.* (2014): Neural progenitor cells orchestrate microglia migration and positioning into the developing cortex. Nat Commun 5:5611.
84. Monier A, Evrard P, Gressens P, Verney C (2006): Distribution and differentiation of microglia in the human telencephalon during the first two trimesters of gestation. J Comp Neurol 499:565–582.
85. Rezaie P, Male D (1999): Colonisation of the developing human brain and spinal cord by microglia: A review. Microsc Res Tech 45:359–382.
86. Verney C, Monier A, Fallet-Bianco C, Gressens P (2010): Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. J Anat 217:436–448.
87. Barger N, Keiter J, Kreutz A, Krishnamurthy A, Weidenthaler C, Martinez-Cerdeno V, *et al.* (2019): Microglia: An intrinsic component of the proliferative zones in the fetal rhesus monkey (*Macaca mulatta*) cerebral cortex. Cereb Cortex 29:2782–2796.
88. Noctor SC, Penna E, Shepherd H, Chelson C, Barger N, Martinez-Cerdeno V, *et al.* (2019): Periventricular microglial cells interact with dividing precursor cells in the nonhuman primate and rodent prenatal cerebral cortex. J Comp Neurol 527:1598–1609.
89. Wolf SA, Boddeke HW, Kettenmann H (2017): Microglia in physiology and disease. Ann Rev Physiol 79:619–643.

90. Bilbo SD, Block CL, Bolton JL, Hanamsagar R, Tran PK (2018): Beyond infection—maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp Neurol 299:241–251.
91. Gurung S, Reuter N, Preno A, Dubaut J, Nadeau H, Hyatt K, *et al.* (2019): Zika virus infection at mid-gestation results in fetal cerebral cortical injury and fetal death in the olive baboon. PLoS Pathog 15: e1007507.
92. Tarantal AF, Hartigan-O'Connor DJ, Penna E, Kreutz A, Martinez ML, Noctor SC (2021): Fetal rhesus monkey first trimester Zika virus infection impacts cortical development in the second and third trimesters. Cereb Cortex 31:2309–2321.
93. Vargesson N (2015): Thalidomide-induced teratogenesis: History and mechanisms. Birth Defects Res C Embryo Today 105:140–156.
94. Coyne CB, Lazear HM (2016): Zika virus—reigniting the TORCH. Nat Rev Microbiol 14:707–715.
95. de Vries LS (2020): Viral infections and the neonatal brain. Semin Pediatr Neurol 32:100769.
96. Parker EL, Silverstein RB, Vera S, Mysorekar IU (2020): Viral-immune cell interactions at the maternal-fetal interface in human pregnancy. Front Immunol 11:522047.
97. Dick GW, Kitchen SF, Haddow AJ (1952): Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 46:509–520.
98. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR (2016): Zika virus and birth defects—reviewing the evidence for causality. N Eng J Med 374:1981–1987.
99. Dudley DM, Aliota MT, Mohr EL, Newman CM, Golos TG, Friedrich TC, *et al.* (2019): Using macaques to address critical questions in Zika virus research. Ann Rev Virol 6:481–500.
100. Newman C, Friedrich TC, O'Connor DH (2017): Macaque monkeys in Zika virus research: 1947–present. Curr Opin Virol 25:34–40.
101. Rice ME, Galang RR, Roth NM, Ellington SR, Moore CA, Valencia-Prado M, *et al.* (2018): Zika-associated birth defects and neurodevelopmental abnormalities possibly associated with congenital Zika virus infection—U.S. territories and freely associated states, 2018. MMWR Morb Mortal Wkly Rep 67:858–867.
102. Narang K, Cheek EH, Enninga EAL, Theiler RN (2021): Placental immune responses to viruses: Molecular and histo-pathologic perspectives. Int J Mol Sci 22:2921.
103. Pereira L (2018): Congenital viral infection: Traversing the uterine-placental interface. Ann Rev Virol 5:273–299.
104. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, *et al.* (2005): Broadly targeted human cytomegalovirus-specific CD4(+) and CD8(+) T cells dominate the memory compartments of exposed subjects. J Exp Med 202:673–685.
105. Zhang T, Scott JM, Hwang I, Kim S (2013): Antibody-dependent memory-like NK cells distinguished by FcRγ-deficiency. J Immunol 190:1402–1406.
106. Méndez-Lagares G, Chin N, Chang WLW, Lee J, Rosás-Umbert M, Kieu HT, *et al.* (2021): Cytomegalovirus mediates expansion of IL-15-responsive innate-memory cells with SIV killing function. J Clin Invest 131:e148542.
107. Mazzarino P, Pietra G, Vacca P, Falco M, Colau D, Coulie P, *et al.* (2005): Identification of effector-memory CMV-specific T lymphocytes that kill CMV-infected target cells in an HLA-E-restricted fashion. Eur J Immunol 35:3240–3247.
108. Barry PA, Lockridge KM, Salamat S, Tinling SP, Yue Y, Tarantal AF (2006): Nonhuman primate models of intrauterine cytomegalovirus infection. ILAR J 47:49–64.
109. Penkler M, Hanson M, Biesma R, Müller R (2019): DOHaD in science and society: Emergent opportunities and novel responsibilities. J Dev Orig Health Dis 10:268–273.
110. Hagberg H, Gressens P, Mallard C (2012): Inflammation during fetal and neonatal life: Implications for neurologic and neuropsychiatric disease in children and adults. Ann Neurol 71:444–457.
111. Lockett GA, Human J, Holloway JW (2015): Does allergy begin in utero? Pediatr Allergy Immunol 26:394–402.
112. Betizeau M, Cortay V, Patti D, Pfister S, Gautier E, Bellemin-Mènard A, *et al.* (2013): Precursor diversity and complexity of lineage relationship in the outer subventricular zone of the primate. Neuron 80:442–457.
113. Li M, Brokaw A, Furuta AM, Coler B, Obregon-Perko V, Chahroudi A, *et al.* (2021): Non-human primate models to investigate mechanisms of infection-associated fetal and pediatric injury, teratogenesis, and stillbirth. Front Genet 12:680342.
114. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, *et al.* (2015): Immune mechanisms at the maternal-fetal interface: Perspectives and challenges. Nat Immunol 16:328–334.
115. Sartoretti J, Eberhardt CS (2021): The potential role of nonhuman primate models to better comprehend early life immunity and maternal antibody transfer. Vaccines (Basel) 9:306.
116. Semmes EC, Chen JL, Goswami R, Burt TD, Permar SR, Fouda GG (2021): Understanding early-life adaptive immunity to guide interventions for pediatric health. Front Immunol 11:595297.
117. Al-Haddad BJS, Oler E, Armistead B, Elsayed NA, Weinberger DR, Bernier R, *et al.* (2019): The fetal origins of mental illness. Am J Obstet Gynecol 221:549–562.
118. Raper J, Kovacs-Balint Z, Mavigner M, Gumber S, Burke MW, Habib J, *et al.* (2020): Long-term alterations in brain and behavior after postnatal Zika virus infection in infant macaques. Nat Commun 11:2534.
119. Venceslau EM, Guida JP, Amaral E, Modena JLP, Costa ML (2020): Characterization of placental infection by Zika virus in humans: A review of the literature. Rev Bras Ginecol Obstet 42:577–585.