Activation of the Maternal Immune System During Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring

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Background: Maternal infection during pregnancy is associated with an increased risk of schizophrenia and autism in the offspring. Supporting this correlation, experimentally activating the maternal immune system during pregnancy in rodents produces offspring with abnormal brain and behavioral development. We have developed a nonhuman primate model to bridge the gap between clinical populations and rodent models of maternal immune activation (MIA).

Methods: A modified form of the viral mimic, synthetic double-stranded RNA (polyinosinic:polycytidylic acid stabilized with poly-L-lysine) was delivered to two separate groups of pregnant rhesus monkeys to induce MIA: 1) late first trimester MIA ($n = 6$), and 2) late second trimester MIA ($n = 7$). Control animals ($n = 11$) received saline injections at the same first or second trimester time points or were untreated. Sickness behavior, temperature, and cytokine profiles of the pregnant monkeys confirmed a strong inflammatory response to MIA.

Results: Behavioral development of the offspring was studied for 24 months. Following weaning at 6 months of age, MIA offspring exhibited abnormal responses to separation from their mothers. As the animals matured, MIA offspring displayed increased repetitive behaviors and decreased affiliative vocalizations. When evaluated with unfamiliar conspecifics, first trimester MIA offspring deviated from species-typical macaque social behavior by inappropriately approaching and remaining in immediate proximity of an unfamiliar animal.

Conclusions: In this rhesus monkey model, MIA yields offspring with abnormal repetitive behaviors, communication, and social interactions. These results extended the findings in rodent MIA models to more human-like behaviors resembling those in both autism and schizophrenia.

Key Words: Animal model, autism spectrum disorder, immune activation, macaque, nonhuman primate, poly IC, schizophrenia

Autism spectrum disorder (ASD) and schizophrenia (SZ) are chronic and disabling brain disorders that each affect approximately 1% of the population (1,2) and are thought to be caused by complex interactions between genetic and environmental factors (3–5). Recent evidence suggests that the prenatal environment, and particularly the maternal immune environment, plays a critical role in some cases of ASD and SZ (6–8). Epidemiologic studies reveal that women exposed to viral, bacterial, or parasitic infections during pregnancy have an increased risk of having a child that later develops SZ (9–14). Likewise, maternal viral and bacterial infections are associated with an increased risk of ASD in the offspring (15–19). The diversity of maternal infections associated with ASD and SZ outcomes suggests that the maternal immune response is the critical link between sickness in the mother and altered neurodevelopment in her child.

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Understanding the mechanism by which maternal immune activation (MIA) during pregnancy increases the risk for SZ and ASD is essential to developing novel preventative or therapeutic strategies. Rodent models have identified molecular, cellular, and behavioral abnormalities associated with prenatal immune challenge (20). Maternal influenza infection (21–24) or injection of the bacterial endotoxin lipopolysaccaride (25–27) yields offspring with behavioral abnormalities, neuropathology, and altered gene expression that are relevant to both SZ and ASD. Similar outcomes are obtained by treating pregnant rodents with the viral mimic, synthetic double stranded RNA (polyinosinic:polycytidylic acid [poly IC]), which stimulates an inflammatory response in the absence of a specific pathogen (28). Offspring born to pregnant dams treated with poly IC at mid-gestation demonstrate repetitive behaviors and deficits in social and communication behaviors that resemble features of ASD, as well as elevated anxiety, deficits in prepulse inhibition, latent inhibition, and working memory that resemble clinical features of both ASD and SZ (21,29–32). Neuropathology observed with ASD (localized loss of Purkinje cells) and SZ (enlarged ventricles) have been reported in poly IC rodent models (33–35), and there are numerous other alterations in brain structure, neurochemistry, gene expression, and immune function (36–39). The deleterious effects on brain and behavior in the mouse MIA model appear to be mediated by the maternal cytokine response, in particular interleukin-6 (40).

While rodent models have laid the foundation for understanding the effects of MIA on fetal brain development, these models have limitations. Extrapolating the timing of fetal brain development between rodents and humans is complicated by the fact that the neural events of the human third trimester occur during the early postnatal period in rodents (41). Moreover, there are challenges in relating the rodent brain to the human brain and rodent behavior

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to human behavior. This is particularly problematic for disorders such as ASD and SZ that are characterized by deficits in a range of complex cognitive, social, and affective functions. Indeed, portions of the human brain, such as prefrontal cortex, which mediate these functions and are heavily impacted in ASD and SZ, are poorly developed in the rodent brain (42). Understanding human disorders involving higher cognitive functions will benefit from studies in animal species more closely related to humans. Nonhuman primates, such as rhesus macaques (*Macaca mulatta*), demonstrate many features of human physiology, anatomy, and behavior, making them an appropriate species to study a variety of human brain disorders (43). The rhesus monkey lives in a complex, hierarchical social system and uses many forms of human-like communication such as facial expressions and social gestures (44). The rich social and cognitive repertoire of rhesus monkeys provides a framework to relate behavioral changes observed in the animal model more directly to human mental illness.

We have developed a novel, nonhuman primate model using a modified form of the viral mimic poly IC, which is adapted for use in primates (polyinosinic:polycytidylic acid stabilized with poly-L-lysine [poly ICLC]). This synthetic RNA is recognized as foreign by the primate immune system and induces a transient innate inflammatory response (45,46). Pregnant rhesus monkeys were injected with poly ICLC over a 72-hour period at the end of the first or second trimester. These gestational ages were selected based on human epidemiologic data identifying the first and second trimesters as vulnerable time points where exposure to MIA increases the risk of autism and schizophrenia (14,17). We evaluated sickness behavior, body temperature, and cytokine responses in the dams to confirm a strong immune activation and then analyzed the behavioral development of the offspring for 4 years. Here, we present our initial behavioral findings through 24 months of age, documenting the emergence of abnormal behavior in rhesus offspring exposed to MIA.

Methods and Materials

All experimental procedures were developed in consultation with the veterinary staff at the California National Primate Research Center. Protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee. Detailed methods are provided in Supplement 1.

Maternal Administration of Poly ICLC

Twenty-four multiparous rhesus monkeys were assigned to one of three experimental groups: 1) first trimester MIA (MIA^1), 2) second trimester MIA (MIA^2), or 3) saline control animals (CON^{Saline}) (Table S1 in Supplement 1). Pregnant animals in the MIA groups were injected with .25 mg/kg synthetic double-stranded RNA (poly ICLC) (Oncovir, Inc., Washington, DC) via intravenous injection while restrained by trained technicians on gestational days 43, 44, and 46 (MIA^1) or 100, 101, and 103 (MIA^2).

Rearing Conditions

Infants were raised in individual cages with their mothers, where they had visual access to other animals at all times. For 3 hours each day, one adult male and four familiar mother-infant pairs were allowed to freely interact in a large cage to provide enrichment and facilitate species-typical social development. Each group consisted of a mixture of male and female offspring of both MIA and control experimental groups. The infants were weaned from their mothers at 6 months of age but continued the same socialization routine.

Table 1. Behavioral Phenotyping Assays

Behavioral Assay	Brief Description	Relevance to Autism Spectrum Disorders and Schizophrenia
6–12 Months of Age		
Mother preference[a]	Following weaning, each infant was tested for 4 days to evaluate one aspect of mother-infant attachment, the infant's preference for its own mother versus another familiar adult female (12 2-minute trials/subject).	Measures of attachment serve as control parameters for species-typical development and response to separation (48).
Postweaning solo observations[b]	At approximately 10 months of age, the animals were observed alone in a large, unfamiliar cage for two 5-minute focal samples on 2 separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (49,53,58).
12–18 Months of Age		
Juvenile Y-maze	At approximately 18 months of age, animals were given visual access to a novel conspecific in one arm of a Y-maze test apparatus. Each animal was tested for six 2-minute trials on 2 separate days, meeting an opposite-sex conspecific on the first day and a same-sex conspecific on the second day.	Initial social assays with novel conspecifics were carried out using the Y-maze testing apparatus and later followed with the three-chambered social approach assay described below.
Juvenile solo observations[b]	At approximately 22 months of age, the animals were observed alone in a large, unfamiliar cage for two 5-minute focal samples on 2 separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (49,53,58).
Juvenile social approach[c]	At approximately 24 months of age, social interactions with a novel conspecific were evaluated using a modified version of the mouse three-chambered social approach assay (20 minutes/subject).	The high-throughput social approach assay used in mouse models (54) paired with the fine-grained focal observations utilized in our nonhuman primate studies (47,48) provide a screen for sociability as indexed by the amount of time spent in a chamber with a constrained, novel conspecific.

ASD, autism spectrum disorders; SZ, schizophrenia.

[a]Assays used to control for changes in physical development, reflexes, fear response development, maternal attachment, and activity levels that are not directly related to the core features of ASD and SZ.

[b]Behavioral assays targeting repetitive behaviors and restricted interests.

[c]Behavioral assays targeting social and communication domains.