Archival Report

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Maternal Immune Activation Delays Excitatory-to-Inhibitory Gamma-Aminobutyric Acid Switch in Offspring

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ABSTRACT

BACKGROUND: The association between maternal infection and neurodevelopmental defects in progeny is well established, although the biological mechanisms and the pathogenic trajectories involved have not been defined.

METHODS: Pregnant dams were injected intraperitoneally at gestational day 9 with polyinosinic:polycytidylic acid. Neuronal development was assessed by means of electrophysiological, optical, and biochemical analyses.

RESULTS: Prenatal exposure to polyinosinic:polycytidylic acid causes an imbalanced expression of the Na^+-K^+-$2Cl^-$ cotransporter 1 and the K^+-Cl^- cotransporter 2 (KCC2). This results in delayed gamma-aminobutyric acid switch and higher susceptibility to seizures, which endures up to adulthood. Chromatin immunoprecipitation experiments reveal increased binding of the repressor factor RE1-silencing transcription (also known as neuron-restrictive silencer factor) to position 509 of the KCC2 promoter that leads to downregulation of KCC2 transcription in prenatally exposed offspring. Interleukin-1 receptor type I knockout mice, which display braked immune response and no brain cytokine elevation upon maternal immune activation, do not display KCC2/Na^+-K^+-$2Cl^-$ cotransporter 1 imbalance when implanted in a wild-type dam and prenatally exposed. Notably, pretreatment of pregnant dams with magnesium sulfate is sufficient to prevent the early inflammatory state and the delay in excitatory-to-inhibitory switch associated to maternal immune activation.

CONCLUSIONS: We provide evidence that maternal immune activation hits a key neurodevelopmental process, the excitatory-to-inhibitory gamma-aminobutyric acid switch; defects in this switch have been unequivocally linked to diseases such as autism spectrum disorder or epilepsy. These data open the avenue for a safe pharmacological treatment that may prevent the neurodevelopmental defects caused by prenatal immune activation in a specific pregnancy time window.

Keywords: Epilepsy, GABA switch, KCC2, Maternal immune activation

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Disruption of correct neurodevelopmental trajectories by maternal immune activation (MIA) is a critical factor in the development of neurological and neuropsychiatric disorders (1–3), especially when maternal infections synergize with a susceptible genetic background or when the prenatal event is followed by postnatal risk factors that act as "second hits" (3,4).

In recent years, the consequences of maternal infections on offspring brain have been explored using mouse models based on prenatal exposure to immune stimuli (5–9). The immunogens most commonly used in rodent models have been lipopolysaccharide, a component of the cell wall of gram-negative bacteria that binds to toll-like receptor 4 and mimics bacterial infection, or polyinosinic:polycytidylic acid (PolyI:C), a double-stranded, synthetic RNA that binds to toll-like receptor 3 like viral nucleic acid. Injection of either immunogen initiates a signaling cascade that leads to the production of inflammatory mediators, such as chemokines, cytokines, and complement proteins (10).

Consistent with epidemiological data, preclinical studies in these models have shown a wide spectrum of long-term behavioral changes, including impaired sensorimotor gating (prepulse inhibition) (8,11,12), increased anxiety-like behavior (13), cognitive deficits (12,14), and altered exploratory behavior (6,12). The phenotypic abnormalities in offspring are frequently accompanied by brain alterations, although with a high degree of variability depending on the specific immune activation paradigm (7,15,16). Injecting the immunogen at different time points during gestation leads to different neuropathological features (6), gene expression profiles (7), and behavioral abnormalities in offspring (6).

Although epidemiological and preclinical evidence indicate that intrauterine exposures elicit enduring effects on offspring,

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the specific molecular targets and the pathogenic pathways involved in brain alterations after prenatal immune activation are still far from elucidated. Recent studies investigated possible alterations in offspring brain transcriptome or proteome, in the attempt to identify mechanisms at the basis of postnatal changes induced by MIA: a widespread dysregulation has been unveiled in several genes linked to neuronal development, mitochondrial function, synaptic vesicle recycling, cytoskeletal structures, energy metabolism, and signal transduction (17,18). However, the possible functional impact of these transcriptomic or proteomic changes is still unknown.

In this framework, we aimed to assess whether prenatal immune activation impacts synapse formation and function, and whether it affects the excitatory versus inhibitory balance in neurotransmission, which is one common trait shared by different neurodevelopmental diseases.

METHODS AND MATERIALS

Animals

All experiments followed the guidelines established by the European Directive 2010/63/EU and the Italian Governing Law 26/2014. Dams were injected with PolyI:C (Sigma-Aldrich, St. Louis, MO) or vehicle intraperitoneally on gestational day 9 (GD9). For the embryo transfer procedure, embryos from interleukin-1 receptor type I knockout (IL-1RI KO) female mice were implanted into wild-type (WT) pseudopregnant female mice, following standard techniques (19). The magnesium treatment protocol was adapted from Hallak *et al.* (20). Kainate treatment was performed as previously described (21), and seizure severity was determined according to Racine's scale (22).

Cell Cultures

Cortical neurons were established from C57BL/6 mice at embryonic day 18 (E18) from vehicle or PolyI:C-treated mothers as previously described (23,24).

Imaging and Electrophysiology

Calcium imaging was performed on cortical cultures as previously described (25,26).

Cultured cortical neurons were recorded at 14 days in vitro (DIV) using an Axopatch 200B amplifier with a Digidata 1440 digitizer (Axon Instruments, Foster City, CA). Whole cell recordings were performed as previously reported (25). Electrophysiological recording from brain slices was performed as described by Lien *et al.* (27).

Biochemistry

Western blot analysis was performed on cortical tissues from E17, postnatal day 20 (P20), and P90 PolyI:C or vehicle prenatally treated mice and on 7 DIV cortical neurons.

Chromatin Immunoprecipitation and Real-time Polymerase Chain Reaction

Immunoprecipitation was performed on cortical tissue from P20 mice incubated with protein G Dynabeads (Invitrogen Corporation, Carlsbad, CA) bound to RE1-silencing transcription factor (REST) (Millipore, Burlington, MA) or methyl-CpG-binding protein 2 (Sigma-Aldrich) polyclonal antibody; K^+-Cl^- cotransporter 2 (KCC2) promoter was then analyzed by quantitative real-time polymerase chain reaction (PCR). For total RNA analysis, real-time PCR was performed on embryonic or P20 tissues.

Statistics

The results are presented as mean ± SEM. Student's two-tailed paired or unpaired *t* tests and one- or two-way analysis of variance, followed by Tukey's or Sidak's multiple comparisons test, were used for normal distributions. The Mann-Whitney nonparametric *U* test was used for non-normally distributed data.

Details can be found in the Supplement.

RESULTS

PolyI:C Mice Show Increased Susceptibility to Epilepsy in the Absence of Chronic Inflammation or Synaptic Alterations

A single PolyI:C exposure performed at GD9 (i.e., at the beginning of cortical neuronal layering) was sufficient to increase offspring susceptibility to seizures at 3 months of age. Response to intraperitoneal administration of 35 mg/kg kainic acid (KA) was compared in offspring of mothers exposed to PolyI:C versus vehicle (control), by behavioral evaluation repeated every 10 minutes over a 3-hour period (Figure 1A). In all mice, KA resulted in immobility and staring followed by head bobbing and isolated limbic motor seizures (Racine scale, stage 4), characterized by forelimb clonus and rearing. While control mice only displayed isolated limbic motor seizures, PolyI:C mice rapidly progressed to stage 5 (status epilepticus) and showed continuous generalized activity lasting for about 80 minutes (Figure 1A, left). One hundred eighty minutes after KA injection, PolyI:C mice still displayed a significantly higher mean Racine score relative to control mice (Figure 1A, right). PolyI:C mice did not display seizures either during normal activity or upon manipulation at any age; seizures were only evident after KA treatment. Contrary to prenatal exposure to the inflammatory hit, adult exposure did not result in any long-lasting increase of circuit hyperactivity. Indeed, a single PolyI:C treatment in adult mice failed to increase susceptibility to seizures (Supplemental Figure S1B). In line with the higher susceptibility to seizures, layer 5 neurons recorded by cell-attached patch clamp in acute cortical slices of PolyI:C-exposed progeny displayed a higher spike frequency than controls (Figure 1B).

Epilepsy may be the consequence of inflammatory processes (28), and therefore we investigated whether the hyperactivity and increased susceptibility to epilepsy observed in mice that were prenatally exposed to PolyI:C was associated with enhanced brain inflammation. Brains of 3-month-old mice that were prenatally exposed to either PolyI:C or vehicle were examined by confocal microscopy; the number and morphological appearance of microglial cells and the expression of proinflammatory markers were quantified. No differences were observed between PolyI:C and control mice, either in the number of microglia—revealed by the specific marker Iba1 (Supplemental Figure S2A)—or in the expression of CD11b (Supplemental Figure S2A) or glial fibrillary acidic protein (Supplemental Figure S2C). Unlike activated microglia,