



Cytokine-dependent bidirectional connection between impaired social behavior and susceptibility to seizures associated with maternal immune activation in mice



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ABSTRACT

Maternal immune activation (MIA) results in the development of autism in the offspring via hyperactivation of IL-6 signaling. Furthermore, experimental studies showed that the MIA-associated activation of interleukin-1 β (IL-1 β) concurrently with IL-6 increases the rate and the severity of hippocampal kindling in mice, thus, offering an explanation for autism–epilepsy comorbidity. We examined whether epileptic phenotype triggered by prenatal exposure to IL-6 and IL-1 β combination is restricted to kindling or whether it is reproducible in another model of epilepsy, whereby spontaneous seizures develop following kainic acid (KA)-induced status epilepticus. We also examined whether in mice prenatally exposed to IL-6 and IL-6 + IL-1 β , the presence of spontaneous seizures would exacerbate autism-like features. Between days 12 and 16 of pregnancy, C57BL/6J mice received daily injections of IL-6, IL-1 β , or IL-6 + IL-1 β combination. At postnatal day 40, male offspring were examined for the presence of social behavioral deficit, and status epilepticus was induced by intrahippocampal KA injection. After 6 weeks of monitoring for spontaneous seizures, sociability was tested again. Both IL-6 and IL-6 + IL-1 β offspring presented with social behavioral deficit. Prenatal exposure to IL-6 alleviated, while such exposure to IL-6 + IL-1 β exacerbated, the severity of KA-induced epilepsy. Increased severity of epilepsy in the IL-6 + IL-1 β mice correlated with the improvement of autism-like behavior. We conclude that complex and not necessarily agonistic relationships exist between epileptic and autism-like phenotypes in an animal model of MIA coupled with KA-induced epilepsy and that the nature of these relationships depends on components of MIA involved.

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1. Introduction

Common bidirectional connection between autism and epilepsy [1–3] has prompted numerous studies of autism–epilepsy comorbidity using animal models. Like in clinics, the association between autism-like and epileptic phenotypes varies significantly in experimental systems. For example, SCN1a haploinsufficient mice, which serve as a model of Dravet syndrome, present with both autism-like behavior and spontaneous seizures [4]. Conversely, mice which carry a missense mutation of a gene encoding neuroligin-3 (a mutation that has been implicated in autism [5]) and display autism-like behavior [6], have increased resistance to primary generalized seizures [7]. At the same

time, inbred BTBR mice, which are characterized by many behavioral and anatomical abnormalities consistent with autism [8], show no epileptic phenotype [9]. Identifying animal models appropriate for exploring autism–epilepsy connections and reflecting a variety of etiologies and mechanisms of both disorders are important for understanding the mechanisms of the comorbidity and for the development of its effective therapies.

Maternal immune activation (MIA) may represent a system suitable for exploring autism–epilepsy comorbidity. Epidemiological studies suggest that MIA, particularly when triggered by viral infections, represents a risk factor for the development of autism in the offspring [10,11]. Congruent with clinical findings, the offspring of mice in which viral infection has been mimicked during pregnancy by means of polyinosinic-polycytidylic acid (Poly I:C), present with a spectrum of behavioral, anatomical, and physiological perturbations consistent with autism [12, 13]. Concurrently, these animals show increased susceptibility to epilepsy in the hippocampal rapid kindling paradigm [14]. Furthermore, components of MIA liable for the evolution of autism-like and epileptic phenotypes have been identified: while autism-like impairments stem

Abbreviations: E, embryonic day; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; KA, kainic acid; MIA, maternal immune activation; P, postnatal day; Poly I:C, polyinosinic-polycytidylic acid; SE, status epilepticus.

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solely from the activation of interleukin-6 (IL-6) [15], parallel propensity to epilepsy requires simultaneous induction of IL-6 and interleukin-1 β (IL-1 β) [14].

In the present study, we further examined the autism–epilepsy connection in the MIA system. We deemed it important to establish that epileptogenicity in the MIA offspring is not model-specific, that is, not restricted to rapid kindling. Such studies appear even more warranted considering somewhat limited relevance of the rapid kindling model, in which no spontaneous recurrent seizures are observed. We chose a model of chronic epilepsy where spontaneous recurrent seizures develop following status epilepticus (SE) induced by intrahippocampal administration of kainic acid (KA) [16,17]. Further, in order to expand on our earlier findings [14], we examined whether the autism–epilepsy connection in the MIA offspring is bidirectional, that is whether the presence of spontaneous seizures in KA-injected mice would exacerbate the severity of autism-like impairments.

2. Material and methods

2.1. Animals

The experiments were performed in C57BL/6J mice. Breeding pairs were obtained from The Jackson Laboratory (Sacramento, CA). Breeding was performed at the UCLA Department of Laboratory Medicine. The procedures complied with the policies of the National Institutes of Health and were approved by the UCLA Office of Research Administration.

The presence of vaginal plug was considered as embryonic day (E) 0. The offspring were weaned at postnatal day (P) 28. Considering the higher prevalence of autism among males [18], and that in the MIA model, autism-like behavior is reserved for male offspring exclusively [19], the experiment proper was conducted in male mice. Animals were maintained at 12-hour light–dark cycle, with free access to food and water. Mice were housed individually, which was necessary for monitoring spontaneous seizures.

2.2. Modeling MIA

Based on earlier findings [14,15], we used recombinant cytokines IL-6 and IL-1 β to mimic MIA in pregnant mice. This offered a cleaner approach as compared with the use of Poly I:C, as the MIA system was limited to factors specifically responsible for the evolution of autism-like and epileptic phenotypes in the offspring and, thus, allowed avoiding wider variability on both seizure and behavioral responses inherent to the Poly I:C protocol. Between E12 and E16, mice received daily intraperitoneal injections of saline ($n = 8$), recombinant IL-6 (20 $\mu\text{g}/\text{kg}$, $n = 11$), recombinant IL-1 β (20 $\mu\text{g}/\text{kg}$, $n = 10$), or recombinant IL-6 + IL-1 β (10 + 10 mg/kg , $n = 13$). Both cytokines were manufactured by R&D systems (Minneapolis, MN) [14].

Cytokine treatment had no observable effects on pregnant mice. Body weight gain was similar to those in saline-treated animals (across all experimental groups, body weight was 30–33 g at E12 and 42–45 g on E16, with no differences among the groups). We did not measure core temperature in these animals, as the insertion of a rectal probe may lead to premature termination of pregnancy. However, in our earlier studies [14], we reported that the applied treatment regimens did not induce hyperthermia, when the temperature was measured in a specially allocated group of mice. After giving birth, cytokine-treated dams did not reject pups at a rate higher than saline-treated ones and did not refuse nursing (occasional rejections and subsequent offspring death occur even in the absence of any manipulations and handling).

From each saline-treated mouse, between 1 and 3 male offspring reached P28. For cytokine treatments, the number of male offspring reaching P28 was 0–3. Survivors from dams subjected to different cytokine treatments showed no noticeable differences in weight gain and general behavior as compared with offspring of saline-treated mice.

2.3. First behavioral testing

Impaired social interaction is a hallmark of autism [20] and is commonly used as a key parameter in characterizing autism-like impairments in animal models [8,21]. We employed a widely used three chamber sociability test [21] adapted in our lab [14] in order to quantify impairments of social behavior in mice. The test was performed between P35 and P40. The apparatus (Noldus, Leesburg, VA) was a 60 \times 40 cm Plexiglas box divided into three connected compartments. Each of the end compartments contained a wired cylindrical enclosure (11 cm high, 10 cm diameter, bar space 1 cm apart). First, a test mouse was placed inside the apparatus, was allowed to explore it for 10 min, and was then removed. An unfamiliar age- and sex-matched mouse (conspecific) was placed inside one enclosure, and an unfamiliar object (cube) was placed inside another enclosure. The placements of the conspecific and of the object were randomized between the two compartments for different test mice. The test mouse was reintroduced into the apparatus and was allowed to explore for 10 min. Behavior was recorded on video and was analyzed off-line by investigators blind to treatments. Total time of direct exploration (i.e., sniffing) by the test mouse of the conspecific ($t_{\text{conspecific}}$) and of the object (t_{object}) was counted. Sociability index was calculated using the formula $[(t_{\text{conspecific}} / (t_{\text{conspecific}} + t_{\text{object}})) \times 100 - 50]$. The index spans from -50 (complete avoidance of the conspecific) to 0 (indifference) to $+50$ (full preference for the conspecific) [12,14].

2.4. Induction and monitoring of chronic epilepsy

Between P40 and P45, animals were anesthetized with isoflurane and placed in the stereotaxic apparatus. Kainic acid (Sigma, St. Louis, MO) was stereotaxically injected in the amount of 50 ng in 0.5 μl of saline into the left ventral hippocampus (coordinates from Bregma: posterior -2.9 mm, lateral -2.8 mm, down -4.0 mm [22]). Control animals received intrahippocampal injection of saline.

In order to avoid litter effect (i.e., that is, offspring from the same litter are identical and, thus, represent a virtual $n = 1$ [23,24]), no subjects belonging to the same litter underwent the same postnatal procedure. For example, if an IL-6-treated mouse produced 3 male offspring reaching P28, one of them received intrahippocampal injection of saline, another received intrahippocampal injection of kainic acid, and the third one was not used in the experiments. This principle was applied to all groups.

Kainic acid injection resulted in the development of limbic SE, the latter consisting of repeated clonic–tonic generalized seizures (rearing and/or rearing and falling; stages 4–5 on the Racine scale [25]) intermittently with focal seizures (motor arrest and/or facial clonus; stage 1 on the Racine scale). Status epilepticus lasted between 3 and 5 h. Only those animals which developed repeated stage 4–5 convulsions were used for further studies.

Three to four weeks after intrahippocampal KA or saline injection, the animals were prepared for seizure monitoring. Under isoflurane anesthesia, wireless transmitter model ETA-F10 (Data Science International, DSI, St. Paul, MN) was placed inside a subcutaneous pocket on the back; its two leads were fed under the skin to the skull surface and fixed with skull screws. The leads were fixed to the skull with dental cement.

Video and EEG monitoring of spontaneous seizures began 1 week after surgery and continued for 6 weeks. Behavioral seizures were recorded using digital cameras focusing on individual cages; data were saved on the digital video recorder. For the acquisition of electrographic seizures, home cages with individually housed animals were placed on top of wireless receivers RPC-1 (DSI) connected to a computer equipped with Harmonie acquisition software (Stellate Systems, Montreal, QC). In order to unambiguously establish the presence of chronic epilepsy, the only seizures considered were secondarily generalized complex partial seizures (stages 4–5 on the Racine scale [25]) with clearly identifiable

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