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# Influence of administration of pro-inflammatory agents on development, individual and social behavior of BTBR mice

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*Motivation and Aim*: Autism spectrum disorder (ASD) is a neurodevelopmental disorder involving 1 out of every 68 children. ASD is defined by two core behavioral characteristics, namely, restricted repetitive behaviors and impaired social-communicative functioning. Accumulating evidence suggests that immune processes play a key role in the pathophysiology of ASD: extensive alterations in immune function have now been described in both children and adults with ASD, including ongoing inflammation in brain specimens, elevated pro-inflammatory cytokine profiles in the cerebrospinal fluid and blood, increased presence of brain-specific auto-antibodies and altered immune cell function. BTBR T+ltpr3tf/J (BTBR) mice provide a valuable animal model for ASD to elucidate the underlying mechanisms of these two behavioral characteristics of ASD. The BTBR mouse was reported to have significantly higher levels of serum IgG, brain IgG deposits and anti-brain IgG, elevated expression of cytokines and an increased proportion of MHC class II-expressing microglia compared to highly social C57BL/6 (B6) mice. The Th2-like immune profile of the BTBR mice and their constitutive neuroinflammation suggests that an autoimmune profile is implicated in their aberrant behaviors, as has been suggested for some humans with autism. Immune activation during early developmental stages has been proposed as a contributing factor in the pathogenesis of neuropsychiatric conditions such as obsessive-compulsive disorder, attention-deficit/hyperactivity disorder, and autism in both human and animal studies. However, the impacts of such an immune challenge on behavior and development are still not clear.

*Methods and Algorithms*: In this study, we used a model of early postnatal immune activation by the application of bacterial endotoxin lipopolysaccharide (LPS, 50 μg/kg), polyinosinic:polycytidylic acid (Poly I:C, 10 μg/kg) which simulates a viral infection, or combination (LPS + Poly I:C) to mice pups BTBR and B6 at postnatal day (PD) 3 and PD 5. The mice were tested in a battery of tasks: development of reflexes in neonatal period (negative geotaxis, grasping and cliff avoidance on 5 and 8 PD), anxiety (light-dark box test, 23 and 39 PD), social behavior (social interaction test, 24 and 39 PD) and stereotypic behavior (marble burying test, 55 PD). A blood test was performed on 24, 40 and 60 PD.

*Results*: Administration of LPS to males led to lower body weight on 5, 8 and 16 PD compared to saline (S) group. Similar effects were determined on 5 and 8 PD for LPS + Poly I:C group both males and females. Analysis of neonatal reflexes revealed a delay

on 8 PD in the formation of negative geotaxis (for LPS group) and grasping (for Poly I:C) for males compared to S group. An interesting effect was found in females: we found a delay in cliff avoidance on 5 PD for all groups compared to control. The analysis of inflammatory blood parameters did not reveal any changes between groups within each age, but changes in dynamics were found: on day 60, the administration of pro-inflammatory agents in males leads to an increase in lymphocytes relative to the control; in control females, no growth was observed after the administration of agents. The analysis of the number of red blood cells revealed an interesting result: on day 40, there was a significant increase in their number relative to other groups for both males and females. Evaluation of the effects of pro-inflammatory agents on the dynamics of platelet parameters showed a decrease in thrombocrit on day 40 and an increase by day 60 after the administration of Poly I:C for both males and females. On the 40 PD of the animals also enzyme immunoassay was performed. Analysis of 8 cytokines (IL-2, IF-γ, TNF-α, IL-5, GM-CSF, IL-10, IL-1β, IL-4) revealed no changes other than a decrease in interleukin-5 in males treated with LPS, Poly I:C and combination compared to control. Next, we assessed the anxious behavior of the animals in the light-dark test and found that the interstrain differences were the most pronounced: BTBR mice spent significantly less time in light arms in 24, 40 and 60 than B6 mice. On 55 PD we analyzed stereotypic behavior in marble burying test and determined number of marble with more than 50 % of sinking. Male BTBR mice have shown to burrow more than B6 mice. Administration of pro-inflammatory agents resulted in decreased stereotypic behavior in male B6 mice but not in BTBR mice.

*Conclusion*: Thus, the introduction of bacterial and viral mimetics at an early age leads to various delayed effects of the immune system of mice in the juvenile, adolescent and adult periods, and also affects the development of animals.

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