DOI 10.18699/SBB-2022-413

Different immune challenges alter hypothalamic microglia and astrocytes activity in the adult BTBR and C57Bl/6 mice

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Key words: BTBR T+Itpr3tf/J, acute inflammation, LPS, Poly I:C, haematological parameters, lymphocyte immunophenotyping

Aim: Autism spectrum disorder (ASD) is a neurodevelopmental disease characterized by aberrant social interactions, deficits in social communication, and stereotyped repetitive behavior. In addition, an impaired immune response including a trend toward high cytokine production and atypical immune cell function is often observed in people with ASD. Therefore, the immune system may play a role in the development of this disorder. The BTBR T+Itpr3tf/J (BTBR) mice model not only demonstrates an autism-like phenotype, but also has impairments in the immune system: they exhibit increased levels of serum antibody and enhanced cytokine expression, that can disrupt the development of the immune response in inflammation. However, the immune response to bacterial and viral infections is still poorly understood. Therefore, we explored differences in immune function in adult (60 days old) male BTBR and C57Bl/6 (B6) mice (used as control) after acute viral and bacterial inflammation.

Methods: BTBR and B6 mice received intraperitoneal injections of either lipopolysaccharide (LPS *Escherichia coli* serotype O55:B5, 50mcg/kg) in pyrogen-free saline or polyinosinic:polycytidylic acid (poly I:C, 10 mcg/kg) in pyrogen-free saline or a combination of them. Equivalent volumes of pyrogen-free saline were administered as control. Blood lymphocyte immunophenotyping and hematological response were conducted at 2- and 16-hours post-injection. In addition, levels of hypothalamic *c-Fos, Gfap* and *Aif1* transcripts were examined at 16-hours post-injection.

Results: In normal state BTBR mice had the same level of WBC and differential, 2-fold increase in T-cells, particularly CD4+ T-helper cells, and 3-fold decrease in B-cells and CD8+ T-killer cells in the peripheral blood compared to B6 mice. LPS, poly I:C and their combination induced physiological responses in both strains.

In BTBR mice LPS injection did not alter the number of cells in the complete blood count (CBC) with differential. Perhaps this is due to an insufficient dose of LPS. But, in B6 mice after LPS injection an increased number of granulocytes and decreased number of lymphocytes was indicated in CBC. Such changes are associated with a bacterial infection. The strongest response was shown after poly I:C administration in both B6 and BTBR mice. The CBC analysis showed that poly I:C administration induced leukopenia associated with sepsis in B6 mice and didn't change cell count in BTBR. However, in both strains CD8+ cytotoxic T-cells decrease (1.6-fold in BTBR vs 2.9-fold in B6) and CD4+ T-helper cells increase (1.15-fold in BTBR vs 1.3-fold in B6) indicates an acute phase of inflammation. Therefore, BTBR mice demonstrated impaired immune

response to bacterial and viral mimetic compared to B6 mice. Combination of LPS and poly I:C does not lead to increased immune response.
It has been shown that peripheral inflammation can change the activity of neurons, microglia and astrocytes in the hypothalamus, as it is the brain area that has been proposed to underlie the different behavioral and metabolic components of cytokine-induced sickness behavior. To determine hypothalamus neurons activation to different mimetics *c-Fos* was measured. Glial fibrillary acidic protein (GFAP) is a cell-specific intermediate filament protein in astrocytes and is widely used as an astrocyte marker. *Aif1* expression indicates microglial activation. In normal state in BTBR mice hypothalamic *c-Fos* is no different from B6. But *Gfap* expression was higher and *Aif1* expression was lower in the hypothalamus of BTBR mice compared to B6 mice. That indicates different base cell activity in the brain of BTBR mice.
Analysis of *c-Fos* expression 16-h after different mimetics administration showed no significant changes in both strains. But, *c-Fos* expression in the hypothalamus was significantly lower in BTBR mice compared to B6 mice. Peripheral inflammation did not change *Gfap* expression in astrocytes in both strains. *Aif1* expression was increased 16-hour after injection of a combination of mimetics in BTBR mice and after injection of LPS or poly I:C in B6 mice. Besides, BTBR mice, even after administration of LPS or poly I:C, retained a reduced level of microglia activation compared to B6.
Conclusion: Thus, poly I:C has been shown to have a more pronounced effect on the immune response and activity of hypothalamic cells than LPS. Co-administration of mimetics does not enhance the induced changes. In the BTBR strain, the injection of poly I:C causes a development of sickness behavior, with prevalence of CD4+ T-helper cells in blood, reduced neuronal and microglial activity. Ultimately, BTBR mice have shown an impairment in immune response compared to B6 mice.
Acknowledgements: This study was supported by Russian Science Foundation 21-15-00142.