ImmunoTools *special* Award 2023

Chiara Cipriani, PhD

Department of Experimental Medicine
University of Rome Tor Vergata, Via Montpellier, 1
00133 Rome, Italy

The role of Endogenous retroviruses' activity in biological processes underlying microglia-dependent over-pruning and the immune dysregulations in Autism Spectrum Disorders.

Autism Spectrum Disorders (ASD) have a multifactorial aetiology attributable to the combination of genetic vulnerability and environmental factors. The involvement of microglial activation in synaptic over-pruning in neurodevelopmental disorders (NDDs) has been speculated [1]. Evidence from post-mortem studies show that cell density and/or the number of ‘activated’ microglia is increased in the brains of individuals with ASD [2]. In mice, molecules of the complement cascade, C1q and C3, are localized in developing synapses, where act as tags for microglia, mediating synaptic pruning; the disrupting of this signalling led to an excess in synapse numbers and connectivity in adulthood. The chemokine CX3CL1 also seems to play a key role in microglia-mediated pruning, since deficient mice showed a transient decrease in synapse pruning and deficits in synapse number and/or function [3-8]. In the intriguing view known as the "over-pruning hypothesis" of NDDs [9], the role of endogenous retroviruses (ERVs) could be speculated. ERVs are genetic elements, integrated into the mammalian genome during evolution, as remnants of ancient viral infection by exogenous retroviruses [10,11]. Due to the long co-evolution with mammalians, some ERVs have been co-opted for physiological functions, especially during pregnancy [12,13]. Growing evidence links altered ERV expression to different human pathological conditions including NDDs [14]. In ASD, an altered expression profile of human ERVs (HERVs), closely related to a more severe clinical phenotype has been described [15]. Interestingly, ASD children and their mothers shared common expression levels of some HERVs and cytokines in peripheral blood mononuclear cells (PBMCs) [16], and by an *in vitro* study it has been demonstrated that the treatment with the anti-retroviral drug, Efavirenz specifically restored HERV activity with a concomitant modulation of cytokines [17]. By means of different ASD mouse models (inbred BTBR T+tf/J and valproate-treated CD1 mice) it has been demonstrated high levels of ERVs, beginning from intrauterine life till adulthood and across generations.

Moreover, the levels of ERVs positively correlated with the expression of pro-inflammatory cytokines and TLR-3 and TLR-4 in embryos and brain tissues [18,19]. Similar results were also obtained in maternal immune activation (MIA) mouse model of ASD, showing an abnormal expression of ERVs and immune mediators in mouse off-spring in a sex-dependent fashion [20].

With the present research project, we aimed to investigate whether ERV deregulation could be linked to pathophysiological events underlying the derailed neurodevelopment, including the microglial cell-dependent over-pruning and the immune dysregulation. To this purpose, in an ASD mouse model induced by the prenatal exposure to valproic acid, we will isolate primary newborn and adult microglia cells at 7 and 60 post-natal days. The cell separation will be obtained through Magnetic-activated cell sorting (MACS) using the anti-CD11b immunomagnetic beads. The cell cultures will be stimulated by using different inflammatory mediators: GM-CSF, IL-1β, IL-2, IL-6, IL-10, IL-21, TNF-α and IFN-γ. In the same culture conditions, we will assess the relative expression of different ERVs (MusD, IAP, Syn-A, Syn-B, ARC, and GLN), interferons, TLRs, cytokines, chemokines, microglial markers (Aif1, Cx3cr1, Tmem119, Ccr2, Ly6c2) and molecular pruning signals (CX3CL1, CX3CR1, C1q, C3) by RT-real time PCR.

Essential bibliography:

1. Pardo et al., 2005, doi: 10.1080/02646830500381930. PMID: 16401547; 2. Bilimoria and Stevens, 2015, doi: 10.1016/j.brainres.2014.11.032; 3. Stevens et al., 2007, doi: 10.1016/j.cell.2007.10.036; 4. Schafer and Stevens, 2010, doi: 10.1042/BST0380476; 5. Chu et al., 2010, doi: 10.1073/pnas.0913449107; 6. Schafer et al., 2012, doi: 10.1016/j.neuron.2012.03.026; 7. Stephan et al., 2013, doi: 10.1523/JNEUROSCI.1333-13.2013; 8. Rogers et al., 2011, doi: 10.1523/JNEUROSCI.3667-11.2011; 9. Thomas et al., 2016, doi: 10.1111/desc.12303; 10. Bock and Stoye, 2000, doi: 10.1016/s0959-437x(00)00138-6; 11. Dewannieux and Heidmann, 2013, doi: 10.1016/j.coviro.2013.08.005; 12. Balestrieri et al., 2015, doi: 10.1155/2015/164529; 13. Roberts et al., 2021, doi: 10.1016/j.placenta.2021.01.006; 14. Gruchot et al., 2023, doi: 10.1016/j.bbi.2022.10.007; 15. Balestrieri et al., 2012, doi: 10.1371/journal.pone.0048831; 16. Balestrieri et al., 2019, doi: 10.3389/fimmu.2019.02244; 17. Cipriani et al., 2022, doi: 10.1186/s12977-022-00603-6; 18. Cipriani et al., 2018, doi: 10.1038/s41598-017-19035-w; 19. Tartaglione et al., 2019, doi: 10.1007/s12035-018-1328-x; 20. Cipriani et al., 2022, doi: 10.3390/ijms232213930.