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Commentary on "Association between NKG2/KLR gene variants and epilepsy in Autism Spectrum Disorder"

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with multifactorial etiology. The development of ASD is suggested as a consequence of interactions between genetic, epigenetic and environmental factors[1,2]. Immune dysregulation is commonly reported in ASD children, both at cellular and systemic levels. In this sense, imbalances between pro- and anti-inflammatory factors have been addressed as potential contributors to the pathogenesis of the disorder[3-10]. Recently, advances in ASD research have revealed an intriguing interconnection between the immune system and neurological development[10]. Therefore, studies approaching immunological aspects in ASD individuals are needed to provide a better understanding of such disorder.

Natural Killer (NK) cells are essential actors in different immunological processes, including accurate immune responses against viral infections, inflammatory processes, programmed cell death (apoptosis), and antitumor responses[10-13]. Considering the potential influence of inflammatory responses in ASD children, NK cells may play a key role in such disorder. Recent studies have revealed that children with ASD exhibit increased levels of NK cells as well as differentiated patterns of cytotoxicity compared to individuals from the general population[10]. This association is supported by transcriptomic analyses that have highlighted an enrichment in genes related to the NK cell-mediated cytotoxicity pathway in ASD subjects[3,10,14,15]. Nevertheless, decreased activity of NK cells was also reported in children with ASD[16]. This set of evidence assists the hypothesis of an imbalanced immune system with a strong genetic component contributing to the outcomes reported in ASD.

Considering the study by Kaminski and collaborators[17], an association is described between *KLRC2* gene deletion, *KLRK1* rs1049174 and *KLRK1* rs2255336 with epilepsy in ASD subjects from a sample of Southern Brazil. As discussed, prior research suggests that individuals with ASD have a decreased NK cell activity and cytotoxicity, which may be related to their findings regarding the *KLRC2* deletion. This deletion is functionally significant, as the KLRC2 receptor plays an important role in activating and regulating NK cells[18]. However, the role of KLR genes and its variants on ASD development and symptomatology still requires further investigation.

The role of NK cells in ASD is rather complex, and deserves to be evaluated. Arteaga-Henríquez and colleagues[19], for instance, conducted a comprehensive meta-analysis concerning lymphocyte and leukocyte

subpopulation dynamics in individuals diagnosed with ASD. The analysis revealed an elevation in NK cell levels among ASD subjects compared to typically developing (TD) controls. In light of the systemic inflammation often observed in ASD, the authors hypothesize that NK cells might restrain their activity as a compensatory mechanism to mitigate inflammation. Additionally, they highlight evidence suggesting that NK cells might migrate to the brain in order to regulate microglial activation, thus revealing a multifaceted role for NK cells in the pathophysiology of ASD. On the other hand, Ashwood et al.[7] suggest that higher counts of NK cells in ASD subjects are possibly related to a compensatory mechanism for possible deficits in NK cell function. They also consider that high levels of cytokines due to altered immune responses may lead to an increase in NK cell numbers.

Furthermore, the interaction between NK cells and the nervous system appears to play a crucial role in the pathogenesis of ASD. Recent research suggests that NK cells can influence synaptic plasticity and brain function by directly modulating neuronal and microglia activity, and that overactivation of NK cells can negatively influence myelination and neuronal transmission[19-22]. Moreover, evidence suggests NK participation in the regulation of the intestinal microbiota, related to the gastrointestinal symptoms common in ASD[10]. Another important piece of the puzzle involving the function of NK cells in the central nervous system is the finding of differential expression of NK cell marker genes in the cerebrospinal fluid in inflammatory processes related to neurological outcomes, such as multiple sclerosis[23].

An interesting literature review addressing the role of NK cells in the pathogenesis of ASD approached several pieces of evidence already discussed here[24]. Among these topics, the authors call attention to NK cell receptors, HLA alleles and KIR/HLA interactions during pregnancy as risk factors for ASD. Specifically concerning NK cell receptors, NKG2C receptor overexpression and an inversely correlation with NKp46 expression, as well as a higher expression of *KIR2DS2* and *KIR2DL1* genes, were features observed in high functioning (hf) ASD adults[25]. Besides, *KIR2DL1*, HLA-DR and NKp46 were associated with IQ ($p = 0.008$), scores of social reciprocity and communication, and structural language ($p = 0.007$) and social awareness ($p = 0.0007$) scores, respectively[25].

HLA-G alleles, a receptor that regulates NK cells and CD8 T cells activity, were also already related to children with ASD[26]. Interestingly, our research group published an article analyzing the 14 bp indel variant in *HLA-G* gene[27], although no association between this variant and susceptibility or symptomatology was reported in our ASD sample.

Considering the ASD symptomatology discussed by Kaminski and collaborators[17], epilepsy is a common comorbidity, affecting around 12.1% (range: 1.8-60%) of ASD cases[28]. The etiology of epilepsy can be associated with a single genetic cause or due to a complex combination of genetic and epigenetic factors[29]. Of note, epileptogenesis shows correlation with immune dysregulation. Several reports found significant association between epileptic patients and a bias towards a pro-inflammatory profile, Th17 activation, CD8 T cell- mediated cytotoxicity against astrocytes and microglial activation[30-32]. In addition, reduced activity in NK cells was reported in epileptic patients[33] and Luo and collaborators[34] found a negative correlation of EGFR expression in NK cells from epileptic patients.

Approaching the connections of the immune and the nervous systems during the development, Ebrahimi Meimand and colleagues[24] hypothesized about impairments regarding KIR/HLA interactions during pregnancy and how these alterations could be linked to maternal immune activation (MIA) and risk for ASD. Actually, a previous study of our research group also addressed the MIA hypothesis and the risk for ASD in the light of extracellular vesicles (EVs) or exosomes[35]. EVs are engaged in several signaling processes, they carry a wide range of molecules, such as immunoglobulins (Igs) and a variety of immune cell receptors, including MHC, and are also capable of crossing tissue barriers. Keeping these features in mind, we hypothesized that EVs may promote a pro-inflammatory milieu at the maternal-fetal interface. In this context, MIA could be ascribed as a mechanism predisposing the progeny for a wide range of neurodevelopmental disorders, such as ASD and epilepsy[36].

Finally, NKs are important for the regulation of immune responses and prevention of autoimmunity (reviewed by Zitti & Bryceson[12]; Liu et al.[37]). Interestingly, a recent longitudinal, population-based birth cohort study including 31,220 individuals concluded that ASD cases had increased risk of autoimmune disease compared with matched referents[38].

In conclusion, examples of connections between NK cells and ASD risk and symptomatology are widely represented in the scientific literature. Certainly, the immune component of ASD includes other alterations in the immune system, such as the presence and levels of brain-reactive antibodies, altered levels of cytokines in the Central Nervous System (CNS), and a Th1/Th2 imbalance (for review see[39,40]). Actually, all such features can be connected, and eventually could share origin, mechanisms and/or consequences. Considering the increased NK cell counts, reduced NK cell cytotoxicity and variants in NK-related genes, it is possible to conclude that altered NK cell activity is linked to the immune dysregulated phenotype observed

in ASD individuals. Nevertheless, ASD is characterized by a complex interplay between genetic and environmental factors, which extends the specific mechanisms of immune system that influence ASD etiology and symptomatology. Therefore, further research is necessary to elucidate additional genetic and epigenetic components contributing to the immunogenetic architecture of ASD.

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