European Journal of Neurodegenerative Diseases
ISSN 2974-6345 / 2023 vol. 12, ISSUE N. 2, May-August

AUTISM SPECTRUM DISORDERS (ASDs): NEW RESEARCH AND POSSIBLE NOVEL THERAPIES

G. Ronconi[1*], F. Carinci[2]

[1] Territorial Patient Clinic, Fondazione Policlinico Gemelli, Rome, Italy;
[2] Department of Translational Medicine, University of Ferrara, Ferrara, Italy.

**Correspondence to*:
Gianpaolo Ronconi,
Territorial Patient Clinic,
Fondazione Policlinico Gemelli,
Rome, Italy.
e-mail: gianpaolo.ronconi@policlinicogemelli.it

ABSTRACT

Autism spectrum disorder (ASD) encompasses a collection of brain disorders involving neurodevelopmental and functional disabilities of the brain. It is a disorder of unknown pathogenesis, begins in childhood, and can have different degrees of severity. Additionally, there has been a high incidence of ASD in the last 10 years. The blood-brain barrier (BBB) protects the brain, however in children it is not yet formed, and for subjects with ASD, this allows the passage of harmful inflammatory substances that could be generated by ASD-induced stress. In ASD, inflammatory substances such as cytokines (IL-1 and TNF) and chemokines (CXCL8) could be generated by brain microglia and mast cells (MCs). Here, we report that the anti-inflammatory cytokines IL-37 and IL-38 may be involved in ASD, which could offer new therapeutic aspects for this disorder of unknown pathogenesis that mainly afflicts children.

KEYWORDS: *autism spectrum disorder, blood-brain barrier, inflammation, immunity, cytokine*

INTRODUCTION

Autism, or autism spectrum disorder (ASD), is a neurodevelopmental disease with functional disabilities caused by brain dysfunction that usually begins before the age of 3 years old. Between the years 2012-2021, 1 in 100 children were affected by ASD and the incidence is increasing (1). In fact, today it is estimated that in the United States, approximately 1 in 60 children are affected by the disease (2). Furthermore, the diagnosis of ASDs has increased more than tenfold in the past 20 years (3).

Individuals with ASD have impaired communication and social interaction, display abnormal behaviors due to sensory hyper-reactivity or hypo-reactivity, and can show pervasive developmental disorder, epilepsy, and intellectual disability (4). The disease can have varying degrees of severity and the causes are not yet known. People with ASD have a normal phenotype that does not distinguish them from other individuals. Some affected individuals may have sufficient verbal communication skills, while others may be nonverbal and need help in their daily lives. Individuals with ASD may also have mental health problems such as anxiety, hyperactivity, depression, and attention deficit disorder, although these disorders can also occur in individuals without ASD (5-7). Subjects with ASD do not have altered diagnostic signs, such as different clinical analyses compared to normal subjects, and therefore diagnosing the disease is difficult. Since there is no biochemical or genetic screening test at the moment, the only available diagnosis seems to be based on the anomalous behavior of the subject affected by the disease.

Received: 01 July 2023
Accepted: 11 August 2023

2974-6345 (2023)

The cause of ASD is unknown, although genetics plays a strong role, and many hypotheses and theories have been reported suggesting the risk factors for disease development (8,9). The idea that vaccines could cause ASD has been disproved by several major studies (10,11). Moreover, since the disease is of unknown etiology, there is no specific pharmacological therapy and as ASD manifests with different degrees of severity, behavioral therapies should be applied individually.

Pathogenesis of autism spectrum disorders (ASDs)

The blood-brain barrier (BBB) is formed by endothelial cells, pericytes, and immune cells, and protects the brain from the entry of toxic molecules transported by the blood. Although there is no correlation between intestinal symptoms and ASD, children with ASD often have gastrointestinal symptoms such as pain, diarrhea, and intestinal dysfunction, which can lead to the absorption of harmful substances that could pass through the still-unformed BBB to reach the brain, somehow causing damage and leading to ASD (12). Autistic subjects are more prone to stress, with greater hypothalamic-pituitary-adrenal axis activity and higher cortisol levels than non-autistic subjects (13).

There are different genetic, environmental, allergic, and infectious factors that could increase the risk of ASD, which could involve immune cells such as mast cells (MCs) (14). MCs derive from marrow cells and reside in vascularized tissues (15). They are activated through the binding of IgE on the FcεRI receptor, which by aggregating allows the release of biologically active compounds (16). MCs mediate innate immunity, including inflammatory disorders, and acquired immunity (17). FcεRI activation leads to cell degranulation and the immediate release of preformed mediators, including tumor necrosis factor (TNF), and subsequently, after some hours, MC activation generates cytokines and chemokines by *de novo* synthesis (18). Several immune cells, including MCs, produce IL-1 which recruits neutrophils, induces TNF and IL-6, and increases inflammation (19). In addition, TNF derived by MCs activates immune cells such as macrophages that participate in the inflammatory reaction (20).

Brain MCs are activated in a state of acute stress and can release corticotropin releasing hormone (CRH) which increases vascular permeability and facilitates the passage of harmful substances to the central nervous system (CNS) (21). Experiments on rodents have shown that stress stimulates intestinal MCs, an event that can also happen in some allergic reactions (14,22). The activation of intestinal MCs in children with ASD, who have an undeveloped BBB, would lead to the release of vasoactive and inflammatory molecules, such as cytokines that could cross the BBB to reach the brain and damage it (14). Thus, in autistic children, several allergens could trigger and activate gastrointestinal MCs, producing molecules such as cytokines and pro-inflammatory toxins that would increase BBB permeability, and then subsequently reach the CNS, causing neuroinflammation. However, these hypotheses still need to be confirmed by further studies. Histamine, serotonin, prostaglandins, TNF, vascular endothelial growth factor (VEGF), and vasoactive intestinal peptide (VIP) are molecules produced by activated MCs that could increase vascular permeability. The increase of these immune molecules in the brain could contribute to ASD.

From literature data, the immune system seems to be increasingly involved in the pathogenesis of ASD (23-29). In fact, it seems that in subjects with ASD, pro-inflammatory molecules such as IL-1, TNF, and some chemokines, including CXCL8, are increased both in serum and in cerebrospinal fluid (30,31). The increase of neuropeptides such as neurotensin stimulates the gene expression and secretion of the cytokine IL-1 and the chemokine CXCL8 in microglia (32). The activation of microglia can lead to brain abnormalities that contribute to ASD (33).

IL-37, which was previously known as IL-1F7, is an IL-1 family member whose pro-IL-37 is cleaved by caspase-1 into biologically active mature IL-37. If IL-1 is important in the pathogenesis of ASD, the inhibition of IL-1 with IL-37, a naturally occurring IL-1 immunosuppressant cytokine, could represent a new therapeutic strategy for this neurological disease. Microglia activation leads to increased gene expression of IL-18 and its receptor IL-18R, mediating the inflammatory phenomenon (Fig.1).

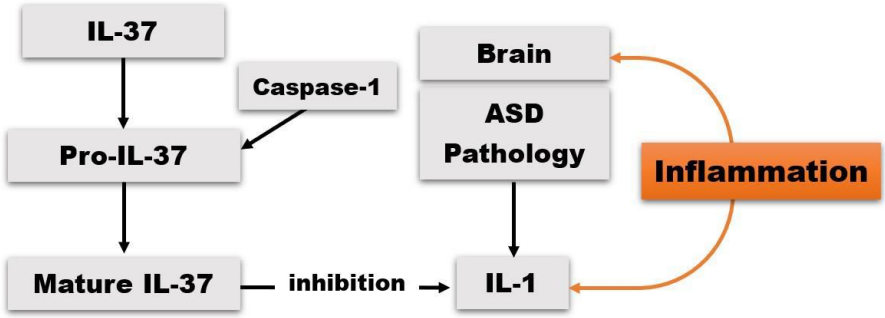

Fig. 1. *The autism spectrum disorder (ASD) brain may secrete IL-1 which induces inflammation, an effect that could be inhibited by mature IL-37.*

The increase in IL-37 expression may have a protective action against some pro-inflammatory cytokines such as IL-1. IL-37 gene expression is increased in ASD, a reaction that attempts to suppress inflammation (32) (Fig.2).

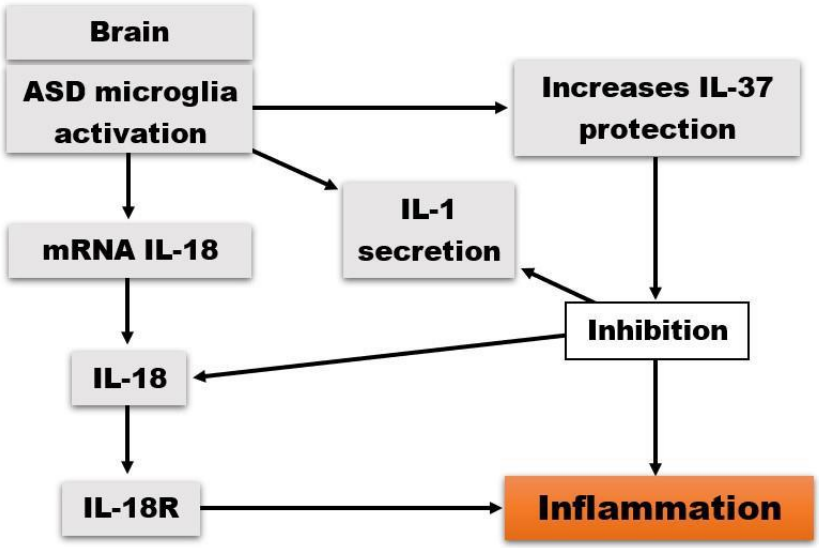

Fig. 2. *Microglia from the autism spectrum disorder (ASD) brain may release inflammatory cytokines such as IL-1 and IL-18, which can be inhibited by the anti-inflammatory cytokine IL-37, which gives protection against IL-1 family members.*

The cytokine IL-38 also derives from the IL-1 family and has an anti-inflammatory power. This cytokine is found within the macrophage and must be cleaved at the N-terminal portion before being secreted extracellularly in an active form (34). The activity of this anti-inflammatory cytokine is carried out after binding to the IL-36 receptor (IL-36R) and the coreceptor IL-1 receptor 9 (IL-1R9) (35). It has been recently reported that IL-38 can inhibit the secretion of stimulated pro-inflammatory molecules from cultured human microglia (36). IL-38 is a more potent inhibitor than IL-37, and in the brains of children with ASD, there is reduced gene expression of IL-38 and its receptor IL-36R, demonstrating that this cytokine plays a key role in the inhibition of microglial activation (36) (Fig.3).

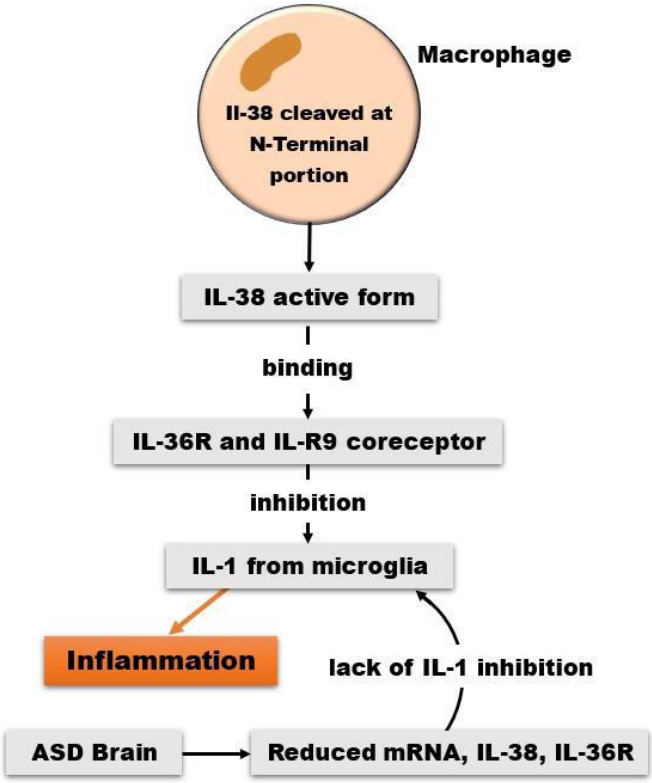

Fig. 3. *Inside the macrophage, IL-38 is cleaved at the N-terminal portion and is released extracellularly as its active form. Mature IL-38 binds the IL-36 receptor and the IL-R9 coreceptor, inhibiting IL-1 secreted by microglia. In the autism spectrum disorder (ASD) brain, mRNA, IL-38, and the IL-36R are reduced, causing the lack of inhibition of IL-1 from microglia, and therefore, inflammation.*

The pretreatment of cultured human microglia with recombinant IL-38 inhibits the neurotensin-stimulated secretion of two important pro-inflammatory molecules: IL-1β and CXCL8 (36). Additionally, gene expression of IL-38 and its receptor IL-36R was decreased in the amygdala of ASD patients (36).

However, increased IL-38 levels could signify a physiological opposition to IL-1-induced inflammation, while a decrease in IL-38 could favor the effect of IL-1. Therefore, variations in the level of IL-38, both in a positive and negative sense, could indicate an inflammatory process in progress.

CONCLUSIONS

In conclusion, ASD is a neurodevelopmental disease with brain disabilities including impaired verbal communication and social interaction, even if affected individuals do not have impaired diagnostic signs. The pathogenesis of this disease is unknown, although it is hypothesized that harmful substances developed in the gastrointestinal tract during childhood could cross the BBB, reach the brain and cause neuroinflammation. Moreover, allergies and intestinal inflammation could activate MCs, leading to the release of pro-inflammatory substances. Activated MCs and brain microglia cause inflammatory substances, such as the cytokines and chemokines IL-1, TNF, and CXCL8, to be released.

Here, based on the data reported in the literature, we hypothesize that the anti-inflammatory cytokines IL-37 and IL-38 may be involved in ASD and might have an inhibitor effect on inflammatory cytokines, which could be useful for therapy treating this disorder.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Zeidan J, Fombonne E, Scorah J, et al. Global prevalence of autism: A systematic review update. *Autism Research*. 2022;15(5):778-790. doi:https://doi.org/10.1002/aur.2696
2. Elsabbagh M, Divan G, Koh YJ, et al. Global Prevalence of Autism and Other Pervasive Developmental Disorders. *Autism Research*. 2012;5(3):160-179. doi:https://doi.org/10.1002/aur.239
3. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators; Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. *MMWR Surveill Summ*. 2012;61(3):1-19.
4. Hodges H, Fealko C, Soares N. Autism spectrum disorder: Definition, epidemiology, causes, and clinical evaluation. *Translational Pediatrics*. 2020;9(1):S55-S65. doi:https://doi.org/10.21037/tp.2019.09.09
5. Chang YC, Quan J, Wood JJ. Effects of Anxiety Disorder Severity on Social Functioning in Children with Autism Spectrum Disorders. *Journal of Developmental and Physical Disabilities*. 2012;24(3):235-245. doi:https://doi.org/10.1007/s10882-012-9268-2
6. Mason D, Mackintosh J, McConachie H, Rodgers J, Finch T, Parr JR. Quality of life for older autistic people: The impact of mental health difficulties. *Research in Autism Spectrum Disorders*. 2019;63:13-22. doi:https://doi.org/10.1016/j.rasd.2019.02.007
7. Hargitai LD, Livingston LA, Waldren LH, Robinson R, Jarrold C, Shah P. Attention-deficit hyperactivity disorder traits are a more important predictor of internalising problems than autistic traits. *Scientific Reports*. 2023;13(1). doi:https://doi.org/10.1038/s41598-022-26350-4
8. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. *Autism and Related Developmental Disorders*. 2012;14(3):281-292. doi:https://doi.org/10.31887/dcns.2012.14.3/pchaste
9. Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. *Cellular and Molecular Life Sciences*. 2018;76(7):1275-1297. doi:https://doi.org/10.1007/s00018-018-2988-4
10. Davidson M. Vaccination as a cause of autism—myths and controversies. *Autism Spectrum Disorders*. 2017;19(4):403-407. doi:https://doi.org/10.31887/dcns.2017.19.4/mdavidson
11. Mohammed SA, Rajashekar S, Giri Ravindran S, et al. Does Vaccination Increase the Risk of Autism Spectrum Disorder? *Cureus*. 2022;14(8). doi:https://doi.org/10.7759/cureus.27921
12. Geir B, Lyudmila P, Maryam D, et al. Gastrointestinal Alterations in Autism Spectrum Disorder: What Do We Know? *Neuroscience & Biobehavioral Reviews*. 2020;118:111-120. doi:https://doi.org/10.1016/j.neubiorev.2020.06.033
13. Tani P, Lindberg N, Matto V, et al. Higher plasma ACTH levels in adults with Asperger syndrome. *Journal of Psychosomatic Research*. 2005;58(6):533-536. doi:https://doi.org/10.1016/j.jpsychores.2004.12.004
14. Theoharides TC, Letourneau R, Patra PB, et al. Stress-induced rat intestinal mast cell intragranular activation and inhibitory effect of sulfated proteoglycans. *Digestive diseases and sciences*. 1999;44(8 Suppl):87S93S.
15. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nature Reviews Immunology*. 2008;8(6):478-486. doi:https://doi.org/10.1038/nri2327
16. Nagata Y, Suzuki R. FcεRI: A Master Regulator of Mast Cell Functions. *Cells*. 2022;11(4):622. doi:https://doi.org/10.3390/cells11040622
17. Katsoulis-Dimitriou K, Kotrba J, Voss M, Dudeck J, Dudeck A. Mast Cell Functions Linking Innate Sensing to Adaptive Immunity. *Cells*. 2020;9(12):2538. doi:https://doi.org/10.3390/cells9122538
18. Moon TC, Befus AD, Kulka M. Mast Cell Mediators: Their Differential Release and the Secretory Pathways Involved. *Frontiers in Immunology*. 2014;5. doi:https://doi.org/10.3389/fimmu.2014.00569
19. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *International Journal of Molecular Sciences*. 2019;20(23):6008. doi:https://doi.org/10.3390/ijms20236008
20. Parameswaran N, Patial S. Tumor Necrosis Factor-α Signaling in Macrophages. *Critical ReviewsTM in Eukaryotic Gene Expression*. 2010;20(2):87-103. doi:https://doi.org/10.1615/critreveukargeneexpr.v20.i2.10

21. Kempuraj D, Selvakumar GP, Thangavel R, et al. Mast Cell Activation in Brain Injury, Stress, and Post-traumatic Stress Disorder and Alzheimer's Disease Pathogenesis. *Frontiers in Neuroscience*. 2017;11. doi:https://doi.org/10.3389/fnins.2017.00703
22. Cao J, Papadopoulou N, Kempuraj D, et al. Human Mast Cells Express Corticotropin-Releasing Hormone (CRH) Receptors and CRH Leads to Selective Secretion of Vascular Endothelial Growth Factor. *The Journal of Immunology*. 2005;174(12):7665-7675. doi:https://doi.org/10.4049/jimmunol.174.12.7665
23. Meltzer A, Van de Water J. The Role of the Immune System in Autism Spectrum Disorder. *Neuropsychopharmacology*. 2016;42(1):284-298. doi:https://doi.org/10.1038/npp.2016.158
24. Zerbo O, Qian Y, Yoshida C, Grether JK, Van de Water J, Croen LA. Maternal Infection During Pregnancy and Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*. 2013;45(12):4015-4025. doi:https://doi.org/10.1007/s10803-013-2016-3
25. Brown AS, Surcel HM, Hinkka-Yli-Salomäki S, Cheslack-Postava K, Bao Y, Sourander A. Maternal thyroid autoantibody and elevated risk of autism in a national birth cohort. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2015;57:86-92. doi:https://doi.org/10.1016/j.pnpbp.2014.10.010
26. Goines PE, Croen LA, Braunschweig D, et al. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. *Molecular Autism*. 2011;2(1):13. doi:https://doi.org/10.1186/2040-2392-2-13
27. Abdallah MW, Larsen N, Grove J, et al. Amniotic fluid inflammatory cytokines: Potential markers of immunologic dysfunction in autism spectrum disorders. *The World Journal of Biological Psychiatry*. 2011;14(7):528-538. doi:https://doi.org/10.3109/15622975.2011.639803
28. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral DG, Van de Water J. Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. *Brain, Behavior, and Immunity*. 2009;23(1):64-74. doi:https://doi.org/10.1016/j.bbi.2008.07.007
29. Garbett KA, Ebert PA, Mitchell A, et al. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiology of Disease*. 2008;30(3):303-311. doi:https://doi.org/10.1016/j.nbd.2008.01.012
30. Zimmerman AW, Jyonouchi H, Comi AM, et al. Cerebrospinal Fluid and Serum Markers of Inflammation in Autism. *Pediatric Neurology*. 2005;33(3):195-201. doi:https://doi.org/10.1016/j.pediatrneurol.2005.03.014
31. Li X, Chauhan A, Sheikh AM, et al. Elevated immune response in the brain of autistic patients. *Journal of Neuroimmunology*. 2009;207(1):111-116. doi:https://doi.org/10.1016/j.jneuroim.2008.12.002
32. Tsilioni I, Patel AB, Pantazopoulos H, et al. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. *Proceedings of the National Academy of Sciences*. 2019;116(43):21659-21665. doi:https://doi.org/10.1073/pnas.1906817116
33. Patel AB, Tsilioni I, Leeman SE, Theoharides TC. Neurotensin stimulates sortilin and mTOR in human microglia inhibitable by methoxyluteolin, a potential therapeutic target for autism. *Proceedings of the National Academy of Sciences*. 2016;113(45):E7049-E7058. doi:https://doi.org/10.1073/pnas.1604992113
34. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *International Journal of Molecular Sciences*. 2019;20(13):3328. doi:https://doi.org/10.3390/ijms20133328
35. van de Veerdonk FL, Stoeckman AK, Wu G, et al. IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proceedings of the National Academy of Sciences*. 2012;109(8):3001-3005. doi:https://doi.org/10.1073/pnas.1121534109
36. Tsilioni I, Pantazopoulos H, Conti P, Leeman SE, Theoharides TC. IL-38 inhibits microglial inflammatory mediators and is decreased in amygdala of children with autism spectrum disorder. *Proceedings of the National Academy of Sciences*. 2020;117(28):16475-16480. doi:https://doi.org/10.1073/pnas.2004666117