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Editorial

Autism and neuro-immune-gut link

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Abstract: Recent evidences sustain the hypothesis that host-bacteria interactions play a critical role in regulating tissue and body homeostasis. Gut microbiota and the brain are strongly interconnected and share communication pathways. Modifications in gut bacteria compositions are correlated to changes in behaviors. Indeed, autism spectrum disorders (ASD) are linked to dysfunctions of the gut bacteria-brain axis. Possible therapeutic strategies in ASD management will aim to restore dysbiosis and gut bacteria imbalance.

Keywords: autism; gut brain axis; intestinal dysfunctions; neuroinflammation

1. Intestinal physiology: Brief overview

The intestinal luminal mucosa is constituted by mucous membrane that consists of an epithelium, a lamina propria, and a muscularis mucosae. It forms the interface between the external and internal environments of the host. It acts like a filtering barrier using to complex epithelial function and secretory mechanisms, so it has a double function:

(1) It acts as a barrier to prevent the passage of harmful compounds, including foreign antigens, microorganisms and their toxins [1,2].

(2) It acts as a selective filter for the passage of nutrients, electrolytes and water from the intestinal lumen into the circulation [1,3,4].

Several processes regulate the intestinal mucosa crossover; among them, the protecting mucous layer, secretion of defensins and mucosal IgA, the cell-to-cell junctions (i.e. tight junctions), and the resident microbiota are components of a delicate homeostatis and participate in its controlling.

Recently, a significant interest has been gained on the gut microbiota. It is constitute from a complex community of microorganisms that colonizes a particular location; microbiota includes not only bacteria, but also other microbes, such as fungi, archaea, viruses, and protozoans. Recent studies speculate that the gut microbiota bears significant functional role in maintaining the equilibrium between gut homeostasis and human health; indeed it acts like a physical barrier for the pathogens by competitive exclusion, consumption of nutrient sources, and production of antimicrobial substances. The mucosal surface is coated by several cellular types that contribute to the creation of the "intestinal barrier". Among them, enterocytes play a key role. Through diffusion and co-transport of the bioproducts of hydrolysis of food components, these cells are able to perform the filter function for all the substances that are entering into the blood mainstream. In contrast to the absorptive enterocytes, microfold (M) cells act as an antigen sampling system with a high capacity for transcytosis of a wide range of microorganisms and macromolecules. Indeed, M cells contribute to transport antigens and microorganisms from the lumen to the inner layers of mucosa, deactivating the dangerous substances during the intracellular transport. Intestinal epithelium integrity gives to the intestinal barrier the characteristics of a polymeric, impermeable membrane, this function is achieved by the presence of a complex protein network that links adjacent cells and seals the intracellular space. Among the involved proteins of the complex, key importance is due to the apical junction complexes (AJC), that form two separate zones: Tight junction (TJ) and the adherent junction (AJ); and desmosomes localized at the basolateral membranes, which support epithelial stability. The AJC have the role of polarizing epithelial cells; indeed, above the AJC, the apical membrane is located (in front of the intestinal lumen), while basolateral membrane is below AJC, which is in contact with the lamina propria [5,6].

The tight junctions are an intricate network of proteins, it is constituted by over 50 proteins, such as occludins, claudins and junctional adhesion molecules (JAMs) linked to the peripheral membrane (scaffolding) proteins (e.g., zonula occludens (ZO), afadins), which in turn are connected with actin and microtubules by the protein linkers cingulin and non-muscle myosin [7]. These complex structures are able to keep close or open the intercellular passage with a sort of zipper mechanism. TJs are dynamic structures; in physiological conditions, the intercellular passage is strictly regulated by the remodeling of the TJs induced by external and intercellular stimula, coming from the crosstalk among intestinal epithelial cells, gut contents, and commensal microbiota; to do this, signaling proteins, protein phosphorylation, and toll-like receptors are utilized [8]. If one or more components of the mucosa are damaged or deregulated, any kind of antigenic substances is able to cross the intestinal barrier and reach the inner layer of mucosa, triggering an immune reaction and permitting systemic absorption of substances, which could adversely affect systemic and/or brain function [9].

2. Intestinal pathology: The leaky gut and autism spectrum disorders

The pathological alteration of intestinal permeability is known as "leaky gut", when the "leaky gut" condition is chronic and is interfacing with the individual genetic predisposition, it is able to trigger an immune-mediated reaction as it happens in celiac disease [10]. Recent studies show that the proper function of the intestinal barrier plays a key role in the conditions where a gut-originated "pollution" of the body is suspected, such as in the autism spectrum disorders (ASD). Indeed, when intestinal permeability has been evaluated in individuals with celiac disease

and in individuals with ASD, both of them showed increased presence of intestinal inflammation, caused by an abnormal intestinal permeability [11,12]. These individuals show inflammation because, when the intestinal permeability is altered, through increased expression of proteins (i.e. zonulin) that control the TJ opening [12], the antigenic substances freely cross the intestinal barrier and enter in the systemic circle, causing release of pro-inflammatory cytokines. For these motivation, nowadays, the concept of "functional food" has been established. According to this theory, the food is considered as a modulator of the physiological functions of the gut and the body. Modern food additives, such as sugars, salt, organic solvents, emulsifiers, gluten, microbial TG, and nanoparticles used in the food industries, are factors that can potentially induce autoimmune diseases through a provoked "leaky gut" [13].

3. Gut-brain axis in ASD

Autism spectrum disorders have multifactorial features [14]; children with ASD show, in addition to gastrointestinal symptoms, also biochemical, metabolic, and immunologic abnormalities [15]. Considering the common clinical features between hepatic encephalopathy and autistic phenotype, it is hypothesized that in both diseases there is a similar mechanism of toxic encephalopathy [16]. Based on this hypothesis, the gut-brain axis plays a key role in the development of these pathologies [17]. Indeed, both these diseases/disorders have an impaired intestinal permeability, that causes absorption of peptides of larger dimension as gluten and casein. The hydrolytic digestion of casein (a major milk protein) and gliadin (a wheat-derived protein) releases opioid-like peptides [18]. These proline-rich opioid peptides are able to modulate the cysteine uptake in cultured human neuronal and gastrointestinal (GI) epithelial cells [19]. The availability of cysteine strongly limits the intracellular synthesis of antioxidant glutathione (GSH), a powerful antioxidant molecule [20]. Emerging evidence from biochemical investigations indicates that ASD is associated with significantly lower levels of antioxidant GSH. Indeed the GSH/GSSG ratio is considered a marker of cellular toxicity and has been used as a clinical test to assess the presence of autism-related oxidative stress [18,21]. Food-derived opioid peptides produced in the gut are also neuroactive compounds. These molecules can exert their effects on the gut epithelium, through an increased permeability of the intestinal membrane and are able to cross the blood-brain barrier, entering the central nervous system [18]. When the opioid-like peptides arrive to the central nervous system, they are able to influence neurotransmission and interfere with neuro-regulatory mechanisms, causing psychiatric, cognitive and behavioral disorders [22]. Furthermore, in the intestinal lamina propria of ASD children, the dietary-derived peptides are able to cause an altered immune response. Several cell subtypes are involved and dysregulated: Monocytes, macrophages, antigen-presenting cells, and T and B cells. In particular, monocytic cells are strongly dysregulated in ASD [23,24]. As these cells are able to move through the body, they could deliver pro-inflammatory signals to the higher centers in the nervous system, through an altered and permeable blood-brain barrier [25]. Indeed, panenteric infiltration of activated lymphocytes, monocytes, natural killer cells and eosinophils into the walls of the gastrointestinal tract in ASD children with GI symptoms has been recently reported [26]. Another hypothesis states that gluten-derived peptides that cross the blood-brain barrier (BBB) could trigger an innate immune response in the brain, that causes the exposure of transglutaminase enzyme from neuronal cells [26]. Increased autoantibodies toward this enzyme could have a role in the pathogenesis of the neuro-psychiatric disorders [27]. Access of these

gluten peptides and/or activated immune cells to the brain may be facilitated by a breach of the blood brain barrier [27]. Indeed, BBB function and integrity is compromised in ASD [25]. This study demonstrated increased expression (as well as protein levels) of several genes associated to BBB integrity and neuro-inflammation on post-mortem autistic brain slices [25]. Recent studies suggest that abnormalities in the gut-brain interactions may be central to ASD. An interesting fact is that children with ASD have high blood levels of serotonin (5-HT). This could explain the GI motor abnormalities of children with ASD. Indeed, serotonin performs its function at both terminals of the axis, between the central nervous system and the gastrointestinal tract [28]. In an experimental model of autism, the mouse valproic acid (VPA) model, an increased expression of neuro-inflammatory markers in the brain of in utero-exposed male offspring has been found. It is hypothesized that during inflammatory processes enterochromaffin cells and intestinal inflammatory cells (neutrophils infiltration through epithelial cell loss) release serotonin [29]. This neurotransmitter induces an increase in the secretions, vasodilatation, and vascular permeability, causing functional dysmotility, stool alterations (diarrhea or constipation), and infiltration of leukocytes in the intestinal wall. Brain serotonin levels will be reduced, because the cells that release serotonin utilize much tryptophan, this means that less tryptophan will be available to cross the blood-brain barrier. The low levels of cerebral serotonin in ASD children are responsible of cognitive dysfunctions found in these individuals. Furthermore, the neurons releasing serotonin allow a communication between the immune system and the brain; in this way, they contribute to innate and adaptive immune responses [30].

Several studies confirm that abnormal lipid metabolism is also implicated in autism. Indeed, in a recent study, the fatty acid contents between 121 autistic children and 110 non-autistic children has been analyzed, the results have shown that the percentage of total polyunsaturated fats (PUFA), essential components of cellular membranes, was lower in autistic patients than in controls. This mean that ASD children have an abnormal ratio of ω-6/ω-3 fatty acids, that could contribute to the changes in brain connectivity, synaptogenesis, cognition, and behavior [31]. In addition, in the brain, oxidative stress, trough quinones/semiquinones production, could deplete the GSH [32], the antioxidant molecule already seen decreased in ASD [21], and affects PUFA levels. Radical molecules are able to react with arachidonic acid, in this way accelerating PUFA degradation [32]. This reaction, in turn, produces more harmful PUFA-derived peroxyl radicals. Given that, a single initiation process is able to generate, through a chain reaction, multiple peroxide molecules [32]. Brain cholesterol/isoprenoid homeostasis was altered in the VPA rat model of autism [33]. Alteration in metabolism of cholesterol triggers decrease in oligodendrocyte density and in myelin levels in the hippocampus of VPA-rats [33]. Another powerful brain-low molecular mass antioxidant, is the ascorbate (vitamin C) [32]. Of note, vitamin C is decreased in ASD children [24]. Furthermore, the decreased levels of arachidonic acid could affect the endocannabinoid (EC) system, a complex network of lipid signaling molecules comprised of endogenous arachidonic acid-derived compounds anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), their G-protein-coupled receptors (cannabinoid receptors CB1 and CB2) and the related enzymes. This EC system has been demonstrated to be implicated in ASD [34]. EC system has been demonstrated to be dysregulated in monocytes and macrophagic cells from ASD [23]. Both mRNA and protein for CB2 receptor are significantly changed in these ASD cellular models, contributing to immunological disruption [23,24]. Dysregulation in EC signaling could also affect the gut-brain axis. Indeed, this system is also involved in controlling the immune gut

homeostasis [35]. Direct/indirect activation of CB1 and CB2 receptors exerts anti-inflammatory activities on intestinal inflammation and reduces gastrointestinal damage [36].

It has been demonstrated that gluten/casein free (GFCF) diet is able to improve the quality of fat intake in ASD children. ASD children under GFCF diet also showed lower body mass index, weight and total energy intake compared to ASD children in regular diet [37]. Recently, a low-mild gut inflammation, dysbiosis, GI symptoms, and increased intestinal permeability have been demonstrated in ASD children. Autism severity was parallel to the presence of yeast infection [38]. By means of cultural approach, Candida spp. was found in 57.5% of ASD samples and none in controls. The colonizating yeast cells showed an aggressive, metabolic active form, indicated by the presence of pseudo-hyphae that facilitate adhesion to intestinal mucosa [38]. High Colony Forming Units (CFU) counts of Clostridium spp. were more abundant in stool samples of ASD than healthy controls, positively correlating with autism severity [38]. Dysbiosis was further indicated by decreased presence of Lactobacillus spp. in ASD samples [38]. Increase in yeast infections in ASD subjects could be also due to decrease in immune functions, as recently reported [39]. Decreasing in Natural Killer (NK) activity in ASD could predispose to opportunistic infections and chronic inflammatory conditions [39].

Altered gut microbial colonization was also found in a murine model of ASD [40], further confirming that gut microbioma is able to affect host social behavior through the alteration of brain neural circuits [41].

4. Conclusions

Gut microbiota show a key role in controlling brain development and behavior, also influencing responses to neuroinflammation, brain injury, autoimmunity and neurogenesis [42]. ASD subjects with GI symptoms have altered intestinal microbiota. Restoring gut homeostasis in ASD could be a possible specific therapeutic strategy in ASD management, as GI dysfunctions could affect ASD symptoms, such as aggressivity, irritability and sleep problems [43]. Some limitations should be addressed before adopting definitive effective treatments. Additional experimental data on animal models and randomized controlled trials will be needed to further confirm gut-brain axis alterations in ASD. Gut dysbiosis, GI symptoms and the following dietary assessments, if any, should be analyzed individually.

Conflict of interest

Authors declare no conflict of interest.

References

1. Blikslager AT, Moeser AJ, Gookin JL, et al. (2007) Restoration of barrier function in injured intestinal mucosa. *Physiol Rev* 87: 545.
2. Podolsky DK (1999) V. Innate mechanisms of mucosal defense and repair: The best offense is a good defense. *Am J Physiol* 277: G495–G499.
3. Kunzelmann K, Mall M (2002) Electrolyte transport in the mammalian colon: Mechanisms and Implications for disease. *Physiol Rev* 82: 245–289.
4. Ferraris RP, Diamond J (1997) Regulation of intestinal sugar transport. *Physiol Rev* 77: 257–302.

5. Groschwitz KR, Hogan SP (2009) Intestinal barrier function: Molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 124: 3–22.
6. Bischoff S, Barbara G, Buurman W, et al. (2014) Intestinal permeability—A new target for disease prevention and therapy. *BMC Gastroenterol* 14: 189–214.
7. Van Itallie CM, Holmes J, Bridges A, et al. (2008) The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* 121: 298–305.
8. Ulluwishewa D, Anderson RC, Mcnabb WC, et al. (2011) Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* 141: 769–776.
9. De Magistris L, Picardi A, Sapone A, et al. (2014) Intestinal barrier in autism, In: Patel VB (ed.), *Comprehensive guide to autism*, New York: Springer, 123.
10. Catassi C, Fasano A (2008) Celiac disease. *Curr Opin Gastroenterol* 24: 687–691.
11. Bjarnason I, Macpherson A, Hollander D (1995) Intestinal permeability: An overview. *Gastroenterology* 108: 1566–1581.
12. Fasano A (2011) Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity and cancer. *Physiol Rev* 91: 151–175.
13. Lerner A, Matthias T (2015) Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. *Autoimmun Rev* 14: 479–489.
14. Siniscalco D, Cirillo A, Bradstreet JJ, et al. (2013) Epigenetic findings in autism: New perspectives for therapy. *Int J Environ Res Public Health* 10: 4261–4273.
15. Siniscalco D, Antonucci N (2013) Possible use of Trichuris suis ova in autism spectrum disorders therapy. *Med Hypotheses* 81: 1–4.
16. Wakefield AJ (2002) The gut-brain axis in childhood developmental disorders. *J Pediatr Gastroenterol Nutr* 34: S14–S17.
17. Siniscalco D (2014) Gut bacteria—brain axis in autism. *Autism* 4: e124.
18. Siniscalco D, Antonucci N (2013) Involvement of dietary bioactive proteins and peptides in autism spectrum disorders. *Curr Protein Pept Sci* 14: 674–679.
19. Trivedi MS, Shah JS, Al-Mughairy S, et al. (2014) Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences. *J Nutr Biochem* 25: 1011–1018.
20. Frustaci A, Neri M, Cesario A, et al. (2012) Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. *Free Radic Biol Med* 52: 2128–2141.
21. Melnyk S, Fuchs GJ, Schulz E, et al. (2012) Metabolic imbalance associated with methylation dys-regulation and oxidative damage in children with autism. *J Autism Dev Disord* 42: 367–377.
22. Shattock P, Whiteley P (2002) Biochemical aspects in autism spectrum disorders: Updating the opioid-excess theory and presenting new opportunities for biomedical intervention. *Expert Opin Ther Targets* 6: 175–183.
23. Siniscalco D, Sapone A, Giordano C, et al. (2013) Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. *J Autism Dev Disord* 43: 2686–2695.
24. Siniscalco D, Bradstreet JJ, Cirillo A, et al. (2014) The *in vitro* GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages. *J Neuroinflammation* 11: 78.
25. Fiorentino M, Sapone A, Senger S, et al. (2016) Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Mol Autism* 7: 49.

26. Rose DR, Yang H, Serena G, et al. (2018) Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain Behav Immun* 70: 354–368.
27. Lionetti E, Leonardi S, Franzonello C, et al. (2015) Gluten psychosis: Confirmation of a new clinical entity. *Nutrients* 7: 5532–5539.
28. O'Mahony SM, Clarke G, Borre YE, et al. (2015) Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res* 277: 32–48.
29. Theije CGMD, Koelink PJ, Korte-Bouws GA, et al. (2014) Intestinal inflammation in a murine model of autism spectrum disorders. *Brain Behav Immun* 37: 240–247.
30. Baganz NL, Blakely RD (2013) A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem Neurosci* 4: 48–63.
31. Van Elst K, Bruining H, Birtoli B, et al. (2014) Food for thought: Dietary changes in essential fatty acid ratios and the increase in autism spectrum disorders. *Neurosci Biobehav Rev* 45: 369–738.
32. Halliwell B (2006) Oxidative stress and neurodegeneration: Where are we now? *J Neurochem* 97: 1634–1658.
33. Cartocci V, Catallo M, Tempestilli M, et al. (2018) Altered brain cholesterol/isoprenoid metabolism in a rat model of autism spectrum disorders. *Neuroscience* 372: 27–37.
34. Brigida AL, Schultz S, Cascone M, et al. (2017) Endocannabinod signal dysregulation in autism spectrum disorders: A correlation link between inflammatory state and neuro-immune alterations. *Int J Mol Sci* 18: 1425.
35. Acharya N, Penukonda S, Shcheglova T, et al. (2017) Endocannabinoid system acts as a regulator of immune homeostasis in the gut. *Proc Natl Acad Sci USA* 114: 5005–5010.
36. Gyires K, Zádori ZS (2016) Role of cannabinoids in gastrointestinal mucosal defense and inflammation. *Curr Neuropharmacol* 14: 935–951.
37. Marí bauset S, Llopisgonzález A, Zazpe I, et al. (2016) Nutritional Impact of a Gluten-Free Casein-Free Diet in Children with Autism Spectrum Disorder. *J Autism Dev Disord* 46: 673–684.
38. Iovene MR, Bombace F, Maresca R, et al. (2017) Intestinal Dysbiosis and Yeast Isolation in Stool of Subjects with Autism Spectrum Disorders. *Mycopathologia* 182: 349–363.
39. Siniscalco D, Mijatovic T, Bosmans E, et al. (2016) Decreased Numbers of CD57 + CD3- Cells Identify Potential Innate Immune Differences in Patients with Autism Spectrum Disorder. *Vivo* 30: 83–89.
40. de Theije CG, Wopereis H, Ramadan M, et al. (2014) Altered gut microbiota and activity in a murine model of autism spectrum disorders. *Brain Behav Immun* 37: 197–206.
41. Needham BD, Tang W, Wu WL (2018) Searching for the gut microbial contributing factors to social behavior in rodent models of autism spectrum disorder. *Dev Neurobiol* 78: 474–499.
42. Fung TC, Olson CA, Hsiao EY (2017) Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci* 20: 145–155.
43. Santocchi E, Guiducci L, Fulceri F, et al. (2016) Gut to brain interaction in Autism Spectrum Disorders: A randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. *BMC Psychiatry* 16: 183.