Anorexia Nervosa and Autism Spectrum Disorders: Future Hopes Linked to Mucosal Immunity

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Highlights

- Anorexia nervosa and autism spectrum disorders present features such as impaired cognitive function, dysbiosis, and immune biomarkers alterations. There are still gaps to fill in our understanding of the underlying mechanisms for each disorder.
- The mucosal immune system appears to be a crucial interface for the microbiota-gut-brain axis and may represent a target of interest.
- Research in psychiatry must turn to a mechanistic understanding of patients' gut immune system, its interaction with the microbiota, and the way information is relayed to the brain.

Keywords

Anorexia nervosa · Mucosal immunity · Autism spectrum disorder · Seventh sense · Microbiota · Gut immunity

Abstract

Mental health is becoming a public health priority worldwide. Anorexia nervosa and autism spectrum disorders are 2 important types of childhood disorders with a bad prognosis. They share cognitive impairments and, in both cases, the microbiota appears to be a crucial factor. Alteration of the microbiota-gut-brain axis is an appealing hypothesis to define new pathophysiological mechanisms. Mucosal immunity plays a key role between the microbiota and the brain. The mucosal immune system receives and integrates messages from the intestinal microenvironment and the microbiota and then transmits the information to the nervous system. Abnormalities in this sensorial system may be involved

in the natural history of mental diseases and might play a role in their maintenance. This review aims to highlight data about the relationship between intestinal mucosal immunity and these disorders. We show that shared cognitive impairments could be found in these 2 disorders, which both present dysbiosis. This literature review provides details on the immune status of anorexic and autistic patients, with a focus on intestinal mucosal factors. Finally, we suggest future research hypotheses that seem important for understanding the implication of the gut-brain-axis in psychiatric diseases.

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Introduction

The global burden of mental illnesses has increased by 41% between the last 2 Global Burden of Disease studies (1990–2010) led by the World Health Organization

(WHO). It represents 7.3–10.4% of the global disease burden [1]. The absence of revolutionary explanations for the pathophysiology of mental diseases is locking patients in stigmas, prejudice, and fear. Mental health research must thus be considered a priority. As such, understanding the etiopathology of these disorders will aid in the achievement of the following previously identified aims: development of new treatments and improvement of the precocity of intervention [2]. Psychiatric diseases appear to be linked to environmental factors, interacting with genetic susceptibility determining disease emergence and severity. These factors could – in part – be microbiological information derived from the intestinal microbiota [3]. The emerging big data from microbiota analysis in psychiatry has opened an exciting field in that links with intestinal microbiota actually appear to be crucial for mental health [4]. Food intakes, and particularly their diversity, are determinant for microbiota's health [5]. The “microbial super-organism” hosted in our gut is under the influence of the environment through our diet [5], xenobiotics ingestion, and the microorganism panel of our living environment, and it is conditioned by our behavior [3, 6]. Intestinal commensal bacteria were recently revealed to be essential for human physiological processes and optimal nervous system development and functioning [7]. Pre-clinical models have shown that germ-free mice present impaired neurodevelopment [8] and that behavior seems to be partially determined by the microbiota in term of stress reaction [9]. In the last decade, the microbiota has emerged as a substantial player in the preexisting concept of a gut-brain axis developed for nutrition and now modeled as the microbiota-gut-brain (MGB) axis [10]. This concept of gut-brain talk is strengthening the popular idea of a gut feeling, explaining the emotional manifestations experienced at the abdominal level and the significance of intestinal wellbeing influencing feelings of comfort/(optimal) mental comfort. This MGB axis is composed of 3 main routes engaged in bidirectional communication.

The nervous system connects the gut to the brain via the vagus nerve [4]. Messages from the enteric nervous system are primarily integrated into the spinal cord and then relayed to the autonomic nervous system at the encephalic level [11].

The second route involves a network of biological mediators, collected through blood flow for humoral signaling in the brain, forming the neuroendocrine axis. Hormones from the hypothalamic-pituitary-adrenal axis are regulated by resident bacteria of the gut, modeling stress responses. Inversely, encephalic hormonal balances reflect the microbiota composition at gut level [4]. Mole-

cules as small as neurotransmitters, trophic factors, short chain fatty acid (SCFA), and peptides secreted by bacteria [12] have been shown to be essential for optimal neurodevelopment, regulation of stress, and social behavior [13].

The last pathway involves the immune system itself [14]. Innate and adaptive immunity could interact with neurological structures at the enteric level, directly transmitting information to the enteric nervous system through cytokines and neuroimmunological dialog [15].

Communication between those systems enables the integration of information from the environment. This are transmitted locally to the enteric nervous system or through the vagus nerve directly to the central nervous system [16]. Distal communication is ensured either systemically (secretion of cytokines and immunomediator release into the blood) or through the migration of primed intestinal cells to the blood-brain barrier. The blood-brain barrier is not an impermeable wall as previously established; it participates in the integration of distant immune messages in CNS [12]. This cross-talk is conceptualized as the “seventh sense” [17].

The immune system interacts tightly with microbes necessary for its maintenance [18]. The mucosal immune system has emerged as a hub of bidirectional pathways between the microbiota, the intestinal epithelium, systemic immunity, and the nervous system. Its implication in the physiopathology of functional gastrointestinal disorders which involve a substantial psychological component has already been described [14]. It represents a potential reserve for biomarkers and therapeutic targets [19] that are much needed in the care of psychiatric diseases [2].

The aim of this work is to review elements regarding anorexia nervosa (AN) and autism spectrum disorders (ASD), with a focus on the mucosal immune system and its possible implication in pathological mechanisms. Interestingly, both of these disorders present cognitive impairments and microbiota alterations, and observing the immunological conditions in parallel could enable the development of assumptions.

Publications were found using PubMed and the phrases “anorexia nervosa” and “autism trouble disorders” associated with immunological vocabulary in terms of interaction and factors.

Clinical Overlap in AN and ASD

AN is a major disease in the range of adolescent psychiatry. It has high mortality [20] and morbidity rates and is marked by functional and social impairment [21]. This

eating disorder is characterized by dysmorphophobia, associated with voluntary weight loss with no regard for consequences as well as an intense fear of weight gain [22]. Recently Himmerich and Treasure [23] reviewed genomic studies in AN and proposed a new pathophysiological model based on extracorporeal factors and involving a social dimension including one's lifestyle, the ideal concept of beauty, and psychosocial stressors but also the biological environment, diet (influenced by social habitus), and use of medication or probiotics. These factors can influence the emergence of the disease by directly initiating the restrictive eating behavior or influencing the body's physiology. Biological factors identified from a genetic point of view as important for the development of AN are the microbiome, the metabolic system, and the immune and nervous systems. All of these factors potentially influence each other, thus conditioning the occurrence of AN [23]. Changes in eating behaviors caused by AN are thought to deeply alter the intestinal microbiota [24], and this has been previously reviewed by Mack et al. [25]. Their conclusions emphasize the difficulties in interpreting results from current studies. Moreover, SCFA abundance and profile alterations were recently reported alongside differences at the phylum and genome levels of microbial taxa in AN [25].

ASD are neurodevelopmental diseases. A multifactorial etiology characterizes these childhood psychiatric disorders. ASD are defined by a strong genetic substratum, but environmental factors are thought to trigger the emergence of the disease. The latest study conducted in the USA reports a 2.41% prevalence in children [26]. The main features of ASD are cognitive impairment in social interactions and facilities, repetitive behaviors, and restricted interests or activities [22]. Abdominal pain and transit disorders [27], and particular eating behaviors such as food selectivity, as well as overweight, are frequently associated symptoms [28]. The microbiota has recently appeared as a critical factor for neurodevelopment, and evidence is accumulating for a role in intestinal physiology and pathologies [7]. Alterations of the microbiota have been identified in ASD patients [29, 30] and are currently being considered as therapeutic targets for the treatment of behavioral and abdominal disorders [31]. In any case, the microbiota appears to play an essential role in ASD [32].

Because they share cognitive impairments, some hypotheses clinically associate AN with ASD. They also have mental traits in common, specifically extreme rigidity, and environmental factors that trigger a genetic susceptibility [33]. Cognitive profiles were compared and results

showed similarities in empathy abilities, executive functions, and central coherence [34]. Studies have investigated ASD prevalence in populations of AN patients. An overrepresentation of ASD by 23% in AN patients in a Swedish cohort has been described [35]. Moreover, autistic traits appear to be more associated with the purely restrictive subtype of AN [36]. The identification of autistic traits in AN remains difficult, and the lack of an established physiopathology does not allow for a definite link between the 2 pathologies [33]. A common endophenotype, identified by overlaps in cognitive and temperament profiles found in AN and ASD patients, has been put forward by Zhou et al. [33]. This allowed them to define a neurocognitive endophenotype based on the following 3 altered domains: impaired mental flexibility evidenced by attentional set-shifting impairments, dysfunctional central coherence, and empathy and theory of the mind alterations. They also defined a temperament endophenotype, based on the following 5 aspects, reflective of the clinical phenotype of the diseases: perfectionism, impulsivity, obsessiveness, low self-regulation, and a high negative affectivity [37]. Similarities seem to be explained by altered cerebral morphology of the grey matter [38] and by genetic determinants that can constitute another part of the endophenotype.

Specificities of the Immune Response in AN and ASD

The intestinal mucosal immune system is multilayered. Its function is to defend the integrity of the body against threats to the intestinal lumen but also to not overreact and tolerate microbiota (recently discovered as essential for human physiology). The epithelial barrier plays an important role in a passive defense function with tight junctions, mucus secretion, and antibacterial molecule production. It is also a safeguard, with the ability to start an inflammation reaction against threats, and promotes resolution of inflammation. The mucosa thickness hosts immune cells shared in 2 subtypes of innate immune cells and adaptive immune cells. In the innate side, mastocytes are common, involved in guarding the barrier integrity and the defense against parasites. Some eosinophils are involved in parasite defense but also in inflammation regulation, participating in the tolerogenic environment of intestinal mucosa. Dendritic cells (DC) are professional antigen-presenting cells that are extremely important for driving the reaction of adaptive immune cells. Macrophages are highly involved in maintaining the tolerogenic environment of the mucosa. Some cells are

called “innate-like” – (issues of the lymphoid line). They present a special T receptor with an invariant α chain, specializing the cell in detection in nonpeptidic antigens as follows: lipids for innate natural killer T cells or vitaminic for mucosal associated innate T cells. Some cells lack the T receptor (so-called innate lymphoid cells; ILC). They are able to quickly react against antigens to initiate inflammation [39]. The adaptive system is called gut-associated lymphoid tissue. Some lymphocytes are distributed in the epithelium, preferentially with the CD8+ phenotype, and with special T-cell receptors composed of a γ and δ chain. Deeper in the mucosa, we find the lymphoid follicles and Peyer’s patch, composed of B and T lymphocytes, surrounded by DC. These are sites of production of immunoglobulin A, involved in the protection of the gut barrier [40].

Chronic starvation of patients suffering from AN invites us to pay attention to their infectious risk, classically increased in the case of undernourishment [41]. Surprisingly, AN patients do not show an increased acute infection risk [42]. Data on immune status in AN patients have been reviewed by Słotwińska and Słotwiński [43] and no immunodeficiency could be highlighted. At the humoral level, plasmatic IgG levels have been reported as decreased in AN patients (0.879 g/dL) versus healthy controls (1.119 g/dL) [44]. Complement levels have been measured in patient’s serum and reveal a decrease suggesting a hypoproduction rather than a consumption [45]. Immunophenotyping studies remain controversial. Leukopenia is common among AN patients, but lymphocyte subset rates appear to be inversely correlated to BMI. CD4+ and CD8+ T lymphocyte numbers were lower or unaffected in the acute phase, returned to normal rates after refeeding, and then stabilized at a subnormal rate during the outpatients’ treatment [46]. No study revealed a profound immunodeficiency; it is rather suggested that there is an impaired ability to respond to pathogens. Vaccine efficiency for H1N1 pandemic prevention was also tested in AN patients and did not reveal an altered immunization potential [47]. Food allergy appears to be a risk factor for the development of eating disorders, more in a biopsychological way [48]. Autoimmune diseases and eating disorders appear to be linked in a bidirectional way in AN patients, with higher HR for celiac disease and lupus [49]. Patients with eating disorders display a higher prevalence of autoimmune diseases (8.9%) than controls (5.4%) [50]; this was recently confirmed in a Danish cohort of AN patients (HR = 1.64%) [51]. Cytokine secretion seems impaired in the acute phase of starvation, and its recovery does not appear to be solely linked

to nutritional status in patients [52]. A large body of work has been done on inflammation, investigating levels of cytokines in the peripheral blood of patients [53]. A meta-analysis revealed differences in AN cytokine profiles. IL-1 β was increased in the restrictive subtype, and higher levels of IL-6 and TNF- α were shown in AN patients versus controls [54]. The discovery of anti- α -MSH antibodies in the blood of patients gave rise to an autoimmune hypothesis. α -MSH is an anorexigenic peptide involved in appetite regulation but also in emotion regulation. Molecular mimicry of ClpB from *Escherichia coli* could generate these cross-reacting antibodies [55], arguing for the involvement of the microbiota in an altered appetite regulation in AN.

Regarding ASD, immune dysregulation appears to be canonical in a subset of patients. Genetic studies have revealed significant immune gene variants associated with ASD, notably HLA genes [56]. In patients, IgG and IgM levels have been reported to be reduced [57], while increases in immune circulating proteins levels have also been reported [58]. DC frequencies have been shown to be elevated in ASD children, but no study has focused on intestinal DC subsets [59]. Neopterin levels were elevated in patients and correlated with an increase in circulating monocyte rates [60]. Both were associated with the severity of symptoms as measured by the Childhood Autism Rating Scale [61], suggesting that innate immune pathways are also involved. A potential role has also been described for mast cells given the frequent comorbidity of allergic-like disorders [62].

Immunophenotyping studies in patient blood described an abnormal frequency of CD4+ T lymphocytes, more from the memory phenotype than from the naive phenotype [63]. In adult ASD, the CD8+ lymphocyte percentage appeared to be higher than that of CD4+, resulting in a lower CD4+/CD8+ ratio than in controls [64]. NK cells have extensively been studied and their circulating frequency in patient blood remains inconsistent, while a genetic alteration of function has emerged in ASD [65]. Patient cytokine analysis and immunophenotyping revealed a Th1 polarization of low-grade inflammation that seemed to be associated with higher rates of behavioral disorders [66] and further suggested an immune imbalance within this spectrum. A recent study showed a dysregulation in lymphocyte expression of transcriptional factors resulting in a reduced frequency of regulatory cells [67]. This is further strengthened by an increase in proinflammatory molecules in ASD patients, with a strong correlation between inflammatory cytokine levels and disorder severity [65]. A significant increase in total

B lymphocytes has also been described. Activated (CD38+) and memory (CD5+) B cells in ASD patients were also shown to have increased [68]. B-cell-expressing IgE-specific receptors (CD23+) also seem to be more frequent among child samples [69]. However, ASD children do not show abnormal responses to vaccination [70]. The autoimmune hypothesis offers new hope in ASD research, with potential roles for auto-antibodies reactive to components of the nervous system. Autoimmune events during pregnancy have also been associated with an increased ASD risk [65], and ASD patients face a higher risk of developing autoimmune diseases and allergic diseases but a lower risk of asthma [71].

Microbiota-Derived Metabolites Appear to be Determinant in AN and ASD Disorders

Many environmental outcomes associated with ASD can affect the microbiota, such as the frequency of caesarian sections for delivery or a high prevalence of antibiotic therapy in early life [72]. Differences in fecal microbiota have been shown in both conditions.

In ASD patients' stool samples, elevation of *Clostridium*, *Lactobacillus*, *Bacteroides*, *Desulfovibrio*, *Caloramator*, and *Sarcina* proportions, and a reduction of the *Bifidobacterium/Firmicutes* ratio, were reported [73, 29]. ASD patients with gastrointestinal disorders showed a reduced presence of *Prevotella*, *Coprococcus*, and *Veillonellaceae* compared to neurotypical children without gastrointestinal symptoms [74]. A recent microbiota study also identified a lower frequency of *Faecalibacterium* and *Haemophilus* [29]. Microbial metabolites were also measured and revealed an elevation of isopropanol and p-cresol and lower GABA levels, while no differences were observed between ASD and neurotypical children regarding propionate and butyrate (2 major SCFA) levels [29]. However, some trials reported an association between propionic acid and autism symptoms in animal models [75]. Modulations in the microbiota composition thus appear to be potential therapeutic strategies in the treatment of behavioral dimension disorders and their associated gastrointestinal manifestations. Therapeutic strategies can either drive indirect modifications through the use of probiotics, prebiotics, or diet intervention or involve direct intervention such as microbiota transfer therapy [76]. More evidence is currently under development in human models after the establishment of proof symptom reduction in ASD mouse models [31, 72].

In AN patients, sequencing data in the context of AN-driven dysbiosis were recently reviewed [25]. *Methanobrevibacter smithii* was shown to be increased in AN subjects, but in a variable proportion. Diversity itself was not altered but structural differences were identified. *Firmicutes* and *Roseburia* were decreased as SCFA metabolites. Longitudinal studies on stool sample evolution during refeeding showed differences in microbial richness in comparison to controls. These differences appeared alongside refeeding at the genus level. Mack et al. [77] identified higher levels of mucin-degrading end-protein-degrading taxa, whereas carbohydrate degraders were lower. As for metabolites, BCFA were elevated and butyrate was lower in patients' feces. Differences in taxonomy were also identified between restrictive and binge-purge AN subtypes [77]. These microbiota alterations are partially shared with inflammatory bowel disease [78] and celiac disease [79], notably in reduction of diversity and reduction of *Bifidobacterium*.

Communication between the Immune and Nervous Systems in the Intestinal Mucosa

Intestinal lumen microorganisms are recognized, and their characteristics are integrated by the immune system. Its role is to orchestrate an adapted response. Stimuli from our environment are also received and integrated into the central nervous system to elaborate an adapted response. For a long time, the nervous and immune systems, both sharing abilities to communicate in various ways, were considered as 2 distinct entities. The immune part of the MGB axis concept involves a means of communication with the nervous system. This interaction would constitute a new sense, with the integration of environmental changes which could be translated into signals to the brain. The concept of this as a seventh sense was introduced by Jonathan Kipnis [17]. Evidence has shown that T-cell depletion in mice engenders abnormal behavior and cognitive dysfunctions, and that those deficits are reversible by T-cells restoration [80]. These findings suggest an important role of the adaptive immune system for optimal cortical functions. Neuroimmune interactions are mostly biochemical, involving ligand-receptor pathways (neurotransmitters, cytokines, and peptides) [81]. This communication takes place inside the mucosa, involving the enteric nervous system for a bidirectional local response. Neurons respond to cytokines in secreting neurotransmitters received by immune cell membrane receptors. These mechanisms constitute a

neuroimmune synapse by close localization. This is particularly relevant in Peyer's patches and in the plexus where a large population of macrophages and mast cells are encountered. The microbiota regulates this communication by regulating the immune cells [82]. In animal models, mast cells are closely associated with neurons producing substance P and calcitonin gene-related peptide [83]. It was shown that mast cells interact with neurites by N-cadherin and β -catenin molecules in vitro [84]. Hyperexcitation of neurons is mediated by histamine released from mast cells, received by the H2 receptor. Triggering of proteinase-activated receptor 2 causes long-lasting neuronal activation and the release of substance P and calcitonin gene-related peptide [83]. These 2 substances regulate the cytokine release by mast cells. Macrophages available in intestinal muscularis appear to be essential for intestinal function based on studies of depletion in mice. These cells establish a bidirectional cross-talk with mucosal plexus neurons, thus regulating motility [85]. These macrophages are influenced by commensal bacteria which regulate the growth factor production of macrophages with enteric neurons (colony-stimulating factor 1 and bone morphogenetic protein 2). Muscularis macrophages present β_2 adrenergic receptors. These are involved in norepinephrine signaling, suggesting an anatomical functioning unit [86]. This subpopulation is known for distinct gene expression and morphological specificities from lamina propria macrophages [87]. In mice, they present a tissue-protective phenotype whereas lamina propria macrophages exhibit a more proinflammatory profile [86]. ILC, specifically ILC2, are abundant in the mucosa and were shown to be located close to neurons, which exert a direct regulation on the neuromedin U peptide, thus triggering a strong reaction in type 2 cytokine production [88]. Inflammatory mediators such as TNF and IL-1 β are detected by sensitive vagus neurons. Enteric neurons are sensitive to alarmines and able to produce stimulation of ILC [88]. T and B cells express the β_2 -adrenergic receptor. In vitro studies have shown abilities of catecholamines to suppress Th1 and promote Th2, Th17, Treg, and B cell production of antibodies. In vivo studies are missing and efforts have been made to define lymphocyte-neuron interactions [89]. An inflammatory "reflex" (like a nervous one) have been described in the spleen: peripheral vagal sensory fibers are stimulated by pro-inflammatory mediators triggering a parasympathetic efferent signal to inhibit cytokine production by macrophages. Action potentials activate celiac ganglion decrease in preganglionic motor fibers. Postganglionic adrenergic neurons are activated and release norepineph-

rine to stimulate T cells producing acetylcholine, down-regulating the production of TNF- α from macrophages. Specific patterns of vagal activation by cytokines have been identified and termed "cytokine neurograms," and they appear to be neuroimmune signals able to be integrated by the brain [90]. Vagal stimulation is a new therapeutic target for bowel inflammatory diseases [91]. On a central level, the brain is able to sense immune messages by systemic mediators, released in blood or in lymph circulation [92]. It appears that no brain structure is specialized in the integration of immune sensory information. In an animal model, it was shown that ventromedial hypothalamic nucleus and hypothalamic noradrenergic neurons change their activity during the immune response. Some areas are sensitive to synergy of signals, which are able to trigger a neuroendocrine response, via the hypothalamo-pituitary axis [93]. This continuous communication is substantially influenced by the microbiota, notably through microbe-derived SCFA and neuropeptides [82, 94].

Mucosal Disruption in AN and ASD

Dysbiosis induces dysregulation of the mucosal barrier and intestinal and systemic disorders [95].

Regarding AN, data found in the literature are restricted to the description of some abnormalities at the mucosal level. Leaky-gut state studies in patients revealed inconsistent results. The use of a lactulose/mannitol absorption assay showed a reduced excretion of lactulose in urine, accounting for an unexpected reduced permeability [96]. Diamine oxidase activity reflects the maturity and integrity of small intestine mucosa. Lower serum levels (8.2 U/L) were reported in restrictive AN subjects compared to AN binge-purging type patients (12.3 U/L) and controls (12.1 U/L). This suggests intestinal structural abnormalities resulting in chronic fasting [97]. Moreover, zonulin dosages were positively correlated with the BMI of patients [98]. An activity-based anorexia murine model revealed alterations of the intestinal and colonic architecture and increased colonic permeability, associated with a decrease in junction protein expression [99].

In ASD patients, hypotheses regarding the leaky-gut state have been formulated and are supported by the high prevalence of gastrointestinal disorders in this population [27]. In a subgroup of ASD patients, increased intestinal permeability evidenced by the lactulose/mannitol test and elevated levels of fecal calprotectin were ob-

served. These findings were similar in first-degree relatives, suggesting a genetic cause [100]. However, intestinal biopsies revealed histological inflammation that was not correlated to differences in fecal calprotectin levels [101]. A study revealed overexpression of the zonulin genotype Hp2-2 in ASD patients presenting gastrointestinal symptoms [73]. Significantly elevated levels of zonulin were correlated to Childhood Autism Rating Scale (CARS) scores assessing the severity of ASD symptoms [102]. However, recent data do not confirm significant differences in zonulin levels in sera [103]. The leaky-gut state could result from an impaired barrier which is often intricately connected to dysbiosis.

Immunoglobulins A (IgA) are key molecules regulating the microbiota [104]. It also protects the epithelium from bacteria migration and adhesion by coating microbes during their migration in the mucus layer ("immune exclusion"). IgA also inhibit bacteria replication in the lumen ("enchained growth"). Their production is related to microbial stimulation during neonatal colonization. It is essential for the persistence of an optimal commensal balance and it is termed antibody-mediated immunoselection [105]. In AN patients, oral IgA levels have been shown to be higher (158.98 µg/mL) compared to controls (97.35 µg/mL) in the acute phase (BMI <15) [106]. In ASD patients, Zhou et al. [107] reported increased levels of fecal IgA, possibly associated with microbiota alterations. This could be a mucosal response to dysbiosis or a mucosal dysregulation involved in the dysbiosis set.

The microbiota is sensed directly or indirectly through microbial or dietary metabolites via Toll-like receptors and the aryl hydrocarbon receptor. Microbial metabolites are involved in the homeostasis of immune cell populations and the expression of regulatory cytokines or in immediate inflammatory outbreak [18, 94]. Models in which the pattern recognition receptor response is inactivated display dysbiosis, pointing to a critical role for the adapted immune response in microbiota homeostasis [108]. In an activity-based anorexia murine model, TLR4 was increased in epithelial cells and macrophages [109]. In ASD patients, lymphocytes were also found with elevated TLR4 expression [110].

The microbiota is required for the development of the mucosal immune system and guides the development of cell subsets. Under microbial homeostasis, most of the proinflammatory functions of effector T cells are inhibited by regulatory cells and factors. In animal models deficient of regulatory actors, Th2 profiles gain the upper hand and are associated with the establishment of dysbio-

sis [108]. ILC are involved in homeostasis of the barrier through constant dialog with CD4+ T cells to ensure the immune reaction. ILC1 produce type 2 IFN and TNF under stimulation of IL-12 or IL-18. ILC2 are close to Th2, reacting against helminths. ILC3 are a counterpart of the Th17 subset, and they are mostly located in intestinal mucosa. These cells appear to be essential for integration of signals from the microbiota through TLR to attune the innate and adaptive immune effector response [111]. To date, no extensive study has been performed in AN to assess intestinal lymphocyte populations [111]. Regarding ASD, a study collected precious data by investigating immune cells on colonic biopsies and revealed a lymphocytic colitis in children suffering from ASD and intestinal disorders. The ensuing immunohistochemistry study revealed a particular colitis with an increased colonic infiltration of T cells (specially γδ T cells), an increase in plasma cells associated with epithelium and basement membrane abnormalities [112]. Th17 cells were not studied, but IL-17 serum levels have been shown to be elevated in ASD patients and correlated to symptom severity [73]. In vitro stimulation of peripheral blood mononuclear cells from ASD children revealed a deficit in FoxP3+ Treg cells and an elevated amount of RORγt+/GATA3+/T-bet+ cells (respectively, Th17, Th2, and Th1 cells) compared to controls [67]. Decreased populations of Treg have also been previously described [113]. These data are consistent with those found in animal models affected by autism [114].

Perspectives of Research

The MGB axis is emerging as a challenging field to make psychiatry more integrative. The behavioral role of microbiota and the immune system benefits of translational research approaches the span from microbiology to neurosciences. Mechanisms of interaction must be studied between vagal and ENS interactions with immunity, as well as their close integration into the central nervous system. Mucosal cells should be studied to define their inflammatory or tolerogenic abilities to establish immunologic messages sent to nerves. Understanding how innate immune cells receive and integrate messages from the microbiota is a first step to strengthen the seventh-sense hypothesis. These studies will also help to better characterize how microbiota and the mucosal immune system interact in bidirectional regulation.

AN and ASD share the following common impairments in neuropsychological dimensions: social process-

Fig. 1. Mucosal disruption and the seventh-sense alteration hypothesis to explain cognitive impairment in AN and ASD. Dysbiosis is determined by environmental and behavioral factors, such as feeding (1). Mucosa rupture leads to antigen penetration and local inflammation reaction (2). An impaired antibody-mediated immunoselection phenomenon leads to microbial pullulation and microbial invasion (3). Impaired PRR recognition or signaling for

immune sense of microbiota (4). Immune balance dysregulation, tuned to inflammation (5). Immune cells impaired communication with nerves (chemical mediators/direct interaction/microvesicles) (6). Impaired nervous transmission of immune signals (7). Impaired central integration of immune sensitive signals (seventh sense) (8).

es of perception and understanding of self and others, mental flexibility, and central coherence. In both disorders, the microbiota appears to be altered. A relation in a bidirectional way may exist between these cognitive alterations and the dysbiosis condition, possibly through the MGB axis. Our hypotheses on potential impairment of the MGB axis are shown in Figure 1.

To our knowledge, abnormalities take place at the epithelium level in both conditions. Evidence remains inconsistent in AN and thus most findings have not yet been exposed. However, the main assumption is barrier weakness. Furthermore, investigations about passive mechanisms of microbiota regulation should also be conducted. Knowledge on microbicidal peptides and IgA profiles may provide new insights into microbiota regulation associated with psychiatric conditions. Antibody-mediated immunoselection also appears to be an interesting way to investigate mechanisms of microbiota regula-

tion [105]. Impaired IgA-mediated regulation of the commensal can enable the establishment of dysbiosis. Imbalance between regulatory and effector T-cell populations could lead to inflammation described in these 2 disorders. We showed consistent results in AN in unpublished preliminary studies. Humoral immunity also appears activated with an increase of the percentage of circulating B cells in AN [46]. Investigations to precisely characterize the cellular immune response have to be conducted in plasma but also in feces to understand the role of the GALT. The modulation of innate immunity in these 2 pathologies has not yet been described. However, macrophages seem to be activated in both conditions. Future studies of mucosal cells (DC, mast cells, ILC, and macrophages) in these 2 pathologies could be of great interest. Targeting of microbiota or immune cells as biomarkers or therapeutic opportunities in psychiatry is enthralling. As such, a potential treatment could be the

modulation of the microbiota, offering behavior modifications with something as simple as a diet intervention or pre-/probiotic administration or, more drastically, fecal microbiota transplantation [76].

Animal models of AN and ASD dysbiosis will allow the investigation of how modulation of the MGB axis impacts behavioral dimensions of psychiatric disorders. Modeling efforts must be made to offer new opportunities to understand the pathophysiology of psychiatric disorders and thus develop better-suited therapeutic strategies.

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