Review Article

Altered Microbiota-GALT Communication in IBD and ASD: Changes in IELs and AhR/ARNT Gene Polymorphism

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Abstract

Growing recognition of the microbiota and gut-associated lymphoid tissue (GALT) as a significant component of human health calls for a better understanding of the mechanisms involved in the host-microbiota interactions. The communication between the microbiota in the external milieu of the gut lumen and the host across the gastrointestinal barrier (GIB) involves recognition, selective response to the commensal vs. the pathogenic microorganisms and antigens, and adaptation, and is orchestrated by the GALT. In health, GALT assures GIB integrity and microbiota symbiosis; in disease, altered GALT's functions compromise GIB integrity and lead to dysbiosis associated with gastrointestinal immune

pathologies such as inflammatory bowel diseases (IBD) and neurodevelopmental disorders such as autism spectrum disorder (ASD). These pathologies are often accompanied by a "leaky gut syndrome" defined as increased intestinal permeability to pathogens. This review focuses on the microbiota-GALT communication involving intraepithelial lymphocytes (IELs) and their aryl hydrocarbon receptors (AhRs). It posits that changes in the IELs or their aryl hydrocarbon receptor (AhRs) jeopardizes GIB integrity and contribute to pathologies such as IBD and ASD. Hence, AhRs activity is regulated by the antiinflammatory dietary ligands present in cruciferous vegetables and fruits, further research is warranted into diet-derived

immunotherapies targeting both gastrointestinal immune and neurodevelopmental disorders.

1. Introduction

Communication between the microbiota in the gut lumen and the gut-associated lymphoid tissue (GALT) of the host involves recognition, response, and adaptation and under health conditions results in mutually beneficial partnership. Altered microbiota GALT crosstalk across the gastrointestinal barrier GIB results in gastrointestinal immune pathologies such as inflammatory bowel disease (IBD) and neurodevelopmental disorders such as autism spectrum disorders (ASD). Human gut microbiota, composed of bacterial, archaeal, fungal and viral cells, is involved in many physiological processes [1] and is responsible for most of the metabolites found in blood [2], as well as innate and adaptive immunity [3]. Microbiota-GALT communication is linked to our behavior as suggested by the results of studies involving germ-free mice [4] and by now supported by the link between the composition of gut microbiota and several neuropathologies including autism [5-7].

Humans are born with an immunologically underdeveloped and relatively sterile gut that slowly matures, completing the formation of the GIB and attaining adult profiles of microbiota by 3-5 years [8], but undergoes many transitions during the lifespan. Importantly, the composition of the gut microbiota is impacted by many environmental factors with both diet and stress being the major ones. The shift from health-promoting bacteria to spore- and neurotoxin-producing bacteria is associated with increased gut permeability, while changes in diet, such as a switch to a high-fat diet, promotes the outgrowth of proinflammatory species [9] and contributes to disruption of GIB.

The gut microbiota is intimately involved in the maturation of both innate and adaptive immune responses as well as the GALT, the largest component of our body's immune system containing 70% of the immune cells. GALT is also the major component of the GIB involved in the regulation of the absorption of nutrients while excluding the pathogens and toxins [10]. GALT abnormalities have been reported in IBD that includes ulcerative colitis and Crohn's disease, and celiac disease [11], and ASD [5, 6, 12].

GALT distinguishes between commensal bacteria and food-derived antigens and pathogens [13]. Disruption in GALT activity disrupts the pro-and anti-inflammatory balance, resulting in abnormal response to commensal bacteria or inadequate response against pathogens. The gut microbiota symbiosis is defined as a mutually beneficial relationship between various microorganisms in the gut. An essential aspect of maintaining gut symbiosis is continuous communication with external microbiota of the gut lumen. Additionally, food-derived ingested bacteria that reach the small intestine in an active form alter the gut metabolism [14] and microbiota composition directly [15]. Changes in diet and stress induce physiological changes and dysbiosis that lead to greater gut permeability allowing antigens, bacterial metabolites and bacteria themselves to enter the systemic circulation. This scenario referred to as the "leaky gut syndrome" [16-18] is associated with typical gastrointestinal disorders including IBD [19] and neurodevelopmental disorders such as ASD [20].

This review provides a synopsis of the major established GALT components, their function in the selective

interactions with microbiota, and abnormalities in gastrointestinal immune and neurodevelopmental disorders. It focuses on the initial communication between intraepithelial lymphocytes (IELs) and their aryl hydrocarbon receptors (AhRs) and the microbiota. It posits that the unique sensitivity of AhRs to environmental ligands and the position in the epithelial barrier underscore its key significance in microbiota communication. Furthermore, the polymorphism of the AhR and/or aryl hydrocarbon nuclear translocator (ARNT) may contribute to neurodevelopmental pathologies such as autism but also the "leaky gut syndrome". This supposition is supported by abnormalities in the IELs in both IBD [21] and ASD [22], as well as genetic abnormalities in AHNT in autism [23]. It concludes with a discussion of the anti-inflammatory properties of AhR's dietary ligands, abundant in cruciferous vegetables and fruits [24], and points to their potential target as immunotherapies for both gastrointestinal immune and neurodevelopmental disorders.

2. Gut-associated lymphoid tissue (GALT) in a nutshell

Human GALT is organized as lymphoid structures and more diffusely scattered lymphocytes with specialized functions, extensively reviewed elsewhere [11, 13, 25, 26] a summary of the most important details regarding GALT structure and functions is presented below. The lymphoid structures include Peyer's patches (PPs), the site of initiation of immune response, and cryopatches (CPs) that are aggregates of 1000 cells composed of lymphoid tissue inducer (LTi) cells, and chemokine producing dendritic cells (DCs), and the stromal cells found around the crypts of the small intestine [25]. The diffusely scattered lymphocytes in the epithelium and the lamina propria (LP) defend against pathogens and mount an immune response against them. The lymphoid cells also exist as isolated lymphoid follicles (ILFs) that

are responsible for the immune sensing of luminal bacteria and macrophages [27] and where antigen-primed T and B cells are activated before migrating to LP.

GALT is separated from the intestinal lumen by a monolayer of epithelial cells, which are interspersed with subpopulations of intraepithelial lymphocytes [28]. IELs are therefore the first immune cells that come in contact with microbiota and food allergens. They interact with the epithelial cells and together mount the immune response. In the intestinal crypts, responsible for epithelial cell renewal, are both typical enterocytes and Paneth cells that release antibacterial substances, such as lysosomes involved in the control of infections. Underneath is a loose connective tissue called LP where most of the immune response takes place and which is connected through the lymphatic circulation to the mesenteric lymph nodes [29, 30], where the immune processes are initiated in response to the presence of immune cells in the epithelial cell layer and the LP. Immature transitional T2 B cells are activated in GALT to the naïve B cells through the process that includes removal of autoreactive cells and leads to the generation of IgA-producing plasma cells [31] that play a crucial role in inhibiting bacterial penetration and maintenance of the symbiotic relationship between commensal microbiota and the host.

Different components of GALT are involved not only in the protection against pathogens, but most importantly can discriminate between commensal and pathogenic microorganisms, and respond with immune tolerance to friends and mount an immune response to foes [27]. The lumen of the human GI tract is colonized by a broad spectrum of symbiotic bacteria that play a crucial role in the proper development and functioning of the organism. GALT comes in contact with many pathogens and food-derived antigens through specific DCs and the

M-cells in the PPs that present the antigens to the T lymphocytes that in turn mount the cytokine-driven immune response [27]. The response of the GALT is a controlled physiologic inflammation that regulates the population of T helper (Th2 vs. Th1 response). In addition to the antigen-presenting M cells, under pathophysiological conditions, the major histocompatibility complex (MHC) cells serve as non-professional antigen-presenting cells that activate regulatory T-cells (Tregs). M cells pass their material to the antigen-presenting macrophages and DCs that are communicating with B cells for antibody production, and the intestinal IgA and are responsible for tolerance towards the ingested material [32].

GALT samples luminal bacteria and other antigens to evoke an immune response against them, leading to differentiated plasma cells producing IgA specific for bacteria. GALT does not possess afferent lymphatics; instead, it receives antigens directly from the mucosal surface across the intestinal epithelium overlying the GALT lymphoid follicles, called follicle-associated epithelium (FAE). M cells take up luminal microbiota- and food-derived antigens through the endocytosis and transport them intact to PPs, where they are processed by antigen-presenting cells (APCs) the innate immune cells such as DCs or macrophages. DCs receive intact antigens and perform antigen processing and presentation [33]. DCs also can extend their pseudopodia through the tight junctions to sample the antigens in the intestinal lumen [34]. APCs migrate to PPs or mesenteric lymph nodes and present processed antigen to naïve T cells, which after acquiring the gut-homing ability, migrate to the lamina propria and epithelium [29].

The adaptive immune system maintains health through a process of recognition of specific pathogens by cell-unique antigen receptors on B and T lymphocytes. It has been suggested that GALT could be determining the fate of immature B cells [31]. To maintain homeostasis, the mature GALT establishes the balance between Tregs and Th1/Th17 lymphocytes. This balance assures the production of IgA by B cells, migrating from PPs to gut epithelium after contact with presented by APC antigen [35].

Microbiota-GALT communication relies on a mechanism allowing to distinguish between symbiotic and pathogenic bacteria that takes place at the level of PPs. The M cells are optimized for antigen and microorganism uptake and handling, and viruses that are transported by adhering to M cells [36]. Pathogen-associated molecular patterns (PAMPs) present on commensal and pathogenic bacteria are recognized by the Pathogen Recognition Receptors (PRRs) present in the host cells. Some of them like the nucleotide oligomerization domain (NOD) are expressed mainly in follicle-associated cells such as DCs. Common NOD2 variants are expressed in Crohn's disease. Nod2v seems to play a pivotal role in GALT development and homeostasis in response to commensal bacteria. Nod2 can modulate the immune response toward bacteria by limiting the development of a Th1 immune response. In the absence of Nod2 PPs present a higher rate of Th1 and Th2 cytokines associated with increased paracellular permeability. Nod2 modulates the adaptive immune response of PPs and may promote immune tolerance and plays a role in inner immunity. PRRs are key players of the mucosal immune response toward gut antigens and bacteria [36]. Binding a ligand to PRR activates the intracellular signaling pathways, influencing the production of cytokines, and co-stimulatory molecules. Depending on the nature of the antigen, symbiotic or pathogenic, stimulation of PRRs results in the induction of tolerance or inflammation, respectively [37].

DCs play a vital role in the inflammatory response against pathogenic microorganisms. In the presence of inflammatory mediators, DCs produce the co-stimulatory molecules, which are essential for the T cells activation. During the inflammation, newly recruited DCs differentiate towards the pro-inflammatory phenotype, rather than the pro-tolerogenic [37]. However, it is important to realize that the communication between microbiota and the GALT is initiated at the structures most closely located to the lumen, the IELs.

3. The unique nature and functions of the IELs

IELs are unique in terms of their location, antigen specificity, and their innate and adaptive specialization, and development. The proximity of the IELs to the lumen, the capacity to tolerance and reactivity, and the unique set of the receptors make the IELs candidates for being the gate-keepers of the GIB and the key immune players in the 'leaky-gut syndrome". Below is a summary of the most important concepts deemed necessary for the present discussion.

3.1 Location

Residing in the gastrointestinal epithelium, most proximally to the lumen, IELs provide the first line of defense, and, at the same time, they protect the integrity of the mucosal membrane. The gastrointestinal epithelium is made up of a single layer of cells, which prevents the passage of foreign antigens, microorganisms, and toxins while allowing the entry of nutrients, electrolytes, and water to the circulation. IELs reside between intestinal epithelial cells (IEC) and participate in the formation of the GIB. They are an integral part of the epithelial barrier and have bidirectional interaction with IEC and other immune cells [21].

3.2 Classification

In humans, IELs are a heterogeneous group of antigen-experienced innate and adaptive intestinal T lymphocytes of thymic origin classified into subsets based on activation mechanisms and on antigens recognition belonging to both the TCRγδ and TCRαβ+ lineages [38]. They do not require priming like the other T-cells, and in contact with antigens immediately release cytokines that attack and kill infected target cells. Among the TCRαβ IELs are the conventional CD8αβ heterodimer-bearing cytotoxic T cells and unconventional homodimer-expressing CD8αα T cells; TCRαβ IELs are further divided based on the nature of CD8. A significant number of CD8αβ T cells migrate with age and increase the number of IELs. [39]; while the number of γδ IELs increases under allergic or inflammatory conditions. The composition of the small intestine consists of 75% CD8αβ IELs, 10% CD4αβ IELs, and 15%γδ IELs [21].

3.3 Functions

Distinct IEL subsets serve a range of functions: regulate intestinal homeostasis, maintain epithelial barrier function, rapidly respond to infection, and mount as well as regulate adaptive and immune responses [39]. They exhibit both the microbicidal and cytotoxic activity and produce both cytokines and chemokines [21]. The TCRγδ+ IELs cells express CD44 and CD69 and do not recirculate. These IELs can mount cytotoxic activity and express cytokines such as interferon IFNγ, interleukins IL-2, IL-4, IL-17, as well as the innate natural killer (NK) receptors [40].

Natural or innate-like IELs originate in the thymus and seed the intestine as a precursor population. Induced or adaptive IELs home to the intestinal barrier upon meeting cognate antigen in the intestine; they accumulate with age and replace the natural IELs. IELs have characteristics of naive, effector and memory cells

involved in the bidirectional cross-talk with IEC [21]. IEL homeostasis is regulated by commensal microbiota and the increase in IELs is induced by microbiota dysbiosis [41]. The γδ-IELs communicate with IECs that sense the pathogens and adjust their inter-epithelial movement dynamics and energy utilization in response to infection [42].

IELs provide immediate immune protection preventing the initial entry and spread of pathogens. However, natural IELs express self-reactive TCRs which under uncontrolled inflammatory conditions may trigger autoreactive cytotoxicity jeopardizing the integrity of mucosal barrier. Excessive activation of the cytotoxic activity of IELs results in chronic inflammation disorders such as IBD and celiac disease, while a lack of IELs leads to impaired protection against bacterial infection. Natural IELs express self-reactive TCRs which under uncontrolled inflammatory conditions may trigger autoreactive cytotoxicity and jeopardize the integrity of the mucosal barrier. The coordinated EC-IEL response to the luminal microorganisms regulates γδ-IELs metabolism and movement that assures the integrity of the barrier [42].

Thus, various IEL subpopulations of thymic (natural) and peripheral (induced) differentiated IEL subpopulations are both beneficial in preserving and repair of epithelial barrier, but can also contribute to immune pathology and inflammatory diseases [43]. Elevated IEL level is associated with inflammation of the mucosa [44]. IELs mediated immune recruitment, and autoimmune response can destroy intestinal epithelial cells period. They can also modulate oxidative stress, which is involved in the breakdown of the gut-brain barrier [39], and thus contribute to the ASD pathology [45].

3.4 IEL development

While human GALT development occurs both prenatally and postnatally, IELs develop during gestation and populate gut before birth in preparation for microorganism colonization [46]. All IEL subsets are the progeny of bone marrow precursor cells that initially develop in the thymus. All natural IELs go through self-antigen-based thymic maturation and migrate directly to the intestinal epithelium. They do not depend on exogenous antigen-driven differentiation, and so they are the first type of antigen-experienced T cells to populate gut before birth. They provide a tolerant response to dietary antigens and colonizing microbiota. Induced or adaptive IELs are the progeny of conventional T cells that are selected in the thymus and as mature thymocytes home to the intestinal barrier upon cognate antigen in the intestine. Induced IELs are sparse early in life and gradually accumulate in response to exogenous antigens and replace the natural IELs. After birth, the number of intestinal IELs increases reaching adult levels by two years of age [21]. IELs number varies with age, circadian cycle, and gut localization, with IEL numbers, decreasing from duodenum to ileum, with few found in the colon [21]. IELs usually do not exceed 5-10 IELs per 100 epithelial cells in healthy individuals, with the pathological cut-off of 20-25 IELs per 100 epithelial cells. An increase in duodenal IELs has been observed in immunological disorders such as celiac disease (CD), lymphocytic colitis, infections, H pylori gastritis, but also following the use of non-steroidal-antiinflammatory drugs [47].

3.5 IEL-aryl hydrocarbon receptors

It has been proposed that subpopulations of human IELs (TCRαβ+CD8αβ+) have the dual capacity to recognize modified self via natural killer (NK) receptors (autoreactivity) as well as foreign antigen via the T cell innate and adaptive receptor (TCR). Furthermore, it has been suggested that local environmental factors dictate

the specificity of IEL responses [48]. What could be the nature of these local intestinal factors? It turns out that changes in microbiota and diet-derived antigens modulate IELs through the host-specific aryl hydrocarbon receptors (AhRs) that have been implicated in the regulation of adaptive immune activity [40]. Interestingly AhR activity can be regulated by diet, and the ligands for AHR are present in cruciferous vegetables [46]. It turns out that IELs express high levels of AHR and the ligand-dependent activation of AhRs affects the maintenance, homing or proliferation of IELs. The deficiency or lack of AhR ligands results in increased immune activation dominated by type 1 response characterized by the production of IFN-γ and more susceptible to increased vulnerability to epithelial damage [40, 46].

4. Microbiota-GALT communication in health and disease

The crosstalk between gut microbiota and GALT is essential for the proper development and functioning of the organism. In health, microbiota-GALT communication involves selective recognition and reaction to the commensal vs. pathogenic microorganisms and antigens, and assures gut symbiosis. Immune dysfunctions associated with abnormalities of the GALT lead to either reduced ability to deal with infections or overactivity producing excess of the proinflammatory factors TNFα, such as found in IBD [49] and autoimmune diseases. GALT is critically important in health maintenance by providing a selective barrier between the host and microbiota in the intestine lumen. Several factors such as increased intestinal permeability, genetic polymorphism in genes involved in barrier factions and environmental factors may interfere with these processes [50]. Several identified diseases such as necrotizing enterocolitis (NEC), IBD, autoimmune diseases, asthma, ASD and many others [11], share in common dysbiosis,

gastrointestinal problems, gastrointestinal inflammation, and dysfunctional GIB referred to as a "leaky gut syndrome." While the mechanisms involved in these pathologies are not clear, it can be assumed that GALT plays an important role in the failure to recognize and respond to the antigens.

4.1 Inflammatory Bowel Disease

IBD describes a group of immune digestive disorders involving chronic inflammation of the digestive tract represented by Crohn's disease, and ulcerative colitis. Dysfunctional GALT has been suggested to contribute to the IBD based on the observations of abnormal interactions between DC and T lymphocytes involving a defect in antigen recognition. Normal interactions between DC and T cells in the gut produce a subset of T lymphocytes that have an immunosuppressive function. In IBD, inappropriate antigen uptake and presentation to naïve cells in MLN may lead to T cell tolerance in the GALT allowing pathogen infiltration and resulting in dysbiosis [51]. The pathogenesis of IBD has also been linked with abnormal expression of inflammatory factors [52]. Several defects in bacterial recognition and processing have been documented in Crohn's disease patients carrying gene polymorphism of PRRs such as NOD2/CARD15. It has been suggested that the lack of feedback between mutated PRRs and microbiota could lead to the breakdown of tolerance [53]. The impairment of the intestinal barrier function is one of the critical events in the pathogenesis of IBD [53]. It has been proposed that IBD is caused by an excessive immune/inflammatory processes in the gut wall; however, the debate exists whether the state of inflammation causes impaired barrier function or barrier damage is an independent event [54].

One of the hypotheses implicates the dysregulation of immune response triggered by microbial antigens that eventually become autonomous in the barrier damage.

The resulting leukocytic infiltrate within the mucosa releases enzymes, reactive oxygen species, and cytokines initiating and perpetuating tissue damage and immune deregulation, loss of immune tolerance, and appearance of tertiary lymphoid tissue [TLT, 23]. This scenario takes place in Crohn's disease characterized by chronic and relapsing inflammation of the digestive tract. Abnormalities in PPs, DCs-T lymphocyte interactions and also IELs have been reported [36]. The number of PPs increases from birth to 15-25 years and then declines, and Crohn's pathology follows the same curve. It is associated with an abnormal T-cell mediated immune response towards gut flora and results in lower bacterial diversity [36]. Animal models of Crohn's disease indicate that gut microbiota is essential for inducing the bowel inflammation as germ-free animals do not develop colitis. However, rather than a single responsible microorganism, it turns out that the mechanism may involve quorum-dependent bacterial proliferation of bacteria and their quorum sensing molecules (QSMs). In turn, QSMs may affect GALT and deregulate the normally present immune tolerance [51].

4.2 Autism Spectrum Disorders

ASD is primarily diagnosed based on the behavioral symptoms that include problems with social interactions, language communication, and repetitive behavior. However, the imbalance of immune responses, increase in proinflammatory cytokines, changes in proinflammatory interleukins, and increased autoimmunity support the notion of significant involvement of immune dysfunctions in ASD. Children with autism display dysregulation in both innate and adaptive immune response that includes altered ratios of Th1/Th2 lymphocytes, with a lower proportion of Th1 cells and a higher proportion of TH2, compared to healthy controls [55]. Decreased level of naïve T cells, an elevated number of circulating monocytes, macrophages, reduced natural killer cell activity, and increased levels of proinflammatory interleukins, decreased cytokine TGFβ1, and lower subpopulation of CD4 and CD8 lymphocytes, have also been observed in autism [32, 55]. A recent report showed that the increased blood concentration of TNFα in ASD children was positively correlated with the severity of symptoms [56]. Increased TNFα can alter the permeability of the intestinal epithelial barrier and contribute to gastrointestinal problems in ASD [57].

The imbalance of immune response and a propensity to impaired gut barrier function are all observed in autism and are associated with differences in microbiome composition [58, 59]. Autism is often comorbid with gastrointestinal disorders, and increased intestinal permeability has been shown in children with autism spectrum disorder [60]. A significant increase in proinflammatory cytokines CD3+CD4+ and analysis of the serum IgG is consistent with the epithelial immunopathology [61]. Intestinal mucosa of children with autism has a higher level of TNF-α, T cells and a lower level of IL10+ T cells further indicating a proinflammatory profile and corroborating increased levels of proinflammatory cytokines in plasma and brain in ASD [62].

The study that examined the 'leaky gut syndrome" in ASD, using gastrointestinal postmortem tissue and gene expression, supported the increased intestinal permeability, reduced GIB-forming components and increased proinflammatory cytokines in the majority of ASD patients [5, 6]. These findings support the hypothesis that inflammation contributes to autism and can affect barrier permeability. Intestinal biopsies in children with regressive autism identified lymphocytic enterocolitis with autoimmune features distinct from other forms of IBD, in which the epithelium appears particularly affected, and CD8+T cell density and IEL

numbers were higher than in Crohn's disease [63]. Immunological imbalance associated with the increased level of proinflammatory cytokines and consistent with autoimmunity was also observed in postmortem brains derived from ASD patients [23]. Thus, the imbalance of immune responses, increased in the proinflammatory cytokines, changes in proinflammatory interleukins, and increased autoimmunity support the notion of significant involvement of immune dysfunctions in ASD.

5. IELs-associated changes in IBD and ASD

Considering the uniquely important role the IELs may play in microbiota-GALT communication, their involvement in IBD and ASD has not been adequately explored. Netherthless, the existing evidence supports the changes in the overall IEL number and the phenotypic profile in the gastrointestinal immune and neurodevelopmental pathologies.

5.1 IELs abnormalities in IBD

Both quantitative and functional abnormalities in IELs have been reported in IBD [36]. Increased IELs levels have been observed in lymphocytic colitis and Crohn's disease, the pathologies that share the "leaky gut syndrome." The increase of IELs is one of the earliest and the most sensitive marker of Crohn's disease with 30 IELs per 100 enterocytes indicating pathological lymphocytosis in the duodenum [41]. Also, a strong negative correlation between peripheral lymphocyte counts and IEL number has been observed with increased IL-1β in UC and IL17A and IFNγ in patients with Crohn's disease. A direct correlation between TCRγδ+ cells in the intestinal mucosa and the severity of IBD has also been observed [1]. Furthermore, Crohn's disease and ulcerative colitis have demonstrated unique patterns of IEL phenotype, cytokine expression, and cytokine-microbiome interactions [1]. The functional changes in IELs in IBD

may be due to the microbiome affecting cytokine secretion. IELs may, in turn, contribute to disease-specific pathology, underlying a complex relationship between IEL-produced cytokines, microbial diversity, and dysbiosis. Other studies have shown induction of colitis by IEL secretion of IL-17 [1].

5.2 IEL abnormalities in ASD

Intestinal biopsies in children with regressive autism identified lymphocytic enterocolitis with autoimmune features distinct from other forms of IBD, in which the epithelium appears particularly affected, and CD8+ T cell density and IEL numbers were higher than in Crohn's disease [63]. Interestingly, the results of studies in animal models of autism showed a reduced thymus size, suggesting T and B cell dysfunctions in ASD [23]. The overall production of T cells including IELs may be affected in ASD due to lower thymus activity. Gastrointestinal problems are frequently observed in autistic children [64] and are associated with lymphocytic colitis. Immunohistochemical studies of illium performed in ASD children suggest lymphocytic colitis with significantly increased number γδIELs and CD8 IELs [63].

5.3 IEL abnormalities and the 'leaky-gut syndrome'

The "leaky gut syndrome" associated with gastrointestinal immune and neurodevelopmental disorders, thus both IBD and ASD, has been defined as an increase in the GIB permeability and as the ability of digestion process to enter the blood [27]. The expanded concept of "leaky gut syndrome" suggests that under specific conditions not only the pathogenic microorganisms but also small molecules, bacterial metabolic components or toxins can translocate and diffuse systemically and affect distinct organs including the brain [44]. Recent data suggest that the "leaky gut syndrome" may be due to the interplay between the intestinal epithelial barrier and the GALT [62].

Increasingly, the "leaky gut syndrome" is being associated with autoimmune and inflammatory diseases and abnormal interaction between innate and adaptive immunity [65]. Studies of Crohn's disease have indicated that the "leaky gut syndrome" appears to be secondary to the abnormal immune reaction induced by gluten [66]. It has been suggested that the common element in the "leaky gut syndrome" associated with autoimmune and inflammatory diseases may be an increase in pro-inflammatory cytokines and activation of the innate immune system in response to the translocation of luminal components into the host [67]. The healthy immune system can compensate for a small decrease in "gut leakiness" and prevent inflammation. The hypothesis presented here posits that IELs play a key role in the "leaky gut" syndrome. It is based on the observation that IELs do not require priming like the other T-cells, and in contact with antigens immediately release cytokines that attack and kill infected target cells. It is supported by the discovery of subpopulations of human IELs (TCRαβ+CD8αβ+) whose specificity may be dictated by local environmental factors [48] and the presence of the host-specific AhRs [40]. Thus the microbiota communication with the host would be determined both by the specificity of the AhRs and their ligands.

6. The emerging role of IEL associated -AhR in IBD and ASD

AhR is a ligand-dependent transcription factor activated by a wide range of endogenous and exogenous ligands [68]. Initially known as the mediator of the toxic effect of dioxin, AhR has been shown to bind to dietary compounds, commensal flora, and environmental toxins containing aromatic hydrocarbons. AhR ligands include tryptophan compounds derived both from diet and produced by our organisms. Following the binding with a ligand AhR is translocated from cytoplasm to the nucleus with the help of the aryl hydrocarbon nuclear

translocator (ARNT) where it regulates the expression of genes including those involved in immune regulation. AhR is also involved in the transcriptional process associated with epigenetic regulation and direct regulation of protein kinase and phosphatases, and interaction with estrogen receptors [69]. AhR is increasingly recognized as a regulator of immunity in health and disease [70]. AhRs are expressed on the γδ IELs. They are presumed to mediate the regulation of inflammation and protection of the small intestine by increasing the number of γδ IELs by decreasing their apoptosis [71]. Mice lacking the AhRs are more susceptible to intestinal challenges [72] and show high susceptibility to some infections [73].

Importantly, AhRs ligands present in the diet or produced by gut flora have been implicated in the development of IELs and internal homeostasis [73]. AhR is a crucial regulator of IEL number and AhR deficiency or lack of the ligands results in increased immune activation and epithelial damage. In the absence of AhR, IELs are not impaired during their development, homing and proliferation, but they are no longer maintained in their epithelial site [48]. Mice genetically engineered to lack γδ lymphocytes show increased morbidity and increased susceptibility to colitis [74]. While the studies involving AhR-deficient mice have shown lower survival of IELs, but also the studies of mice fed the AhR-ligand-deficient diet resulted in the disappearance of intestinal IELs that could be restored upon addition of the specific dietary component found in cruciferous vegetables [48]. In that context, the examination of a variety of naturally occurring vegetable-derived dietary compounds, like indole-3-carbinol processed in the stomach, that can directly activate or inhibit AhR signaling pathways [75] seems especially promising.

6.1 IEL-associated AhR in IBD

AhR signaling is involved in the regulation of both pro- and anti-inflammatory processes, as suggested by decreased accumulation of lymphocytes in the spleen and lymph nodes of the AhR knockout mice [74]. AhR abnormalities have been linked to the immune-inflammatory diseases such as Crohn's disease, rheumatoid arthritis, and multiple sclerosis and Celiac disease [76].

The pathogenesis of IBD is associated with elevated inflammatory response and has been linked to abnormalities in AhR and upregulation of IL-22 in an animal model of colitis. It has been suggested that a deficiency in AhR may result in a decrease in resistance to inflammation [52]. Importantly, the deficit in AhR signaling has been detected in IBD and AhR expression is down-regulated in Crohn's disease [77]. Furthermore, AhR activation reduces the production of inflammatory cytokines, suggesting that AhR inhibits the transcription of inflammatory genes.

It has been reported that AhR reduces the release of cytotoxic products in IEL from Crohn's disease, suggesting a regulatory effect of AhR on cytotoxic cell proliferation and functions in the gut [76]. Furthermore, low intake of fruits and vegetables in children correlates with Crohn's disease risk [78]. Thus, of interest is the observation that AhR activation by 1,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) agonist has been shown to induce IELs which display regulatory functions associated with the induction of tolerance to dietary antigens [79].

6.2 The emerging role of IEL associated-AhRs and ARNT polymorphism in ASD

Among the AhR environmental ligands are the endocrine disruptors like polychlorinated biphenyls (PCB) implicated in autistic pathology [80]. Animal studies have linked AhR to the regulation of the developmental toxicity to PCB [81] based on the observation that mice deficient in AhR show increased sensitivity to the developmental exposure to these environmental toxicants. Furthermore, allelic differences in AhR also affect the susceptibility to developmental PCB neurotoxicity and lead to greater impairments in learning and memory and motor function [81].

The polymorphism in AhR-related genes appears to affect the interaction with the environmentally derived ligand and predispose individuals to increase sensitivity to several toxins related to autism. ASD has been reported to be linked to AhR-related gene polymorphism [23]. While no statistically significant difference was found between ASD and control individuals and AhR polymorphism, a significant difference in the symptom severity was observed in rs2228099 polymorphism, and a significant difference in the social communication score of severity was observed in ARNT codon 189 polymorphism, with the individuals with the ARNT GG genotype being more impaired on social communication scores than those with GC genotype [23]. Furthermore, analysis of the ARNT2 gene showed a statistically significant association between Asperger syndrome (AS) and SNP rs17225178 that modifies transcription factor binding sites in the neural cells [82]. These results suggest an important involvement of AhRs in neurodevelopmental disorders. Considering environmental toxins on one hand and health-promoting dietary compounds on the other, the link between AhR-and related genes and ASD has not been sufficiently explored and deserves serious consideration.

7. Summary

This review provides evidence for the essential role of microbiota-GALT communication in both inflammatory

and neurodevelopmental disorders while providing examples of the uniquely specific role of IELs, and IEL-associated AhRs in IBD and ASD. Microbiota-GALT communication is critical during development determining the tolerance and supporting immune function. It also plays an essential role in maintaining a healthy GIB between the external environment and the human organism. GALT deregulation and the resulting dysbiosis may contribute not only to the inflammatory gastrointestinal diseases such as IBD, but also neurodevelopmental disorders, such as ASD that are associated with damaged GIB and the "leaky gut syndrome." IELs emerge as GALT-component that plays a crucial role in health and disease. IELs are in the gut before birth, are in the direct proximity to the GIB, and interact with microbiota and food-derived antigen through the set of AhRs. Increased levels and altered function of IELs can be observed in both IBD and ASD and are associated with changes in the GIB integrity; they may thus be key immune cells of the GALT involved in the 'leaky-gut syndrome."

IELs express high levels of AhR; the deficiency of AhRs or lack of AhR ligands results in a decrease in IEL number and increased vulnerability to epithelial damage. The abnormalities in AhRs both in the quantity and genetic identity are reported in both IBD and ASD. Since AhRs determine host's sensitivity to both the environmental toxins and beneficial ligands and they may determine the initial tone of communication with microbiota. Importantly, AhRs activity can be regulated by dietary ligands in cruciferous vegetables and fruits. Thus, further studies on IEL-AhRs interactions, targeting future immune therapies for gastrointestinal and neuroimmune disorders is warranted.

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Statement of Conflict of Interest

The author has no conflict of interest.

References

1. Wang B, Yao M, Lv L, et al. The human microbiota in health and disease. Engineering 3 (2017): 71-82.

2. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. PNAS 106 (2009): 3698-3703.

3. Blomberg JS, Fricke WF, Brinkman CC, et al. Microbiota - implications for immunity and transplantation. Nat Rev Nephrol 11 (2005): 342-353.

4. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. Gastroenterology 141 (2011): 599-609.

5. Fiorentino M, Sapone A, Senger S, et al. Blood-brain barrier and the intestinal epithelial barrier alterations in autism spectrum disorders. Mol Autism 7 (2016): 49.

6. Quigley EM. Leaky gut-concept or clinical entity? Curr Opin Gastroenterol 32 (2016): 74-79.

7. Sharon G, Cruz NJ, Kang DW. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. Cell 177 (2019): 1600-1618.

8. Rodríguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Heal Dis 26 (2015): 26050.

9. Bibbo S, Ianiro G, Giorgio V, et al. The role of diet on gut microbiota composition. Eur Rev Med Pharmacol Sci 20 (2016): 4742-4749.

10. Anderson RC, Dalziel JE, Gopal PK. The Role of Intestinal Barrier Function in Early Life in the Development of Colitis. Intechopen (2012): 3-31.

11. Koboziev I, Karlsson F, Grisham MB. Gut-associated lymphoid tissue, T cell trafficking, and chronic intestinal inflammation. Ann N Y Acad Sci 1207 (2010): 86-93.

12. Li Q, Han Y, Dy ABC, et al. The gut microbiota and autism spectrum disorders. Front Cell Neuroscience 11 (2017): 120.

13. Bergstrom KS, Sham HP, Zarepour MV, et al. Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. Cellular Microbiology 14 (2012): 475-484.

14. Derrien M, van Hylckama Vlieg JET. Fate, activity, and impact of ingested bacteria within human gut microbiota. Trends in Microbiology 23 (2015): 354-366.

15. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol 33 (2015): 496-503.

16. Sajdel-Sulkowska EM and Zabielski R. Gut microbiome and brain-gut axis in autism-Aberrant development of gut-brain communication and reward circuitry. In INTECH. Recent Advances in Autism Spectrum Disorders V1: Chapter 4 (2013): 61-79.

17. Makowska M, Kasarello K, Bialy M, et al. Autism: "Leaky gut". Prematurity and lactoferrin. Austin J Autism and Related Disabil 2 (2016): 1021.

18. Mu Q, Kirby J, Reilly CM, et al. Leaky gut as a danger signal for autoimmune diseases. Front Immunol 8 (2017): 598.

19. Rapozo DCM, Bernadazzi C, de Souza HSP. Diet and microbiota in inflammatory bowel disease: the gut in disharmony. World J Gastroenterol 23 (2017): 2124-2140.

20. Fowlie G, Cohen N, Ming X. The perturbance of microbiome and gut-brain axis in autism spectrum disorders. Int J Mol Sci 19 (2018): 2251.

21. Konjar S, Ferreira C, Blankenhaus B, et al. Intestinal barrier interactions with specialized CD8T cells. Front Immunol 8 (2017): 1281.

22. Gladysz D, Krzywidzinska A, Hozyasz KK. Immune abnormalities in autism spectrum disorder - could they hold promise for causative treatment? Mol Neurobiol 55 (2018): 6387-6435.

23. Fujisawa TX, Nishitani S, Iwanga R, et al. Association of aryl hydrocarbon receptor-related gene variants with the severity of autism spectrum disorders. Front Psychiatry 7 (2016): 184.

24. Kiss EA and Diefenbach A. Role of the aryl hydrocarbon receptor in controlling maintenance and functional programs of RORγt+ innate lymphoid cells and intraepithelial lymphocytes. Front Immunol 3 (2012): 124.

25. McNamee EN and Rivera-Nieves J. Ectopic tertiary lymphoid tissue In inflammatory bowel disease: protective or provocateur? Front Immunol 7 (2016): 308.

26. Mao K, Baptista AP, Tamoutounour S, et al. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. Nature 554 (2018): 255-259.

27. Viggiano D, Ianiro G, Vanella G, et al. Gut barrier In health and disease: focus on childhood. Eur Rev Med. Pharmacol Sci 19 (2015): 1077-1085.

28. Olivares-Villagomez D and Van Kaer L. Intestinal Intraepithelial lymphocytes: sentinels of the mucosal barrier. Local Immune Responses 39 (2017): 264-275.

29. Reis BS, Mucida D. The role of the intestinal context in the generation of tolerance and inflammation. Clin Dev Immunol (2012): 157948.

30. Bain CC, Mowat AM. Macrophages in intestinal homeostasis and inflammation. Immunol Rev 260 (2014): 102-117.

31. Vossenkamper A, Blair PA, Safinia N, et al. A role for Gut-associated lymphoid tissue in shaping the human B cell repertoire. J Exp Med 210 (2013): 1665-1674.

32. Samsam M, Ahangari R, Naser SA. Pathophysiology of autism spectrum disorders; revisiting gastrointestinal involvement and immune imbalance. World J Gastroenterol 20 (2014): 9942-9951.

33. Ohno H. Crosstalk between the intestinal immune system and gut commensal microbiota, intestinal M cells. J Biochem 159 (2016): 151-160.

34. Farache J, Koren I, Milo I, et al. Luminal Bacteria Recruit CD103+ Dendritic Cells into the Intestinal Epithelium to Sample Bacterial Antigens for Presentation. Immunity 38 (2013): 581-595.

35. Yang H, Duan Z. The local defender and functional mediator: gut microbiome. Digestion 97 (2018): 137-145.

36. Jung C, Hugot J-P, Barreau F. Peyer's Patches: the immune sensors of the intestine. International J Inflammation (2010): 823710.

37. Rescigno M. Dendritic cells in oral tolerance in the gut. Cell Microbiol 13 (2011): 1312-1318.

38. Mayassi T and Jabri B. Human intraepithelial lymphocytes. Mucosal Immunol 11 (2018): 1281-1289.

39. Sheridan BS and Lefrancois L. Intraepithelial lymphocytes: to serve and protect. Curr Gastroenterol Rep 12 (2010): 513-521.

40. Cheroute H, Lambolez F, Mucida D. The light and the dark sides of intestinal intraepithelial lymphocytes. Nat Rev Immunol 11 (2011): 445-456.

41. Cukrowska B, Sowinska A, Bierla JB, et al. Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota - Key players in the pathogenesis of celiac disease.. World J Gastroenterol 23 (2017): 7505-7518.

42. Hoytema van Konijnenburg DP, Reis BS, Pedicord VA, et al. Intestinal epithelial and intraepithelial T cell crosstalk mediates dynamic response to infection. Cell 171 (2017): 783-794.

43. Sajdel-Sulkowska EM, Xu M, Koibuchi N. Increase in cerebellar neurotrophin-3 and [oxidative stress markers in autism. Cerebellum 8 (2009): 366-372.

44. Knox WF. Restricted feeding and human intestinal plasma cell development. Arch Dis Child 61 (1986): 744-749.

45. Van Kaer L and Olivares-Villagomez D.Development, homeostasis, and functions of intestinal intraepithelial lymphocytes. J Immunol 200 (2018): 2235-2244.

46. Obrenovich MEM. Leaky gut, leaky brain? Microorganisms 6 (2018): 107.

47. Sergi C, Shen F, Bouma G. Intraepithelial lymphocytes, scores, mimickers and challenges in diagnosing gluten-sensitive enteropathy

(celiac disease). Worl J Gastroenterol 23 (2017): 573-589.

48. Li Y, Innocentin S, Withers DR, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 147 (2011): 629-640.

49. Nochi T, Denton PW, Wahl A, et al. Cryptopatches are Essentials for the development of human GALT. Cell Rep 3 (2013): 1874-1884.

50. Michielan A and D'Inca R. Intestinal permeability In inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of Leaky Gut. Mediators of Inflammation (2015): 628157.

51. Konig J, Wells J, Cani PD, et al. Human Intestinal barrier function in health and disease. Clinical and Translational Gastroenterology 7 (2016): 196.

52. De Magistris L, Familiari V, Pascotto A, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 51 (2010): 418-424.

53. Patterson ER, Shmidt MD, Oxentenko AS, et al. Normal villous architecture with increased intraepithelial lymphocytes. Am J Clin Pathol 143 (2015): 445-450.

54. Regner EH, Ohri N, Stahly A, et al. Functional intraepithelial lymphocyte changes in inflammatory bowel disease and spondyloarthritis have disease-specific correlation with intestinal microbiota. Arthritis Research and Therapy 20 (2018): 149.

55. Masi A, Glozier N, Dale R, et al. The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci Bull 33 (2017): 194-204.

56. Xie J, Huang L, Li X, et al. Immunological cytokine profiling identifies TNF-α as a key molecule dysregulated in autistic children. Oncotarget 8 (2017): 82390-82398.

57. Gorrindo P, Williams KC, Lee EB, et al. Gastrointestinal dysfunction in autism: parental report, clinical evaluation and associated factors. Autism Res 5 (2012): 101-108.

58. Neu J. Perinatal and neonatal manipulation of the intestinal microbiome: A note of caution. Nutr Rev 65 (2007): 282-285.

59. Sajdel-Sulkowska EM, Bialy M, Cudnoch-Jedrzejewska A. Altered BDNF levels, "leaky gut" and abnormal gut microbiome in autism. In Brain-derived neurotrophic factor BDNF: therapeutic approaches, role in neuronal development and effects on cognitive health. Nova Science Publishers (2015): 147-180.

60. Rose DR, Yang H, Serena G, et al. Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. Brain Behav Immun 70 (2018): 354-368.

61. Ashwood P, Murch SH, Anthony A, et al. Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology. J. Clin Immunol 23 (2003): 504-517.

62. Fasano A. Leaky Gut and Autoimmune Diseases. Clin Rev Allergy Immunology 42 (2012): 71-78.

63. Furlano RI, Anthony A, Day R, et al. Colonic CD8 and γδ T-cell infiltration with epithelial damage to children with autism. J Pediatr 138 (2001): 366.

64. Hsiao EY. Gastrointestinal issues in autism spectrum disorder. Harv Rev Psychiatry 22 (2014): 104-111.

65. Li B, Selmi C, Tang R, et al. The microbiome and autoimmunity: a paradigm from the gut-liver axis. Cell Molec Immunol 15 (2018): 1-15.

66. Heyman M, Abed J, Lebreton C, et al. Intestinal Permeability in Coeliac Disease: Insight into Mechanisms and Relevance to Pathogenesis. Gut 61 (2012): 1355-1364.

67. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterol 14 (2014): 189.

68. Wheeler MA, Rothhammer V, Quintana FJ. Control of immune-mediated pathology via the aryl hydrocarbon receptor. J Biol Chem 293 (2017): 12383-12389.

69. Li Y, Wang K, Zou Q-Y et al. A possible role of aryl hydrocarbon receptor in spontaneous preterm birth. Med Hypotheses 84 (2015): 494-497.

70. Gutierrez-Vazquez C and Quintana FJ. Regulation of the immune response by the aryl hydrocarbon receptor. Immunity 48 (2018): 19-33.

71. Zhang Z, Pu A, Yu M, et al. Aryl hydrocarbon receptor activation modulates $\gamma\delta$intestinal intraepithelial lymphocytes and protects against ischemia/reperfusion injury in the murine small intestine. Mol Med Reports 19 (2019): 3.

72. Sutter CH, Bodreddigari S, Campion C, et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin Increases the Expression of Genes in the Human Epidermal Differentiation Complex and Accelerates Epidermal Barrier Formation. Toxicological Sciences 124 (2011): 128-137.

73. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front Cell Infect Microbiol 8 (2018): 13.

74. Fernandez-Salguero PM, Ward JM, Sundberg JP, et al. Lesions of aryl-hydrocarbon receptor-deficient mice. Vet Pathol 34 (1997): 605-614.

75. Nagy SR and Denison M. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Ann Rev Pharmacol 43 (2003): 309-334.

76. Dinallo V, Marafini I, Di Fusco D, et al. Protective effects of Aryl hydrocarbon receptor signaling in Celiac disease mucosa and in poly I:C-induced small intestinal atrophy mouse model. Front Immunol 10 (2019): 91.

77. Monteleone I, Rizzo AA, Sarra M, et al. Aryl Hydrocarbon Receptor-Induced Signals Up-regulate IL-22 Production and Inhibit Inflammation in the Gastrointestinal Tract. Gastroenterology 141 (2011): 237-248.

78. D'Souza S, Levy E, Mack D, et al. Dietary patterns and risk for Crohn's disease in children. Inflammatory Bowel Diseases 14 (2008): 367-373.

79. Cervantes-Barragan L, Jiani N Chai JN, TianeroMa D, et al. Lactobacillus reuteri induces gut intraepithelial CD4+CD8$\alpha\alpha$+ T cells. Science 357 (2017): 806-810.

80. Sajdel-Sulkowska EM. Environmentally induced oxidative stress and disruption of brain thyroid hormone homeostasis in autism spectrum disorders. IntechOpen (2011).

81. Klinefelter K, Kromme Hooven M, Bates C, et al. Genetic differences in the aryl hydrocarbon receptor and CYP1A2 affect susceptibility to developmental polychlorinated biphenyl exposure in mice: Relevance to studies of human neurological disorders. Mammalian Genome 29 (2017): 112-127.

82. Di Napoli A, Warrier V, Baron-Cohen S, et al. Genetic variant rs17225178 in the ARNT2 gene is associated with Asperger Syndrome. Molecular Autism 6 (2015): 9.