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Review Article

# Developmental significance of early gut-associated lymphoid tissue (GALT)-microbiota interactions in health and disease: Creating balance between tolerance and inflammation in children

## Abstract

The establishment of gut microbiota in humans does not occur randomly but develops after birth through highly organized interactions between microorganisms, the immune processes orchestrated by the Gut-Associated Lymphoid Tissue (GALT), and a selective absorption to the blood regulated by the Gastrointestinal-Blood Barrier (GIB). In term infants, the initial colonization of gut microorganisms depends on the maturation of the GALT and critical closure of GIB and these interactions lead to the establishment of symbiotic conditions defined as a balance between immunity and infections. In preterm infants, development of the GALT is less complete at birth, and the GIB closure is delayed, both of which impact gut microbiota colonization resulting in dysbiosis. Early developmental dysbiosis may underlie non-infectious diseases such as allergies, autoimmune diseases, or inflammatory bowel diseases and contribute to the pathology of neurodevelopmental disorders. This review focuses on the developmental significance of GALT - microbiota interaction while comparing preterm and term infants. It concludes with the premiss that the developmental dysbiosis may have both short- and long-term impact on human health.

## Introduction

The establishment of gut microbiota in humans commences at birth and involves highly organized interactions between microorganisms, the immune processes orchestrated by the Gut-Associated Lymphoid Tissue (GALT) and a selective absorption to the blood regulated by the Gastrointestinal Barrier (GIB). In term infants, the initial colonization of gut microorganisms depends on the maturation of the GALT and critical closure of GIB [1], and these interactions lead to the establishment of symbiotic conditions defined as a balance between immunity and infections. GALT is anatomically developed at birth in term infants but matures functionally through the interactions with microbiota [2]. The timing of the first gut closure occurs during the first 48 hours after birth decreasing GIB permeability [3]. In preterm infants, the anatomy of the GALT is less complete at birth, and the GIB closure is delayed [4], impacting gut microbiota colonization leading to dysbiosis.

This review summarizes the latest findings on GALTs functions, GALT-microbiota communication, and focuses on the developmental significance of GALT-microbiota interaction while comparing preterm and term infants. It concludes with a suggestion that the developmental dysbiosis may be a risk factor for such diseases as Necrotizing Colitis (NEC), Inflammatory Bowel Disease (IBD), autoimmune diseases, and neurodevelopmental disorders such as Autism Spectrum Disorders (ASD) and many others [5–7].

### Gut-Associated Lymphoid Tissue (GALT): structure and functions

Intestinal lumen is part of the external environment that contains potentially harmful pathogens, such as bacteria, viruses or fungi. Gut epithelial cells act as a physical barrier between the intestinal lumen and the inner milieu preventing pathogens from entering the blood [8]. Tight-junction connections between epithelial cells provide mechanical protection against large molecules and microorganisms. Pathogens (e.g., bacteria, viruses) or their metabolites in the lumen may cause damage to the GI tract, eventually destroying the mechanical barrier allowing penetration of microorganisms into the bloodstream and underlying tissues. This points to the importance of proper recognition of antigens on commensal

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and pathogenic bacteria by the Pattern Recognition Receptors (PRRs) present on the surface of specialized cells of GALT and mounting the defense against pathogens.

GALT is composed of lymphoid tissue containing lymphocytes scattered in the epithelium and lamina propria, Peyer's Patches (PPs), Isolated Lymphoid Follicles (ILFs), and M cells [9,10]. GALT is functionally connected to the mesenteric lymph nodes [8,11].

GALT functions include the identification of pathogens from non-pathogenic microorganisms or antigens and defense against pathogens [10]. The lumen of the human GI tract is colonized by a broad spectrum of symbiotic bacteria that plays a crucial role in the proper development and functioning of the organism. Furthermore, the intestines come in contact with dietary antigens, which are foreign to our immune system [8,12]. Evoking an immune response against such antigens would induce constant inflammation in the gut, causing chronic inflammatory diseases observed in food allergies or intolerances [13-15]. The phenomenon of suppression of immune response against non-harmful antigens from gut microbiota or food antigens is called oral tolerance [16,17] (Figure 1).

## Antigen processing and induction of oral tolerance

Food- and microbiota-derived antigens present in the intestinal lumen, undergo endocytosis by M cells located in the gut epithelium and are transported to underlying tissue or to PPs, where they are processed by Antigen-Presenting Cells (APCs) such as Dendritic Cells (DCs) or macrophages. DCs also have the ability to extend their pseudopodia through the tight junctions to sample the antigens in the intestinal lumen [18,19]. APCs migrate to PPs or mesenteric lymph nodes and present processed antigen to naive T cells, which after acquiring the gut-homing ability, migrate to the lamina propria and epithelium [8,20,21]. Clones of the lymphocytes that reach the epithelium or lamina propria will be tolerant of the antigen previously presented by APCs.

Induction of tolerance is related to the dose of antigen presented. In response to low doses of antigen, naive T cells differentiate into regulatory T cells (Tregs) that produce anti-inflammatory cytokines, especially IL-4, IL-10, and TGF-β, which in turn, prevent inflammatory reaction (Th-1 dependent) induction. In response to high doses of antigens presented by APCs, T Cell Receptors (TCRs) are internalized or degraded, leading to the anergy or clonal deletion of lymphocytes [22,23]. Anergic lymphocytes are unresponsive to antigens and cannot migrate, creating the anti-inflammatory environment in the place of antigen presentation by APC [24]. Furthermore, the oral tolerance may be induced in the periphery by the population of migrating DCs, allowing the deletion of antigen-specific T cells (CD4+ and CD8+) in lymph nodes and liver [25-28].

What is the origin of this tolerance? The DCs in GALT, expressing CD103 have a tolerogenic phenotype, resulting from the exposure to TGF-β and retinoic acid produced by epithelial cells during their differentiation [29-31]. CD103+ DCs, in turn, produce anti-inflammatory cytokines, IL-10, and TGF-β that result in the differentiation of naive T cells into Th2 and Tregs and naive B lymphocytes into IgA producing B cells; these processes are also supported by TGF-β and retinoic acid produced by epithelial cells [32,33].

## Recognition of the pathogen from non-pathogen

It has been suggested that the primary mechanism involved in distinguishing between symbiotic and pathogenic bacteria is based on the recognition of the Microorganism-Associated Molecular Patterns (MAMPs) by the pattern recognizing receptors (PRRs) on the surface of DCs. Binding the ligand to PRRs 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 [34].

## Role of the microbiota

Human intestines are colonized by the symbiotic bacteria belonging to about 500 - 1000 species, and in a number equivalent to the human cells; this number has been revised from the previous ratio of 10:1 [35]. Gut microbiota plays an essential role in maintaining the balance between tolerance to foreign antigens, and defense against foreign pathogens [36].

Toll-Like Receptors (TLR), belonging to PRRs, in general, are in general responsible for defense against pathogens, but in a tolerogenic environment in the gut, TLRs interact with symbiotic bacteria and do not evoke immune responses against them. Moreover, stimulation of the DCs' TLRs results in the production of anti-inflammatory cytokine IL-10 [37,38], suggesting a direct interaction between immune cells and microbiota in inducing oral tolerance. Polysaccharide A (PSA) derived from *Bacteroides fragilis* induces differentiation
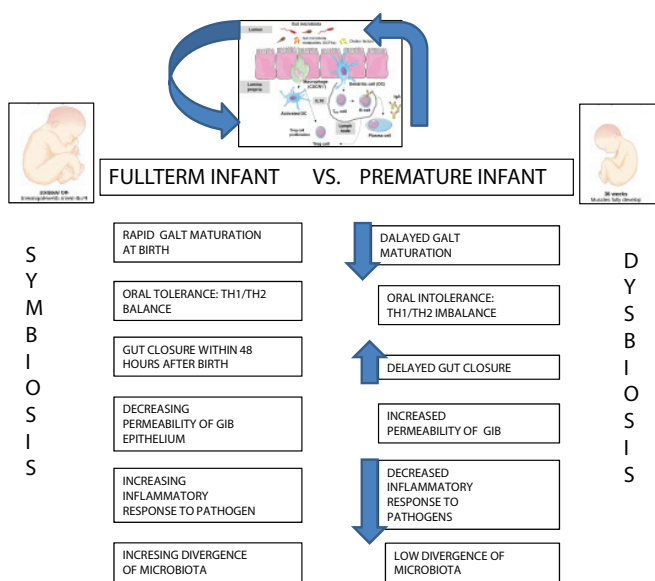


**Figure 1:** Developmental Processes and Galt-Microbiota Interactions.

of naive T cells into Treg subpopulation. PSA, by binding to TLR2 receptors on the surface of T cells, also induces the production of IL-10. Symbiotic bacteria use the TLR-dependent intracellular signaling pathway to inhibit the pro-inflammatory responses [39,40]. Indigenous *Clostridium* species increase the production of TGF-β and influences the accumulation of Tregs in intestines by increasing the level of secreted IL-10 [36]. Also, the metabolites of *Clostridium* species, short-chain fatty acids (SCFAs) promote the differentiation towards Tregs [41].

Microbiota may also influence other aspects of the gut immune response, such as migration of the DCs by regulating the release of chemokines. Immunomodulating mechanisms of microbiota also include elevation in IgA production, reduction of pro-inflammatory cytokines production, inhibition of lymphocyte proliferation and maintenance of gut epithelium integrity [23,42-44].

## Responses to pathogenic microorganisms

Human GI plays a crucial role in the defense against pathogens that involves both specific and non-specific mechanisms. Mucus produced by goblet cells presents the physical barrier for pathogens. Enterocytes produce antimicrobial peptides (AMPs), which disrupt bacterial membranes leading to cell lysis or reduce the amount of the nutrients essential for bacterial cells through the process of so-called 'nutritional immunity [45].

DCs of the GALT play an important role in the inflammatory response against pathogenic microorganisms. In the presence of inflammatory mediators, DCs produce the co-stimulatory molecules, which are essential for T-cell activation. During the inflammation, newly recruited DCs differentiate towards the pro-inflammatory phenotype, rather than the pro-tolerogenic. DCs that are recruited directly from the mesenteric lymph nodes are deprived of the influence of TGF-β and retinoic acid derived from the gut epithelium. Other immune cells residing in the GALT under the influence of the inflammatory mediators also change their phenotype into the proinflammatory one [46-48].

## Regulation of GALT development: Establishing the immune balance in term infants

GALT is structurally developed in term infants, but after birth, undergoes the process of maturation in response to microorganisms colonizing the mucosal surfaces of the newborn [49]. Crosstalk between the immune system and microbiota is essential for the proper maturation of immune functions, establishing the Th1/Th2 balance, thus preventing Th1- or Th2-mediated diseases [2]. Once mature, to maintain gut homeostasis, GALT must maintain the established balance between Tregs and Th1/Th17 lymphocytes and assure the production of IgA by B cells, migrating from Peyer's Patches to gut epithelium after contact with presented by APC antigen [41].

## Immune development

The immune system consists of two distinct arms, innate and adaptive immunity. During inflammatory reactions, innate mechanisms are the first line of defense, followed by adaptive, antigen-specific responses. In newborns, the innate immune system presents the full repertoire of cells, such as neutrophils, monocytes, macrophages, DCs, natural killer cells, but compared to adults, their functions are depressed. Adaptive mechanisms are directed toward the maintenance of tolerance. T cells in newborns under the influence of foreign antigens differentiate into Th2 or Treg subtype, and B cells produce the low-affinity antibodies, both resulting in low-grade immune responses [50-52]. In the GALT of infants, immune reactions rely on the Innate Lymphoid Cells (ILCs), which have a similar function to T cells, but lack antigen-specificity. As adaptive mechanisms mature, CD4+ lymphocytes take over the function [53,54].

The anti-inflammatory characteristic of the immune reactions in neonates may be a life-saving adaptive mechanism in the fetus, allowing it to tolerate maternal-derived antigens. However, after birth, the suppressed and immature immune mechanisms contribute to the increased vulnerability of the newborns, relying on maternal antibodies received during fetal life [transplacental transfer] and breastfeeding [50-52].

## Development of the epithelial barrier

Human fetal gut development begins early in the first trimester of gestation. During pregnancy, several processes resulting in the formation of structurally developed gut take place, including the growth, differentiation, and cell maturation, the formation of specific villi-crypt structures, and the elongation of intestines [55]. The Formation of tight-junctions begins with week 10 of gestation. Nevertheless, fetal gut remains permeable, allowing the exchange of molecules between fetal serum and amniotic fluid. Closure of the membrane, so-called "gut-closure" occurs during the first week of the postnatal period and is essential for the normal functioning of the intestinal barrier [3].

Breastmilk is vital for the proper gut-closure, and postnatal GALT maturation. Breastmilk contains many growth factors stimulating intestinal growth [56]. Nursing is also important for gut-closure, as significantly lower intestinal permeability was observed in breastfed newborns, compared to formula-fed children [57]. Furthermore, similar observations were made for preterm infants; those breastfed or receiving human milk had lower gut permeability than the formula-fed ones [58].

Studies performed using germ-free animals showed that commensal bacteria are also essential for gut maturation. Microbiota regulates the villus growth and proliferation of the epithelial cells [59].

Developmental disorders affecting the GIB barrier and resulting in increased intestinal permeability may contribute to the pathology of several diseases, such as NEC, infectious diarrhea, or allergic gastroenteropathy in newborns. It is also postulated that the disturbances in the gut barrier function during infancy may be a predisposing factor for inflammatory diseases such as inflammatory bowel diseases celiac disease or irritable bowel syndrome in adult life [60].

## Regulation of the immune response by microbiota

The colonization of newborns GI tract by the mother's vaginal bacteria during labor is essential for maturation of the gut immune system [61]. Research performed in germ-free mice showed strongly underdeveloped GALT, with a significantly reduced number of lymphocytes (both T cells and plasma cells) in lamina propria and hypoplastic PPs lacking most of the germinal centers. Interestingly, allowing the proper microbial colonization of germ-free animals reversed those changes, and caused proper maturation of GALT structures [62].

Microbiota exert diverse effects on the differentiation of T cells. Despite the tolerogenic environment in the intestines, the balance between Th1/Th17- and Th2-dependent response must be established and preserved [63,64]. *Clostridium sp.* induces the TGF-β production, and Tregs proliferation, helping to maintain the anti-inflammatory environment [65]. Segmented filamentous bacteria (SFB) induce Th17 cell differentiation, which is essential for defense against invading pathogens [66,67]. Also, the presence of Th1/Th17 cells and inflammatory mediators (IL-17, IL-22) in the gut is vital, while inhibiting excessive Th2-dependent mechanisms expansion is relevant in preventing asthma, allergic reactions, and other Th2-dependent diseases development. In children delivered by C-section, there is a low diversity and abundance in bacteria species colonizing the GI tract, a very low number of *Bacteroides* and *Bifidobacterium* species, and reduced levels of mediators of the Th1-dependent responses. This, in turn, shifts the balance towards the Th2-mediated mechanisms [68,69]. On the other hand, preventing excessive Th17-dependent responses mediating the autoimmunity is necessary to suppress autoimmune diseases, such as rheumatoid arthritis or multiple sclerosis [43,70].

Microbiota influences editing the B cell receptors and shaping its repertoire [71]. Additionally, symbiotic bacteria promote the differentiation of Bregs producing IL-10, helping to maintain the gut-homeostasis [72]. In germ-free mice, the increased levels of IgE, are accompanied by the decreased IgG and IgA levels [73]. Microbiota transplant to the germ-free mice results in increased production of both IgG and IgA [74].

Microbiota regulation of immune development involves the epigenetic mechanisms, such as DNA methylation or histone phosphorylation and acetylation, involved in gene expression and silencing [75,76]. It has been proposed that the insufficient early-life gut colonization by microbiota may result in silencing the IFN-γ genes in naive T cells, shifting the balance in Th1/Th17- and Th2-dependent mechanisms towards Th2, which may result in an allergic response [77]. Immune responses may also be influenced by epigenetic modifications of nuclear factor-κB, TLRs, and many other genes of molecules involved in the modulation of inflammatory processes [78].

Interestingly, a recent report showing the presence of symbiotic bacteria in the placenta, amniotic fluid, and meconium [79,80] challenged the hypothesis of fetal gut sterility. Nevertheless, the data showed a very low diversity and abundance of bacteria [61,81]. However, a critical assessment of the evidence supporting these two opposing hypotheses, pointing to the methodological errors and the ability to reliably derive axenic animals via cesarean sections, strongly supports sterility of the fetal environment in mammals [82].

## GALT development in preterm infants

As discussed above, the crosstalk between gut microbiota and GALT is essential for the proper development and functioning of the organism. However, there are several factors that may interfere with these processes [6]. Depending on the gestational age at the time of birth, the immune system may be more or less mature, and the degree of prematurity causes abnormal response for commensal bacteria evoking the inflammatory response in place of immune tolerance. The timing of GIB closure is delayed in preterm infants and the prematurity of the GIB barrier contributes to the overall pathological state. The underdeveloped physical epithelial barrier in terms of abnormal cell proliferation, disturbed cell differentiation, or tight-junction formation leads to antigen penetration and inflammation [83]. Inflammatory reactions in intestines lead to the damage of the epithelium and disruption of the gut-blood barrier, promoting bacteria penetration into underlying tissues, eventually causing life-threatening conditions like sepsis or NEC [4,84].

Prematurity implies a further risk of gut-microbiota dysbiosis. Preterm children are often delivered by C-section that results in gut colonization by the skin bacteria, instead of vaginal and fecal microorganisms during normal labor. Secondly, preterm infants are often formula-fed, deprived of mother's milk containing nutrients used by microbiota, and also bacteria that colonize the intestines of the infant. Additionally, treatment with antibiotics and the sanitary conditions of intensive care nurseries reduce the diversity of microorganisms colonizing the gut and further contribute to the microbiota dysbiosis in the newborn [69,85]

## Summary

This review provides evidence supporting the concept of underdeveloped functions of GALT, dysfunctional GIB, and altered GALT microbiota interactions in preterm infants. GALT is not only critical during development, determining the tolerance and supporting immune function. It also plays an essential role as a gate-keeper in maintaining a healthy GIB barrier between the external environment and the human organism. Developmental GALT dysregulation, GIB barrier dysfunction, and dysbiosis may all contribute to non-infectious diseases, such as autoimmune diseases, allergies, or other inflammatory diseases. Further studies of the mechanisms involved in the establishment of developmental gut symbiosis are crucial for the proper care of preterm infants and lowering long-term health risks.

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