OPEN ACCESS Freely available online

Immunome Research

Review Article

Gastrointestinal Microbiota Impact on the Development of Food Allergy Immune Phenotype

Ileana Constantinescu[1,2*], Roxana Sfrent-Cornateanu[1]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; [2] Centre for Immunogenetics and Virology, Fundeni Clinical Institute, Bucharesct, Romania

ABSTRACT

Recent technological advances have allowed researchers to more accurately describe gastrointestinal microbiota composition and its interaction with the host in health and disease. Lifestyle changes, including the large-scale use of antibiotics, alter both the composition and function of the intestinal microbiome, which predisposes the host to gastrointestinal and metabolic disease, as well as immune system dysfunction.

The purpose of this review is to highlight some key concepts pertaining to the interaction between the gut microbiome and host, as well as to illustrate how this interaction influences immune system programming, more specifically, its impact on the development of the immune phenotype characteristic for food allergy.

The following discussion will be about the role of the gastrointestinal microbiota, as one of the many factors which contribute to immune dysfunction in food allergy pathogenesis.

Keywords: Food allergies; Immune phenotype; Gut microbiota; Therapeutic interventions

INTRODUCTION

Humans, like all multi-cellular organisms, constantly interact with numerous micro-organisms, including bacteria, viruses, fungi, archaea and other eukaryotes, resulting in a synergistic interdependence between our cells and foreign ones. The number of symbiotic micro-organisms colonizing an adult host exceed the number of human cells by at least 10-fold, while microbial genes at the level of the colon exceed human genes by 100-fold; as a result, they participate in numerous important physiological processes. This particular interaction, occurring at a genetic level between the human host and symbionts, is referred to as a 'microbiome' ("the sum of microbes, their genomic elements, and interaction in a given ecological niche") [1-3].

The most well colonized surface in humans is, by far, the gastrointestinal (GI) tract, greatly exceeding the number of microbial colonies found on the skin, within the respiratory tract or the vaginal mucosa [4]. Compared with other microbiota - defined as "the sum of microbes found in a given ecological niche", the intestinal microflora particularly contributes to maintaining the health of the host by strengthening their defense system locally, by protecting epithelial cells from lesions, as well as systemically, by shaping the immune response [3,5].

Increased interest in the relationship between the GI microbiota and food allergy (FA) pathogenesis is the result of several findings, including: the association of FA phenotypes with gut dysbiosis, the striking similarity between risk factors associated with FA development and dysbiosis, and furthermore, it was observed that manipulation of the gut microbiota (such as vaginal seeding, diet, the use of probiotics) may reduce the risk of FA development [6-9].

Food allergies (FAs) have been defined as "objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus [food] at a dose tolerated by normal subjects" for which an immunologic mechanism has been demonstrated [10,11]. In practical terms, the diagnosis of FA requires a patient history of clinical signs associated with ingestion or exposure to a particular food and a clinically demonstrable immunologic mechanism (usually involving allergen-specific IgE). Furthermore, clinical symptoms should be reproducible with

Correspondence to: Ileana Constantinescu, Centre for Immunogenetics and Virology, Fundeni Clinical Institute, Bucharesct, Romania, E-mail: ileana.constantinescu@imunogenetica.ro

Received: February 14, 2020; **Accepted**: February 27, 2020; **Published**: March 06, 2020

Citation: Constantinescu I, Sfrent-Cornateanu R (2020) Gastrointestinal Microbiota Impact on the Development of Food Allergy Immune Phenotype. Immunome Res. 16:174. Doi: 10.35248/1745-7580.20.16.174

ingestion of the suspected allergen during oral provocation tests. In cases where an immunologic mechanism cannot be demonstrated, or in the absence of an oral provocation test, the condition is termed food hypersensitivity (FHS) [12].

Although not as prevalent as other allergic diseases, their incidence has continued to rise in both developed and developing countries, making FAs a worldwide health issue [6,13,14]. FAs significantly impact quality of life due to mandatory food restrictions, as well as increased risk of anaphylactic shock, since it is currently impossible to predict this outcome or the threshold dose at which it may occur [10,15]. Patients and their families are under constant stress, which limits their daily activities, especially in the case of children, who are most severely affected by FAs [8,16].

While certain details have yet to be elucidated, at present, FAs are considered to be an imbalance in oral tolerance to certain foods, which is the result of immune system dysfunction at multiple levels, based on experimental animal models [16]. The main characteristics defining the FA immune phenotype comprise a decreased T helper 1 cell (Th1) and T regulatory cell (Treg) response, with predominant T helper 2 cell (Th2) activation, excessive production of immunoglobulin E (IgE) and lower levels of secretory immunoglobulin A (IgA) [8].

Various intrinsic factors thought to be involved in FA pathogenesis include genetic background, epigenetic modifications and vitamin D deficiency. Extrinsic factors associated with FAs include specific characteristics of the food allergen, such as, dosage and exposure during the first year of life. Other extrinsic factors such as, the type of birth, diet (of the infant as well as the mother during pregnancy and breastfeeding) and antibiotic consumption may also promote a state of immunological imbalance implicated in the pathophysiological mechanisms of Fas [9].

COMPOSITION AND DEVELOPMENT OF MICROBIOTA IN HEALTHY GUT

The composition of normal adult intestinal microbiota consists of 2 main phyla – Firmicutes (mainly the genera Clostridium, Faecalibacterium, Blautia, Ruminococcus, and Lactobacillus) and Bacteriodetes (Bacteroides and Prevotella) [17]. Less abundant phyla include Actinobacteria (Bifidobacteria), Proteobacteria (Enterobacteriaceae), Verrucomicrobia, Fusobacteria, Spirochaetes, and Lentisphaerae [18-21]. In addition to these anaerobic bacteria, the gut microbiota also comprises Archaea groups, predominantly methanogens, such as Methanobrevibacter and Methanosphaera [22,23]. Various fungi colonize the gut, including Ascomycota (Candida and Saccharomyces genera) and Basidiomycota phyla [24,25]. However, since the bacterial component of the microbiome vastly outnumbers all other elements and is the most well studied, we will focus on gut bacteria in our discussion [26]. It has been demonstrated that dysbiosis, an "imbalance in the microbial ecosystem", for instance, a change in the ratio of Firmicutes and Bacteroidetes abundances, can be linked to numerous diseases, and may even have predictive potential [18].

It is unanimously recognized that optimal development of both innate and adaptive immunity is closely linked to the changes in composition of the intestinal microbiota during early life [27]. Due to the influence of various external factors, the intestinal flora undergoes compositional changes with an extremely high variation rate during the first year of life. The composition of gut microbiota during this period was found to be of major importance to the infant's immune status (healthy, atopic, or at risk of developing FAs), since it coincides with the period of immune response and oral tolerance setting [28].

The exact period of initial intestinal colonization seems to play an important role in the normal development of host immunity, but this aspect is still under investigation. Recent studies have refuted theories suggesting that the amniotic sac and its contents are sterile [29]. Moreover, they have demonstrated the presence of microbial deoxyribonucleic acid (DNA) in amniotic fluid, placental and fetal tissues, as well as blood originating from the umbilical cord during the course of normal pregnancy [30]. Jimenez and colleagues have demonstrated, in an experiment using mice, that newborn meconium is not sterile; in fact, it contains bacterial species (including Enterococcus and Escherichia) found in the GI tract of adults, which suggests that the transfer of these micro-organisms takes place during gestation, from the maternal GI tract [31]. In humans, neonate meconium contains a specific microbiota dominated by Enterobacteriaceae (Escherichia genus). Pre-term neonate meconium, however, comprises mainly bacilli and other Firmicutes different from those normally found in feces after a week of life, which suggests that term status or lack thereof also influences infant gut microbiota [32].

Immediately after birth, the newborn intestine is an aerobic environment, inhabited by facultative anaerobes such as Enterobacteriaceae. Within a few days, the lumen becomes anaerobic, and is colonized with obligate anaerobic bacteria (Bifidobacteria, Clostridium species and Bacteroidetes) [18]. Within the first 3 years of life, the adult microbial flora is established and is relatively stable regardless of the host's geographical location [33].

GI MICROBIOTA COMPOSITION ASSOCIATED WITH FA IMMUNE PHENOTYPE

Children with FA (immune-mediated adverse reaction to food) or FHS, defined as "conditions clinically resembling allergy that cause objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects" [11,12], which encompasses the term FA, as explained previously, have been shown to have a lower overall diversity of gut microbiota, as well as a decreased abundance of the phylum Bacteroidetes more specifically (Table 1) [5,34]. Compared with healthy children, the number of Bacteroidetes was significantly decreased while that of Firmicutes was significantly increased in affected children. Regarding bacterial Genus – children with FHS had increased numbers of Sphyngomonas, Sutterella, Bifidobacterium, Collinsella, Clostridium sensu stricto, Clostridium IV, Enterococcus, Lactobacillus, Roseburia, Faecalibacterium, Ruminococcus, Subdoligranulum, and Akkermansia, while they had decreased numbers of Bacteroides, Parabacteroides, Prevotella, Alistipes, Streptococcus, and

Veillonella. The study found that the most substantially altered taxa were increased abundances of Clostridium IV and Subdoligranulum and decreased abundances of Bacteroides and Veillonella; furthermore, these could be used to identify children with FHS [5].

Another study found a significant correlation between the genus Clostridium sensu stricto and the presence of antigen specific IgE in infants with FA [35]. It is very difficult to describe a gut microbial pattern associated with the various subtypes of FA (related to the trigger allergen, for instance); however, some characteristic patterns have emerged. It was observed that infants with milk allergy had increased microbial diversity and higher numbers of Ruminococcaceae and Lachnospiraceae, both of which are Firmicutes (Table 1) [36]. On the other hand, a study of 226 children with milk allergies noted that higher levels of Firmicutes, including clostridia, during infancy were associated with FA resolution by the age of 8 years [37]. Children with peanut allergies showed a different gut flora pattern than those with milk allergies; their microbiota was less diverse, with higher numbers of Bacteroidetes [38]. In summary, although there seem to be some exceptions, sensitization to allergens has been associated with decreased gut microbiota diversity, decreased Bacteroidaceae/Enterobacteriaceae ratio and/or a low relative abundance of Lachnospiraceae [34,39]. Some theories explaining the association of early dysbiosis in children prone to develop food allergies suggest that an altered intestinal microbiota composition in early life can lead to inadequate immune programming, resulting in an overreaction to antigens or loss of food tolerance [40,41].

Table 1: Comparison of GI microbiota composition of healthy children and children with food allergies/hypersensitivities.

	Healthy Children	Children with FHS	Children with FAs	
			Cow's Milk Allergy	Peanut allergy
Overall bacterial diversity	High (+)	Low (-)	High (++)	Low (-)
Bacteroidetes	Low (-)	Low (-)	Low (-)	High (+)
Firmicutes	Low (-)	High (+)	High (+) Ruminococcaceae, Lachnospiraceae	

FACTORS THAT INFLUENCE THE COMPOSITION OF THE GI MICROBIOTA AND THEIR ASSOCIATION WITH PREDISPOSITION TO FA DEVELOPMENT

Type of birth

One of the most studied factors involved in changes in GI microbiota is the type of birth; whether investigated separately or together with other perinatal factors, type of birth influences both the newborn's gut microbiome composition as well as it's modification during the first year of life [7,42-44]. Alterations of the intestinal microbiota associated with caesarean section (CS) delivery seem to have a remarkable impact on the likelihood of developing FAs later on in life [3,42-45]. Rutayisire and colleagues conducted a large meta-analysis on the differences between the composition and age-related modifications of microbial patterns characteristic for vaginal delivery and CS (Table 2). Despite differences in investigative techniques- culture-dependent and molecular analyses or DNA extraction and 165r RNA gene amplification or fluorescence *in situ* hybridization of bacterial cells – the reported microbial patterns were generally similar [43].

Infants born through CS seem to have a lower abundance of Actinobacteria, Bacteroidetes and Firmicute phyla during the first 7 days after birth, and higher colonization with the Firmicute phylum, especially the genera Clostridium and Lactobacillus, with lower overall bacterial complexity after 30 days of life [18,43,44].

Term infants born through vaginal delivery, which have been breast-fed, benefit from a presumably ideal gut microbiota, on account of having the lowest chances of developing FHS, FA and a variety of other diseases. In the first year of life, Proteobacteria, the initial gut colonizers, decrease in number, being replaced predominantly by Actinobacteria, Firmicutes and Bacteroidetes [42]. These infants tend to have the highest numbers of Bifidobacteria and lowest counts of *C. difficile* and *E. coli* [39]. On the other hand, infants without ideal conditions (born through CS, exclusively formula-fed or exposed to antibiotics early in life) do not show this typical decline in Proteobacteria after 3 months of life and have lower abundances of Bifidobacteria and Bacteroides, with higher numbers of *C. difficile* [8,42]. Increased colonization with Enterobacteria at 3 or 12 months is an established risk factor for food sensitization [34,42]. It is currently presumed that exposing a newly born infant, delivered through CS, to the bacterial flora specific to a vaginal delivery, could result in beneficial changes to their intestinal microbiota, thereby decreasing their risk of Fas [3]. However, little is currently known about the long-term outcomes of this practice [46]. Furthermore, Caberara-Rubio and colleagues (2012) observed a difference between the milk microbiota of women who underwent elective C-section compared to those who underwent non-elective C-section and vaginal delivery. In the case of non-elective C-section and vaginal delivery, which yielded similar milk microbiota, hormonal changes due to labor likely influenced the bacterial composition of the breast milk [47]. This suggests that breast milk microbiota may also play a key role in the bacterial colonization of the newborn.

Breast feeding

Besides type of delivery, breastfeeding – alone or combined with other external factors – is one of the most studied aspects related to gut microbiota composition and function. Human milk was found to play an important role in the establishment of normal infant gut microbiota, which impacts balanced immune response development [3,48]. It appears to influence both innate and adaptive immune system development, including gut barrier function and oral tolerance induction [49].

Human milk has many components, including nutrients, cellular and humoral defense agents, such as immune cells, immunoglobulins (mostly soluble IgA), antimicrobial peptides (for example lysozyme and α-lactalbumin), immunomodulatory agents, human milk oligosaccharides (HMO) and other prebiotics, all of which impact gut microbiota [40,48,50,51]. It is important to note that breast milk undergoes a change in composition from colostrum, secreted several days post-partum and containing higher levels of immunologic and trophic components, to intermediate milk, to mature milk, which is secreted 4 to 6 weeks post-partum and contains a higher level of nutritional components for the infant. However, the individual components, regardless of their relative amounts, are mostly the same [51].

Human milk contains commensal bacteria, some of which act as probiotics or "live micro-organisms that, when administered in adequate amounts, confer a health benefit to the host" [27]. These decrease the infant's risk of developing allergic diseases, including FAs, by increasing anti-inflammatory cytokines, most notably transforming growth factor β (TGF-β), which has been demonstrated to modulate the immune response [52,53]. Higher levels of TGF-β were observed in exclusively breast fed infants, in contrast to formula-fed infants, who showed higher levels of cytokines with pro-inflammatory potential, including tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2) [53]. TGF-β induces colonic Forkhead box P3 (FoxP3) expressing CD4+CD25+ Tregs (we will refer to these as extra-thymic or peripheral Tregs) and can therefore indirectly influence T cell activation, normalizing Th1/Th2 balance [54].

Milk microbiota-derived products, especially short-chain fatty acids (SCFAs), have been shown to increase TGF-β production/secretion by intestinal epithelial cells (IECs) (the exact mechanism is unknown). SCFAs also increase the expression of metalloproteases (integrin αvβ8) on the surface of IECs, which leads to higher levels of functional TGF-β [55]. Microbiota-induced TGF-β also contributes to the development of mucosal immunity by stimulating the production of local specific secretory IgA in infants who initially rely heavily on breast milk as a source [53,56].

Due to its ability to selectively promote the growth of some commensals over others, human milk has an extremely important influence on the infant gut microbiota. The most common bacteria found to colonize breast-fed infants are from the Actinobacteria phylum, specifically the Bifidobacterium longum infantis subspecies, because of their efficient utilization of HMOs [40]. Actinobacteria contribute both directly and indirectly, towards maintaining an appropriate composition and balanced function of the infant's intestinal microbiota. On one hand, Actinobacteria promote an acidic pH between 5 and 6, which reduces the level of harmful species (e.g. Enterobacteriaceae, Clostridia and Bacteroides), on the other hand, these bacteria are better able to metabolize HMO, outcompeting potentially harmful bacteria due to a large genomic cluster allowing them to thrive on α-1,2-fucosylated HMOs which are abundant in human milk [57,58].

HMOs are among the most studied bioactive components of human milk and comprise the third most abundant constituent thereof. They are largely responsible for the typical and less diverse gut microbiota colonizing breast-fed infants compared to formula-fed ones. They can be classified as prebiotics or "selectively fermented ingredients (usually indigestible oligosaccharides) that result in specific changes in the composition and/or activity of gut microbiota, thus conferring benefits to host health". HMOs comprise over 200 different carbohydrate polymers including monosaccharides D-glucose, D-galactose, N-acetylglucosamine, L-fucose and N-acetylneuraminic acid [59,60].

Although HMOs are not digested by the infant, they exert immunomodulatory influence, mostly through α1–2-fucosylated oligosaccharides, such as 2′-fucosyllactose (2′FL) and lacto-N-fucopentaose (LNFP) I components,which especially stimulate the growth of Bifidobacteria, and defend against pathogen adhesion on the intestinal epithelium [50,61].

HMOs are added to most infant formulas based on their ability to contribute directly and indirectly (e.g increasing the amount of beneficial bacteria, such as Bifidobacteria and Bacteroides) towards maintaining intestinal health and barrier function which comprise the first line of defence in innate immunity. They consequently reduce the risk of atopy, as well as FAs [45,61]. However, according to the American Academy of Allergy, Asthma and Immunology (AAAAI) and the European Academy of Allergy and Clinical Immunology (EAACI), prebiotics are only effective when given with the correct composition ratios and dosage [3].

Infant diet, bacterial metabolites and bacterial components

Infant diet has been shown to influence gut microbiota composition, depending on the relative amounts of fats, fibers and sugars ingested [18]. Moreover, the metabolites resulting from digestion of different dietary components contribute to the development of the immune system and determine the predominant immune response type (Table 2).

The gut microbiota composition was found to be related to the intake of complex carbohydrates during infancy. Undigested, complex carbohydrates are converted into organic acids, such as lactate, and short chain fatty acids (SCFAs), including acetate, propionate and butyrate, by anaerobic gut microbes through the process of fermentation and their intake has been associated with a higher fecal concentration of butyric acid, as well as increased levels of secretory IgA (an indicator for the presence of Bifidobacterium in the gut). Bacteroides produce predominantly acetate and propionate, while Firmicutes produce butyrate.

Propionate results from the fermentation of inulin-type fructans (a prebiotic) [62-64].

SCFAs, butyrate being the most potent, act as histone deacetylase inhibitors, making them epigenetic regulators of gene expression [62,63]. They help maintain gut homeostasis and immune tolerance, by promoting the differentiation of peripheral Tregs and resident CD103+ DCs [39]. More specifically, they promote an anti-inflammatory state by decreasing NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) activity, converting macrophages and DCs to a tolerogenic phenotype and decreasing the pro-inflammatory innate immune response (Table 2). SCFAs are also extra thymic Treg cell inducers [62], since they increase the number and function of peripheral Tregs via enhanced Foxp3 expression [63]. Monocyte-derived DCs, when matured in the presence of butyrate, were shown to express higher levels of IL-10 and lower IL-6 and IL-12 [65]. SCFAs act as G-protein coupled receptor (GPCR) ligands, influencing GPCR signalling, including GPR43, GPR41 and GPR109A, which impact numerous cell types (immune cells, IECs). High levels of maternal SCFAs were also shown to have a positive impact on the offspring [63].

Dietary tryptophan is converted into aryl hydrocarbon receptor (AHR) ligands by Lactobacilli species [66]. These co-metabolites bind to host AHR and stimulate group 3 innate lymphoid cell populations (ILC3s) to produce IL-22; the result is resistance to colonization and protection from inflammation. The absence of AHR activity has been associated with enhanced immune activation and immunopathology [63]. Activation of the AHR pathway was demonstrated to suppress the FA response in a mouse peanut allergy model through induction of peripheral Treg cells (Table 2) [67].

Tryptophan is also converted to indoleacrylic acid (IA) by Peptostreptococcus russellii, which promotes the barrier function of the intestinal epithelium, a key component of innate immunity, and reduces the inflammatory response [68].

Furthermore, tryptophan can be metabolized to indoleamine 2,3-dioxygenase (IDO) and Kynurenine (KYN). One study demonstrated the presence of TRP, KYN and IDO in the breast milk of germ-free lactating dams, which had been exposed to gestational colonization with *E. coli*, together with elevated ILC3 and mononuclear cell expression in the newly born rat pups, following a vaginal birth. This suggests that these TRP metabolites permit maternal microbial programming of infant immune system development through breastfeeding (Table 2) [69].

Dietary amino acids are converted into polyamines, such as putrescine, spermidine and spermine, by both host and microbial synthesis, the difference being that their production by the host is far more limited and their richest source in the gut is the microbial flora [70]. They promote the high proliferation rates and rapid turnover of intestinal epithelial cells and are very important for post-natal development of the GI tract. In addition, polyamines modulate both systemic and mucosal adaptive immunity by influencing immune cell subset and function [71].

When administered to rat pups, polyamines stimulated the production of mucus and secretory IgA in the small intestine, while polyamine deficient pups developed intestinal mucosal hypoplasia. This suggests that they have an important role in GI tract innate immunity [63].

Bacterial components can also act as host-microbe signaling molecules. Polysaccharide A (PSA), from Bacteroides fragilis, a Gram negative commensal microbe, interacts with Toll-like Receptor 2 (TLR2) on DCs to enact pleiotropic modulatory effects on both innate and adaptive immune cells [72]. PSA has the ability to correct an imbalance of Th1 and Th2 response observed in germ-free mice [73], by increasing the inducible population of IL-10 producing Treg cells. Clostridium species have also been identified as a source of PSA [63].

Table 2. The effect of dietary and microbial components on immunity and the FA immune phenotype.

	Components	Bacterial Metabolites	Bacterial Source of Metabolites	Effect on Immune System Function	Influence on FA Immune Phenotype
Infant Diet	Complex carbohydrates	SCFA -acetate -propionate -butyrate	Bacteroides : acetate propionate Firmicutes: butyrate	↑ Intestinal Cell maturation ↑ Intestinal barrier function ↑ Peripheral Treg induction ↓ Inflammation	Promotes food tolerance: ↑ Secretory IgA Promotes Th1/Th2 balance ↑ IL-10 ↓ IL-6, IL-12

	Tryptophan (TRP)	aryl hydrocarbon receptor (AHR) ligands	Lactobacilli spp	↑ IL22 production by ILC3 ↓ Microbial colonization ↓ Inflammation ↑ Peripheral Treg induction	↓ Transfer of dietary antigen across the barrier: ↓ Allergic sensitization Promotes Th1/Th2 balance
		Indoleacrylic acid	Peptostreptococcus russellii	↑ Barrier function ↓ Inflammation	↑ Tolerance for food allergens
		TRP-KYN-IDO pathway	*Escherichia coli*	↑ ILC3	↑ Tolerance for food allergens
	Amino acids ornithine agmatine arginine	Polyamines -putrescine -spermidine -spermine	Synthesized by mammilian cells, as well as bacteria	↑ Intestinal immune cell maturation ↑ Intestinal barrier function	↑ Secretory IgA
Bacterial Components	Polysaccharide A (PSA)		Bacteroides fragilis Clostridium spp	↑ Peripheral Treg induction ↓ Inflammation	Promotes Th1/Th2 balance ↑ Tolerance for food allergens ↑ IL-10

Antibiotics

One of the most studied factors shown to influence the composition of GI microbiota, and consequently immune function, is the early and excessive use of antibiotics. Perinatal exposure to antibiotics of term newborns decreases bacterial flora diversity and the quantity of beneficial bacteria, such as Bifidobacterium and Lactobacillus [44,45], while promoting overgrowth of Proteobacteria. Furthermore, it' s been demonstrated that pre and post natal antibiotic exposure is associated with atopy [44]. Other studies, however, show that infants with atopic eczema at 6 and 18 months of age were found to have greater microbial diversity compared to healthy infants, as well as increased counts of Firmicutes, including Clostridium, and decreased Bacteroides [74]. This contrasts previous studies that showed a lack of Bacteroides was associated with decreased bacterial diversity. Taking this into account, the exact nature of the association between antibiotics, microbial diversity and immune dysfunction should be further examined. The destruction of beneficial bacteria by antibiotics reduces gut microbial diversity and gives rise to increased pathologic colonization with C. *difficile* or other aggressive pathogens such as Enterobacteriaceae [40,45]. Although the use of any antibiotic could lead to opportunistic infection with C. *difficile*, some of the most common ones include ampicillin, amoxicillin, cephalosporins, clindamycin and fluoroquinolones [40].

Other environmental factors

In addition to the previously mentioned aspects, other environmental factors, such as pH, water decontamination, food pasteurization, sterilization and uninterrupted cold-chain delivery, ultimately result in decreased exposure to commensal microbes and infections, thus representing possible mechanisms of gut dysbiosis [18,40,42,75]. Even though pathologic microbial patterns acquired after birth become less apparent after 6 months of life, they seem to play a major role in the development and maturation of the immune system, since abnormal GI microbiota during infancy has been identified as a predicting factor for future diseases, including FAs [3,42,43].

GI MICROBIOTA AND IMMUNE TOLERANCE

Mechanisms of oral tolerance for food antigens

Oral tolerance entails a lack of immune response, at the local and systemic levels, following the oral administration of harmless antigens, such as food proteins [76]. It is extremely important for the immune system to be able to differentiate between dangerous antigens and inoffensive materials: an active systemic immune response against commensal micro-organisms can lead to inflammatory and/or autoimmune diseases while failure to tolerate certain food antigens may lead to food allergies and celiac disease [77]. It has been demonstrated, mostly in animal models, that tolerance for food proteins requires several concurrent mechanisms, including the maintenance of an appropriate Th1/Th2 balance, the

suppressive action of Treg cells and the presence of secretory IgA [64,76,77].

Oral tolerance can be subdivided according to antigen uptake method, which depends on the nature of the antigen [77]. The vast majority of food allergens are water-soluble proteins with a molecular weight between 5 kDa and 50 kDa [78]. In the gut, soluble antigens acquire both local and systemic tolerance, whereas particulate antigens, presumably because they are more likely to arise from commensal bacteria, only achieve local tolerance [77]. Immune recognition of particulate antigens occurs through M cells in the epithelium overlying Peyer's Patches or isolated lymphoid follicles, which transport this material from the gut lumen to gut associated lymphoid tissue (GALT) areas. It is unclear whether M cells play a role in the recognition of soluble antigens; the main mechanism, in this case, seems to be uptake by DCs, and to a much lesser extent GALT [77].

Secretory IgA contributes to the induction of oral tolerance by its ability to bind antigens in the gut, thus preventing their systemic uptake. Mucosally induced tolerance is initiated in the mesenteric lymph nodes, and further sustained and expanded in the intestinal lamina propria by antigen-specific Tregs [79,80]. Certain bacterial species may promote or disfavour peripheral Treg induction, which will determine immune reactivity [80].

GI dysbiosis contributes towards setting a FA immune phenotype

Microbial colonization plays an important role in both the morphological and functional development of the immune system [1,3]. The mechanisms by which the predominant type of immune response is established in early life and how this influences an individual's health throughout their lifetime have been the topic of numerous studies, leading to advancements especially in the field of atopy [*3,64,81].*

In the last years, a growing body of evidence shows that the gut microbiota influences immune development at many levels and early dysbiosis, defined as an "imbalance in a microbial ecosystem" , contributes towards setting a FA immune phenotype. From an immunologic point of view, the FA phenotype is characterized by decreased Th1 and Treg response, excessive production of IgE and a lower secretion of IgA, together with impaired oral tolerance to food allergens. Next, we will focus on the effect of the gut microbiota on each of these components [3,8].

GI microbiota effect on peripheral Treg development and Th1/Th2 balance

It has been shown that the GI microbiota plays an important role in promoting the development of balanced CD4+ T lymphocyte sub-types, being a significant inducer of peripheral Tregs, cells which have a major role in maintaining a normal Th1/Th2 ratio [64]. For example, it was demonstrated that normal intestinal colonization in early life allows for the recognition of pathogen-associated molecular patterns (PAMPs), inducing the maturation of Th1 mediated immunity, which is controlled by Treg cells [75,82]. Gut dysbiosis, for example a lack of Bacteroides, contributes towards maintaining a predominantly Th2 type of response known to be associated with the atopic phenotype. Moreover, gut microbiota induce both Tregs cells and proinflammatory Th17 cells, more specifically, a subtype of Tregs expressing the nuclear hormone receptor ROR γ t. A Th2 response predominates in the absence of ROR γ t+Tregs, thus the microbiota balances the mucosal response to pathogens by promoting type 3 ROR γ t+ Treg [83].

Hunter and colleagues considered that decreased peripheral Treg populations, characteristic of the immune phenotype associated with FA, could be due to decreased activation of the inflammatory signalling cascade, leading to down-regulation of the NFkB pathway and decreased production of IL-8. C-Rel, a subunit of NFkB, regulates the expression of Bach2, a transcription factor acting on CD4+ T cells to promote the development of Tregs. The actual mechanism may involve a suppressing effect of cell transcriptional programs84. This is further supported by the fact that certain variations of the Bach2 gene have been associated with increased susceptibility to allergic disease [84,85].

Colonic Tregs seem to be extremely important for maintaining immune homeostasis in the gut, as well as promoting tolerance of commensal microbiota and dietary antigens while eliminating pathogens [86]. It is well known that there are two types of CD4+CD25+FOXP3+ Treg cells: Tregs which develop in the thymus and peripherally derived Treg [86]. The colon is a major site of peripheral Treg development; however, this is only possible in the presence of microbiota [86]. These extra thymic Treg cells have distinct TCR variants and play a crucial role in gut homeostasis [87]. The exact mechanism of peripheral Treg cell induction in the colon has not been elucidated, however, the process seems to be promoted by certain bacterial metabolites, among which are SCFAs, butyrate being the most potent, and PSA, produced by Bacteroides fragilis [86]. Furthermore, peripheral Treg cells activated by food antigens and commensal microbes are crucial for maintaining oral food tolerance and preventing allergic reaction. This is supported by the fact that germ-free and antibiotic-treated mice, which are more susceptible to allergic sensitization, had lower peripheral Treg and secretory IgA levels compared to normal mice [79,88].

GI microbiota effect on IgE production

There is increasing evidence that long-term IgE levels are determined by intestinal microbial diversity in early-life. These antibodies are the main players in allergic disease and confer immunity to parasites [89]. Immunoregulatory signals originating from the gut microbiota are necessary to maintain normally low IgE levels. Furthermore, these must be present early in life, during a critical time window, as they are responsible for establishing baseline immunoregulatory status for life [90].

Cahenzli and colleagues demonstrated that the absence of microbial colonization leads to increased serum IgE levels in germ-free mice and that the presence of elevated IgE levels in these mice was dependent on CD4+ T cells and IL-4. It seems that the default pathway for B cells at mucosal sites is an isotype switch to IgE in the absence of microbial exposure and neonatal

colonization with diverse gut flora is necessary to inhibit the induction of IgE in a critical time window - for rat pups this being approx up to one week after birth. Elevated levels of IgE in germ-free mice resulted in increased binding to mast cells and an exaggerated systemic reaction to orally introduced antigens [90]. These mice studies have demonstrated that a decrease in the variety or quantity of intestinal microbiota early in life can have a significant and lasting negative impact on immune regulation.

GI microbiota effect on secretory IgA production

Frossard and colleagues demonstrated that beta-lactoglobulin (BLG)-specific serum IgA was greatly increased in a murine model of food allergy; in contrast, secretory IgA levels were high, but serum levels were low in tolerant mice. Furthermore, there was an increased number of BLG-specific IgA-secreting cells in Peyer's patches of tolerant mice, and enhanced BLG-induced secretion of IL-10 and TGF-β at these sites of secretory IgA production. This study demonstrated that secretory IgA plays an important role in the mechanism of food tolerance, and that CD3+ cells in Peyer's patches appear to promote IgA secretion by releasing IL-10 and TGF-β [91].

THE RELEVANCE OF MURINE STUDIES IN ELUCIDATING IMMUNOLOGIC PATHWAYS INVOLVED IN HUMAN PATHOLOGY

Despite numerous similarities in the overall structure and function of the immune system in mice compared to humans, there are still some differences which should be taken into account when extrapolating the results of murine experiments [92]. A comprehensive list of these differences is beyond the scope of this review; however, the following may be relevant to the biochemical pathways discussed.

There are some differences in Ig subtypes, between mice and humans, with different abilities to bind FcR and the complement system, however, these are not considered significant. IL-13 has no effect on murine B cells, however, it is known to induce an IgE class switch in humans. Th1/Th2 polarization is a much clearer phenomenon in mice than it is in humans. Furthermore, IFNα stimulates Th1 development in humans, but doesn't have this effect in mice. IL-10 is secreted only by Th2 cells in mice, yet both Th2 and Th1 cells are able to produce IL-10 in humans. Activated human T cells express MHC II, but not those in mice, suggesting differences in Ag presentation. On account of their larger size and longer lifespan, humans generally have a wider variety of B and T cells, which persist for longer compared to mice [92].

Overall, the applicability of murine studies to human pathology has already been established [92]; these may provide valuable information about human immunologic pathways related to food allergies, however, the data must be interpreted with caution.

FA THERAPIES TARGETING THE MICROBIOTA

Immunotherapy has been the cornerstone of allergic disease treatment during the past century, however, due to recent advances revealing the link between the human microbiome and numerous diseases, including allergies, several promising therapies targeting microbiota composition and function have emerged [65].

Oral administration of probiotics with or without immunotherapy

Some major sources of probiotics include dairy products containing Lactobacillus and Bifidobacterium species. Their immunomodulatory role was demonstrated through increased secretory IgA and IL-10 expression, together with the suppression of TNF-α, casein-induced T-cell activation, circulating soluble CD4 and TLR4 signaling [93]. Lactobacillus fermentum and Lactobacillus rhamnosus were shown to improve allergic inflammation and decrease local eosinophil infiltration, as well as stimulate the production of IgA by ILC3s via TNFβ. Bifidobacterium longum 35624, Clostridia and Bacteroides fragilis were demonstrated to induce intestinal Treg cells, promoting their differentiation through PRR activation of DCs [65]. Furthermore, probiotics, such as Lactobacillus rhamnosus, have been successfully used to improve the results of oral immunotherapy for peanut allergy [94].

Recently, there were published two large meta-analyses regarding the efficacy and safety of probiotics in the management of food allergy. Both of them conclude that probiotics may have a moderate effect (limited low-quality evidence) in inducing tolerance to cow milk after a minimum of 2 years of treatment, especially with Lactobacillus rhamnosus GG. There is evidence (moderate certainty) that the use of probiotics can relieve symptoms of children with cow's milk allergy [95,96].

Human umbilical cord mesenchymal stem cell therapy

Modification of the intestinal flora with the goal of modulating oral tolerance for food allergens was tested on animals using human umbilical cord mesenchymal stem cell (hUC-MSC) [39]. Mice with ovalbumin induced food allergy received intraperitoneal administration of hUC-MSC with oral gavage of culture medium; this significantly alleviated their allergic symptoms, decreased IgE levels, Th2 response, inflammatory cytokines (IL-4, TNF-α) in the colon and partly re-established gut flora [39]. Before treatment, after sensitization to ovalbumin, pathologic changes to the microbiota included an increased abundance of Helicobacter and Mucispirillum, and decreased abundance of Bacteroides, Bacteroidales S24-4 and Lachnospiraceae (which are butyrate producing bacteria). However, after treatment, there was recovery of Bacteroidales S24-4 and Lachnospiraceae with partial alleviation of FA symptoms. The mechanism of gut flora restoration requires further investigation, but these promising results suggest its suitability as a potential FA therapy [39].

Microbiota engineering

To date, microbiota engineering comprises three methods of altering gut microbiota composition and/or function in order to alleviate allergic symptoms [65]. Firstly, the administration of commensal bacteria in pathological conditions, via fecal transplantation or vaginal swab, in order to change gut

microbiota composition to resemble that of healthy individuals. Secondly, the engineering of biosynthetic pathways resulting in de novo or enhanced production of compounds promoting gut health and homeostasis by gut bacteria. Lastly, selected commensal strains, such as lactic acid producing bacteria, could be engineered to express or secrete recombinant therapeutic proteins [65]. Cano-Garrido and colleagues described the combined effect of probiotic bacteria (Lactobacillus lactis) engineered to express an antigen involved in FHS (Ara h2, causing peanut allergy), resulting in the successful modulation of excessive Th2 cell specific antibody response. Immune modulation occurred regardless of whether the allergen was expressed as an intracellular, extracellular (secreted) or cell-wall anchored antigen [97].

PRACTICAL APPLICATIONS OF GUT MICROBIOTA MANIPULATION

Clinical studies on human subjects have confirmed the benefits of probiotic use on various GI diseases including irritable bowel syndrome, the elimination of H. Pylori, inflammatory bowel disease (especially ulcerative colitis) and diarrheas, in addition to allergic diseases. This is due to their anti-pathogenic properties, discussed previously in section III. The use of probiotics has also been shown to ameliorate weight loss in obese subjects, insulin resistance in metabolic syndrome, type 2 diabetes and non-alcoholic fatty liver disease. It is important to note that an altered Bacteroidetes/Firmicutes ratio is not limited to allergic diseases, and has also been observed in obese and diabetic patients, as well as those with autoimmune diseases, linking the gut microbiota to a plethora of human afflictions, thus highlighting the immense potential for therapeutic intervention at this level. Furthermore, probiotics may be used in the prevention and treatment of urinary tract infections and bacterial vaginosis. Probiotics have been shown to regulate angiogenesis, while some strains of Lactobacillus fermentum were found to potently suppress colorectal cancer cell growth and promote normal colonic epithelialisation, giving rise to possible anti-cancer applications. The exciting and relatively new field of gut microbiota manipulation is vast and the applications towards improving human health are numerous, and not limited to allergic disease [98,99].

CONCLUSION

The GI microbiota is an extremely complex ecosystem, which comprises diverse commensal micro-organisms. There is a sensitive period from 0-6 months, during which the microbiota is particularly susceptible to internal and external sources of perturbation. Also, during this period, the immune system is programmed, through exposure, to distinguish between pathogenic factors and commensal organisms, as well as food, stimulating the mechanisms of tolerance, where appropriate. Consequently, this period of immune system development presents a window of opportunity for therapeutic intervention, to avoid the development of an allergy-promoting immune phenotype in those at risk by ensuring an optimal GI environment.

The connection between microbial colonization during early life and susceptibility to food allergies is an increasingly studied topic, due to its potential applicability in treating this pathology. More specifically, the incidence of this disease seems to be greatly reduced by addressing modifiable external factors acting on the GI microbiota, such as the use of prebiotics and probiotics, together with avoidance of excessive antibiotics use, as well as more recently described therapies, such as the use of hUC-MSC and microbiota engineering. Improved knowledge of the mechanisms involved may lead to advancements in personalized medicine, targeting specific pathways resulting in the allergic phenotype. Future studies should focus on further development of appropriate therapeutic interventions and their evaluation.

REFERENCES

1. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157:121-141.
2. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59-65.
3. Huang YJ, Marsland BJ, Bunyavanich S, Mahony LO, Leung DYM, Muraro A, et al. The Microbiome in Allergic Disease: Current Understanding and Future opportunities-2017 PRACTALL Document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. J Allergy Clin Immunol. 2017;139:1099–1110.
4. Reynolds LA, Finlay BB. A case for antibiotic perturbation of the microbiota leading to allergy development. Expert Rev Clin Immunol. 2013;9:1019-1030.
5. Chen CC, Chen KJ, Kong MS, Chang H, Huang J, et al. Alterations in the gut microbiotas of children with food sensitization in early life. Pediatr Allergy Immunol. 2016;27:254-262.
6. Gupta R, Sheikh A, Strachan DP, Anderson HR, et al. Time trends in allergic disorders in the UK. Thorax. 2007;62:91-96.
7. Li M, Wang M, Donovan SM. Early development of the gut microbiome and immune-mediated childhood disorders. Semin Reprod Med. 2014;32:74-86.
8. Aitoro R, Paparo L, Amoroso A, Costanzo MD, Cosenza L, Granata V, et al. Gut microbiota as a target for preventive and therapeutic intervention against food allergy. Nutrients. 2017;9:672.
9. Stiemsma LT, Reynolds LA, Turvey SE, Finlay BB. The hygiene hypothesis: current perspectives and future therapies. Immunotargets Ther. 2015;4:143-157.
10. Boyce JA, Assaad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the united states: Summary of the NIAID-sponsored expert panel report. J Allergy Clin Immunol. 2010;126:1105-1018.
11. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski M L, et al. A revised nomenclature for allergy. An EAACI position statement From the EAACI Nomenclature Task Force. Allergy. 2001;56:813-824.
12. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised Nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Imunnol. 2004;113:832-836.
13. Simionescu R, Cherecheanu AP, Voinea L, Sfrenț-Cornățeanu R. TNF-α Gene Polymorphisms and Primary Open Angle Glaucoma in Romanian Population. Allergo Imunol Clin. 2015;12:6-10.

14. Hu Y, Chen J, Li H. Comparison of food allergy prevalence among Chinese infants in Chongqing, 2009 vs. 1999. Pediatr Int. 2010;52:820-824.
15. Pawankar R, Holgate ST, Canonica GW, Lockey RF, Blaiss MS. WAO White book on Allergy 2011-2012. Executive Summary. 2012:4.
16. Chin S, Vickery BP. Pathogenesis of FA in the pediatric patient. Curr Allergy Asthma Rep. 2012;12:621-629.
17. Mariat D, Firmesse O, Levenez F, Guimaraes Vd, Sokol H, Dore J, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC microbial. 2009;9:123.
18. McCoy KD, Köller Y. New developments providing mechanistic insight into the impact of the microbiota on allergic disease. Clin Immunol. 2015;159:170-176.
19. Clavel T, Desmarchelier C, Haller D, Gerard P, Rohn S, Lepage P, et al. Intestinal microbiota in metabolic diseases: From bacterial community structure and functions to species of pathophysiological relevance. Gut Microbes. 2014;5:544-551.
20. Rajilic-Stojanovic M, Smidt H, de Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. Env microbial. 2007;9:2125–2136..
21. Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut. 2008;57:1605-1615.
22. Gill SR, Pop M, Deboy RT, EckburgPB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312:1355-1359.
23. Mihajlovski A, Alric M, Brugere JF. A putative new order of methanogenic Archaea inhabiting the human gut, as revealed by molecular analyses of the mcrA gene. Res microbial. 2008;159:516-521.
24. Scanlan PD, Marchesi JR. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. ISME. 2008; 2:1183-1193.
25. Ott SJ, Kuhbacher T, Musfeldt M, Rosenstiel P, Hellmig S, Rehman A, et al. Fungi and inflammatory bowel diseases: Alterations of composition and diversity. Scan J Gastroenterol. 2008;43:831-841.
26. Pascal M, Perez-Gordo M, Caballero T, Escribese MM, Longo MNL, Luengo O, et al. Microbiome and allergic diseases. Front Immunol. 2018;9:1584.
27. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015;26:26050.
28. Rodrigues, Huang YJ, Marsland BJ, O'Mahony L, Leung DYM, Muraro A, et al. The microbiome in allergic disease: Current understanding and future opportunities. J. Allergy Clin Immunol 2017;139:1099-1110.
29. Moles L, Gómez M, Heilig H, Bustos G, Fuentes S, de Vos W, et al. Bacterial diversity in meconium of preterm neonates and evolution of their fecal microbiota during the first month of life. PLoS One. 2013;8:66986.
30. Jimenez E, Fernandez L, Marin ML, Martín R, Odriozola JM, Nueno-Palop C, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005;51:270-274.
31. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile. Res Microbiol. 2008;159:187-193.
32. Koleva PT, Kim JS, Scott JA, Kozyrskyj AL, et al. Microbial programming of health and disease starts during fetal life. Birth Defects Res C Embryo Today. 2015;105:265-277.
33. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486: 222-227.
34. Azad MB, Konya T, Guttman DS, Sears MR, HayGlass K T, Mandhane P J, et al. Infant gut microbiota and food sensitization: Associations in the first year of life. Clin Exp Allergy. 2015;45:632-43.
35. Ling Z, Li Z, Liu X, Cheng Y, Luo Y, Tong X, et al. Altered fecal microbiota composition associated with food allergy in infants. Appl Environ Microbiol. 2014;80:2546-2554.
36. Berni Canani R, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, et al. Lactobacillus rhamnosus GG-supplemented formula expands butyrate producing bacterial strains in food allergic infants. ISME J 2016; 10: 742-50.
37. Bunyavanich S, Shen N, Grishin A, Wood R, Burks W, Dawson P, et al. Early-life gut microbiome composition and milk allergy resolution. J Allergy Clin Immunol 2016;138:1122-130.
38. Hua X, Goedert JJ, Pu A, Yu G, Shi J. Allergy associations with the adult fecal microbiota: analysis of the American Gut Project. EBioMedicine 2016;3:172-179.
39. Yan N, Xu J, Zhao C. Human umbilical cord-derived mesenchymal stem cells ameliorate the enteropathy of food allergies in mice. Exp Ther Med. 2018;16:4445-4456.
40. Bull MJ, Plummer NT. Part 1: The human gut microbiome in health and disease. Integrative Medicine: Clini J. 2014;13:17-22.
41. Thomas S, Izard J, Walsh E. The host microbiome regulates and maintains human health: A primer and perspective for non-microbiologists. Cancer Res. 2017;77:1783-1812.
42. Yasmin F, Tun HM, Konya TB. Cesarean section, formula feeding, and infant antibiotic exposure: Separate and combined impacts on gut microbial changes in later infancy. Front Pediatr. 2017;5:200.
43. Rutayisire E, Huang K, Liu Y. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016;16:86.
44. Prince BT, Mandel MJ, Nadeau K. Gut microbiome and the development of food allergy and allergic disease. Pediatr Clin North Am. 2015;62:1479-1492.
45. Lee YY, Hassan SA, Ismail IH, Chong SY, Raja Ali RA, Amin Nordin S, et al. Gut microbiota in early life and its influence on health and disease: A position paper by the Malaysian Working Group on Gastrointestinal Health. J Paediatr Child Health 2017;53:1152-1158.
46. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med. 2016;22:250-253.
47. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A, et al. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr. 2012;96:544-551.
48. Newburg DS. Innate immunity and human milk. J Nutr 2005; 135:1308-1312.
49. van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V, et al. Shaping the gut microbiota by breastfeeding: The gateway to allergy prevention? Front Pediatr. 2019;27:47.
50. Murphy K, Curley D, O'Callaghan TF. The composition of human milk and infant faecal microbiota over the first three months of life: A pilot study. Nature. 2017;7:40597.
51. Ballard O and Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatric Clin North Am. 2013;60:49-74.

52. Isolauri E, Rautava S and Salminen S. Probiotics in the development and treatment of allergic disease. Gastroenterol Clin North Am. 2012;41:747-762.
53. Kainonen E, Rautava S and Isolauri E. Immunological programming by breast milk creates an anti-inflammatory cytokine milieu in breast-fed infants compared to formula-fed infants. Brit J Nutr 2013;109:1962-1970.
54. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-β regulation of immune responses. Annu Rev Immunol. 2006;24:99-146.
55. David Bauché, Julien C Marie. Transforming growth factor β : A master regulator of the gut microbiota and immune cell interactions. Clin Trans Immunology. 2017;6:136.
56. Bridgman SL, Konya T, Azad MB, Sears MR. Infant gut immunity: A preliminary study of IgA associations with breastfeeding. J Dev Orig Hlth Dis. 2015;7:68-72.
57. Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, et al. The genome sequence of Bifidobacterium longum subsp infantis reveals adaptations for milk utilization within the infant microbiome. Proc Nat Acad Sci. 2008;105:18964-18969.
58. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. Microbiome. 2015;3:13.
59. Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci. 2011;108:4653-4658.
60. Kunz C, Rudloff S, Baier W, Klein N, Strobel S. Oligosaccharides in human milk: Structural, functional, and metabolic aspects. Ann Rev Nutr. 2000;20:699-722.
61. Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. Ann Nutr Metab. 2016;69:42-51.
62. Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut hemostasis and human diseases. BMC Immunol. 2017;18:2.
63. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016;16:341-352.
64. Molloy J, Allen K, Collier F, Tang ML, Ward AC, Vuillermin P, et al. The potential link between gut microbiota and IgE-mediated food allergy in early life. Int J Environ Res Public Health. 2013;10:7235-7256.
65. Untersmayr E, Bax HJ, Bergmann C. AllergoOncology: Microbiota in allergy and cancer: A European Academy for Allergy and Clinical Immunology position paper. Allergy. 2019;74:1037-1051.
66. Zelante T, Iannitti RG, Cunha C, Luca AD, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013;39:372-385.
67. Pieters R, Schulz V, Bol-Schoenmakers M, Smit J. AhR pathway activation prevents food allergy in mice partly by preserving CD25-positive Tregs in the thymus. Clin Trans Allergy. 2013;3:43.
68. Wlodarska M, Luo C, Kolde R, Hennezel E, Annand JW, Heim CE, et al. Indoleacrylic acid produced by commensal peptostreptococcus species suppresses inflammation. Cell Host Microbe. 2017;22:25-37.
69. Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T. The maternal microbiota drives early postnatal innate immune development. Science. 2016;351:1296-1302.
70. Di Martino ML, Campilongo R, Casalino M. Polyamines: Emerging players in bacteria-host interactions. Int J Med Microbiol. 2013;303:484-491.
71. Perez-Cano FJ, González-Castro A, Castellote C. Influence of breast milk polyamines on suckling rat immune system maturation. Dev Comp Immunol. 2010;34:210-218.
72. Wang Q, McLoughlin RM, Cobb BA. A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. J Exp Med. 2006;203:2853–2863.
73. Mazmanian SK, Liu CH, Tzianabos AO. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122:107-118.
74. Nylund L, Satokari R, Nikkila J. Microarray analysis reveals marked intestinal microbiota aberrancy in infants having eczema compared to healthy children in at-risk for atopic disease. BMC Microbiol. 2013;13:12.
75. Arpaia N, Campbell C, Fan X. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013;504:451-455.
76. Commins SP. Mechanisms of oral tolerance. Pediatr Clin North Am. 2016; 62:1523-1529.
77. Pabst O and Mowat AM. Oral tolerance to food protein. Mucosal Immunol. 2012;5:232-239.
78. Woodfolk JA, Commins SP, Schuyler AJ. Allergens, sources, particles, and molecules: Why do we make IgE responses. Allergol Int. 2015;64:295-303.
79. Stefka AT, Feehleya T, Tripathia P, et al. Commensal bacteria protect against food allergen sensitization. PNAS. 2014;111:13145-13150.
80. Atarashi K, Tanoue T, Shima T. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011;331:337-341.
81. Penders J, Thijs C, van den Brandt PA. Gut microbiota composition and development of atopic manifestations in infancy: The KOALA Birth Cohort Study. Gut. 2007;56:661-667.
82. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504:446-450.
83. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, et al. Mucosal immunology. The microbiota regulates type 2 immunity through RORgammat(+) T cells. Science. 2015;349:989-993.
84. Hunter JE, Butterworth JA, Zhao B, Sellier H, Campbell K J, Thomas H D, et al. The NF-kB subunit c-Rel regulates Bach2 tumour suppressor expression in B-cell lymphoma. Oncogene. 2015;1-9.
85. Raghunath P. Role of Gut Microbiota and Infectious Burden in the Development of Autoimmune and Allergic Diseases. Iran J Allergy Asthma Immunol. 2017;16:77-78.
86. Hoeppli RE, Wu D, Cook L. The environment of regulatory T cell biology: cytokines, metabolites, and the microbiome. Front Immunol. 2015;6:61.
87. Lathrop SK, Bloom SM, Rao SM. Peripheral education of the immune system by colonic commensal microbiota. Nature. 2011;478:250-254.
88. Feehley T, Stefka AT, Cao S. Microbial regulation of allergic responses to food. Semin Immunopathol. 2012;34:10-15.
89. Liston A, Enders A, Siggs OM. Unravelling the association of partial T-cell immunodeficiency and immune dysregulation. Nat Rev Immunol. 2008;8:545-558.
90. Cahenzli J, Köller Y, Wyss M. Intestinal microbiota diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe. 2013;14:559-570.

91. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. J Allergy Clin Immunol. 2004;114:377-382.
92. Mestas J, Hughes CCW. Of Mice and Not Men: Differences between mouse and human immunology. J Immunol. 2004;172:2731-2738
93. Nowak-Węgrzyn A, Sampson HA. Future Therapies for Food Allergies. J Allergy Clin Immunol. 2011;127:558-573.
94. Tang ML, Ponsonby AL, Orsini F. Administration of a probiotic with peanut oral immunotherapy: A randomized trial. J Allergy Clin Immunol. 2015;135:737-744.
95. Tan-Lim CSC, Esteban-Ipac NAR. Probiotics as treatment for food allergies among pediatric patients: a meta-analysis. World Allergy Organ J. 2018;11:25.
96. Qamer S, Deshmukh M, Patole S. Probiotics for cow's milk protein allergy: a systematic review of randomized controlled trials. Eur J Pediatr. 2019;178:1139-1149.
97. Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E. Lactic acid bacteria: Reviewing the potential of a promising delivery live vector for biomedical purposes. Micro Cell Fact. 2015;14:137.
98. Markowiak P, Slizewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017;9:1021.
99. Kerrya RG, Patrab JK, Goudac S, Park Y, Shin H, Das G. Benefaction of probiotics for human health: a review. J food drug anal. 2018; 26:927-939.