Leading Article

Human microbiome and its role in paediatric allergic disease

***Sanath Thushara Kudagammana[1], Gihani Udeshika Vidanapathirana[2]**

Sri Lanka Journal of Child Health, 2020; **49**(2): 102-107
DOI: http://dx.doi.org/10.4038/sljch.v49i2.8956
(Keywords: Microbiome, children, allergy, asthma, atopic dermatitis, dysbiosis, food allergy).

Human microbiome

Microbiome is the group of microorganisms (bacteria, fungi, archae-bacteria and protozoa) and viruses that colonize a specific environment physiologically, or sometimes pathologically, and co-exists in a symbiotic relationship with the body[1]. Though found mainly in the colon, they are also found in other anatomical sites, including respiratory tract, conjunctiva, genital tract, skin, mammary glands, seminal fluid, saliva, oral mucosa, biliary tract, and other parts of the gastrointestinal tract[1].

During the newborn period the baby is exposed to groups of microorganisms for the first time in their lives and starts developing their microbiome[2]. The mode of delivery determines the initial bacterial inoculum of the newborn. Vaginally delivered infants have a microbiome composition more similar to vaginal flora of the mother, containing *Lactobacillus*, *Prevotella*, *Atopobium* and *Sneathia* species[2]. In contrast, infants born via caesarean-section have a less diverse microbiome that is more similar to the maternal skin flora[2].

Breast feeding is considered to be a major factor in the establishment of the infant microbiome and contains both important prebiotics and probiotics to establish the infant's gut microbiome[3]. Further changes in the microbiome are influenced by the type of diet during early infancy[3]. They include breast-feeding versus artificial feeding and the timing of introduction of solid food to the diet[4]. Other factors such as antibiotic use, and the genetics of the infant too have been shown to be of importance[4]. With the diversification of the diet, by the age of 3, the microbiome slowly shifts towards an adult-like configuration[4].

A study conducted in USA, comparing intestinal microbiota in healthy 1–4-year-old children and healthy adults revealed that Actinobacteria, Bacilli, Clostridium cluster IV and Bacteroides are the principal groups differentiating young children and adults[5]. Clostridium cluster XIVa, which is thought to be established at an early age, was equally predominant in young children from adults[5]. American children have significantly more *Bifidobacterium* species than adults from the same locality[5]. Nevertheless, generally, microbiota of young children show less diversity than those of adults[5].

Microbiome and immune modulation

Emerging evidence has proven that microbe colonization in humans during early life plays a crucial role in the establishment and maturation of the developmental pathways[1]. Microbiome influences host immune response in promoting distinct adaptive immune responses related to protecting against or developing a range of chronic inflammatory and autoimmune disorders[6].

Human T helper cells (TH) are a very important subpopulation of immune cells. TH1 is important for the body to fight and eradicate harmful agents and a predominance of TH2 enrolment promotes allergic reactions[7]. The newborn immune system is skewed towards a TH2 phenotype[6]. There is a limited activity of TH1-cell-polarizing cytokines which minimize the potentially harmful pro-inflammatory responses, allowing microbial colonization[6]. This state of impaired immune response predisposes neonates to opportunistic pathogenic infections as well[6]. Transition from TH2 to TH1 polarization takes place following multiple pathogenic encounters by the immune system which occurs in a time and age dependent way. This cascade of events, associated with change of microbiota, reduces the chance of the child developing allergies and atopy[6]. *Bacteroides fragilis* has been shown to modulate the TH1/TH2 balance[6].

Another important sub-population of T cells is the Regulator T cells (Tregs). They modulate immune system activity, maintain tolerance to self-antigens,

[1]*Senior Lecturer in Paediatrics,* [2]*Research Assistant, Department of Paediatrics, Faculty of Medicine, University of Peradeniya, Sri Lanka*

**Correspondence:* sanathusara@yahoo.com
orcid.org/ 0000-0002-5813-5907

The authors declare that there are no conflicts of interest
Personal funding was used for the project.

and prevent the development of autoimmune disease[7]. Recent studies have shown that symbiotic microbes, including the Clostridium species, modulate induction of Tregs[7].

Paediatric allergic diseases and the microbiome

Childhood asthma is the leading entity among a variety of allergic diseases that include allergic rhinitis, eczema, food allergy, drug allergy and chronic urticaria, affecting children[8]. The International Study on Asthma and Allergies in Childhood (ISAAC), the largest international study on allergic diseases, has shown the prevalence patterns in different countries of the world. The highest prevalence seems to be in English speaking developed countries and the lowest among some of the developing countries[8]. The phase three trial, conducted at least 5 years after phase one, has shown that the prevalence of allergic diseases is increasing in many countries especially in those countries shown to have lower prevalence in the phase one trial[8]. All of these allergic conditions have significant morbidity and childhood asthma especially, has an associated mortality as well[8].

In recent years, the possible role of the human microbiome in immune modulation and pathogenesis of allergic disease has been extensively studied. A potential link between the neonatal microbiome and genesis of allergic diseases has been identified in either contributing to or maintaining immune mediated inflammatory processes[9]. The "hygiene hypothesis" initially described the link between microbes and allergy[9]. The "microflora hypothesis", an extension of the "hygiene hypothesis", has been suggested with advances of human microbiome research[10]. The suggestion has been made that early-life environmental exposures have the ability to alter the composition of the microbiome, which causes the maturation of the immune system towards a hypersensitive or hyper-inflammatory state[10].

A dysbiosis or dysregulation of the microbiome in the neonatal period is important in the genesis of allergic disease due to its role in the disruption of immune maturation[6]. Among the TH cells, TH2 and TH17 cells direct an immune response coordinating B cells, eosinophils, mast cells and neutrophils[7]. TH2 cells produce interleukins (IL-4, IL-5, IL-9, IL-10, and IL-13)[7]. These are mediators of immune reactions stimulating B cells to produce eosinophils and IgE antibodies, which then enhance mast cells to release histamine, serotonin and leukotrienes, causing bronchoconstriction, urticaria and angioedema, thereby contributing to the allergic response[7].

Microbiome and asthma

Hyper-activation of the TH2 arm of the adaptive immune response resulting in overexpression of pro-inflammatory cytokines IL-4, IL-5, and IL-13, eosinophilia, and mast cell airway infiltration, are the immunological cascade of allergic asthma[10]. Asthma initiation and exacerbation are dependent on individual susceptibility, viral infection, allergen exposure, tobacco smoke exposure, and air pollution[11]. The possible relationship between the microbiome and asthma has been supported by a number of research findings.

Development of the nasopharyngeal microbiome in the infant has been linked to the risk of development of asthma[11]. Neonates whose oropharynxes were colonized by a large number of *Streptococcus pneumoniae, Haemophilus influenzae,* or *Moraxella catarrhalis* within the neonatal period were at increased risk of recurrent wheeze and asthma in later life[11]. They have significantly more eosinophils, and increased total serum IgE levels[11]. In a longitudinal study of postnasal aspirates of 234 Australian children, 6 separate bacterial microbiota types were identified. Each had a particular dominant genus viz. *Moraxella, Streptococcus, Corynebacterium, Staphylococcus, Haemophilus,* or *Alloicoccus,* which were linked to an increased risk of asthma[12]. Another study conducted using bronchial brushing or nasal and pharyngeal swabs of children, including 17 with asthma and 7 healthy subjects, revealed that asthma patients were colonized with bacteria in phylum Proteobacteria, which includes the genera *Haemophilus*, *Moraxella* and *Neisseria*[13].

There is some emerging evidence suggesting that the bacterial microbiota colonizing the respiratory mucosa can affect the host response to viral infection. There are some asthmagenic viruses, like respiratory syncytial virus (RSV) and rhinovirus that can increase the risk of developing asthma[14]. Mice nasally exposed to two distinct strains of commensal *Lactobacillus rhamnosus* have become susceptible towards subsequent RSV infection. Increased levels of IFN-b, IFN-g, IL-6, and TNF-a, in both BAL and serum samples, have contributed to viral clearance. These mice exhibited increased levels of IL-10, which reduced inflammation-related lung tissue damage[14].

Microbiota in sites other than the respiratory tract, too, have been shown to exert an effect on pathogenesis of asthma. A study carried out in pre-school children with a diagnosis of asthma has shown evidence of gut bacterial dysbiosis which has led to the concept of the 'gut–lung axis'[13]. The mechanism of pathogenesis can be achieved by systemic dissemination of metabolites produced by the gut microbes, like lipopolysaccharides,

peptidoglycans and short-chain fatty acids. These metabolites can be transferred to other anatomical sites through blood, where they may exert their anti-inflammatory properties[15]. Alternatively, bacteria can be directly seeded from the intestinal microbiota into the airways in which these bacteria can attack the local immune cells, shaping their responses[15].

Microbiome and allergic rhinitis (AR)

AR or hay fever is inflammation of nasal mucous membrane giving rise to sneezing, nasal congestion, nasal itching and rhinorrhoea[16]. It is caused by an exaggerated immune response to environmental allergens, most commonly pollen, dust mites and mold[17]. There are two types viz. seasonal, which occurs only during a certain time of the year, and perennial, which occurs throughout the year[16]. There is a high prevalence of AR in the paediatric population, significantly affecting their quality of life[16]. There is growing evidence that dysbiosis in the sino-nasal microbiome is a predisposing factor for the development of AR[17]. Past studies have implicated *Streptococcus pneumoniae* and *Haemophilus influenzae* as the principal pathogens isolated from patients with AR, and also confirmed in animal models[16]. Further, it has been identified that changes in commensal bacteria in allergic subjects can cause effects on sino-nasal epithelia, enhancing or blocking immune responses to allergen or other stimuli, with effects also on lower airway disease[17]. In a murine model, a breakdown in the normal microbial flora has been shown to lead to dysregulation of the IgE-Basophil axis causing high IgE levels and circulating basophils, eventually resulting in AR[17].

Microbiome and food allergies (FA)

Food allergies (FA) are also common in young children due to an altered immune response to some foods. Allergies to milk and egg proteins are the most common and allergies to peanuts, nuts, fish or shellfish become prevalent with age[18]. Most of the studies on microbiota and food allergies have focused on a potential link between the prevalence of some gut bacterial species and the onset of allergy to cow milk proteins. It has been revealed that a reduction in *Bifidobacterium* and *Lactobacillus* species at 1-2 months can be related to the onset of allergies at 5 years of age, thus demonstrating the protective effect of breast milk against food allergies[18]. A Spanish study compared the microbiome of 46 children allergic to cow milk proteins and 46 children with no allergies by analysing their gut microbiome. According to their findings, allergic subjects showed a greater variety of bacterial species compared to healthy subjects. After the supplementation of a 6-month hydrolysed milk diet, the microbiome of allergic subjects showed changes such as a decrease in *Bifidobacteria* and an increase in lactobacilli compared to healthy subjects[19].

Developing an effector or tolerance response to different antigens is a very important function of the gastro-intestinal tract (GIT). It is done by balancing the activities of TH1 and TH2 cells and regulating TH17 and Treg cells in the lamina propria[20]. Most antigens in the GIT originate from dietary sources and gut microbiota[20]. These antigens may affect immune tolerance by promoting activity of Treg cells to these dietary factors[20]. It is important to avoid an immune response to dietary antigens. Dysbiosis in the gut microbiome may disrupt mucosal immunological tolerance, leading to allergic disease including FA[20].

Microbiome and atopic dermatitis (AD)

AD is a chronic inflammatory cutaneous disorder with an impaired skin barrier leading to increased permeability, higher pH, greater risk of allergic sensitization, and lower protection against resident microbes[21]. Diversity of normal cutaneous flora depends on temperature, age, amount of sebum, sweat, etc[21]. Sebaceous sites are filled with lipophilic *Cutibacterium* species, while *Corynebacterium* and *Staphylococcus* species are found in moist areas[21]. *Cuticabacterium acnes* use skin lipids to make short-chain fatty acids which reduce microbial threats[21]. However, the most prevalent staphylococcal species on the skin of healthy subjects belonged to the genus *epidermidis* in association with other coagulase negative staphylococci (CoNS)[22]. These CoNS species can secrete antimicrobials, limiting the overgrowth and biofilm formation of *S. aureus*[22].

Antimicrobial peptides (AMPs) such as defensins, cathelicidin, and dermicidin are less abundant in AD skin due to the presence of TH2 cytokines, which makes the skin permeable to *S. aureus* colonization[22]. Another study has found that CoNS strains that express AMPs are abundant in normal skin but rarely detected in AD lesions[22]. Further, AMPs produced by commensal CoNS species *Staphylococcus epidermidis* and *Staphylococcus hominis* have the ability to minimize the S.aureus colonization[22]. Atopic skin shows a reduced diversity of bacterial species and this difference with healthy skin may contribute to this inflammation[21]. Interestingly, a gut microbiome dysbiosis associated with AD has been identified in several studies[23]. An elevated level of *Clostridium* and a decreased level of *Bifidobacterium* have been reported in the stools of atopic infants[23]. Narrow band ultraviolet B phototherapy and topical corticosteroids, which have the ability to increase microbial diversity and decrease *S. aureus* proportion on skin lesions after treatment, are the common treatments used to treat AD[21].

Microbiome and therapeutic options

There is a strong association of allergic diseases with the status of the microbiome[1,6]. Thus, it is not surprising that the microbiome is identified as a therapeutic target for treating allergic diseases. These therapeutic options manipulating microbiome are capable of developing into preventive therapy by restoring altered microbiome or as an adjuvant in specific immunotherapy[24]. The therapeutic options support the use of microorganisms – probiotics or substances that can be metabolized and contribute to the growth of some bacterial species – prebiotics to alter dysbiosis that cause allergic diseases[24]. A combination of prebiotics and probiotics can produce synergistic health benefits – a symbiotic[24]. In a randomized controlled trial, extensively hydrolysed casein formula supplemented with *L. rhamnosus* GG has been studied in comparison to formula-only as a therapeutic option in children with IgE specific cow milk protein allergy. The acquisition of tolerance at 36 months was greater for infants fed with formula plus probiotic in comparison with the formula only group[25].

Faecal microbiota transplantation is a novel therapeutic modality where faeces from a healthy donor is transferred to the intestinal tract of a recipient patient[24]. Faecal microbial transfers from healthy subjects have been successful in manipulating the microbiome from a state of disease, back to a state of health in the treatment of recurrent *Clostridium difficile* infections[24]. This is another potential therapeutic modality to treat or prevent allergic diseases in children. Probiotics or topical skin prebiotics, which could modulate the growth of protective species and controlling the overgrowth of *S. aureus*, are more widely used as a treatment option of AD[22].

Conclusion

The microbiota is a highly dynamic environment influenced by genetics and multiple external environmental and dietary factors. The preliminary links shown between the immunomodulatory role of the microbiome and occurrence of respiratory, skin and food allergies of children have now been disclosed with firm evidence. Interventions with probiotics, prebiotics, or symbiotics have shown effectiveness for the development of preventive therapies and a treatment option for allergic diseases. This may work either by removing the dysbiosis which originally altered the microbiome or as a boosting of the immunological system in specific immunotherapy. Further studies will be needed to explore this aspect of management of allergic diseases which carries a lot of promise for the future with the advantage of having a minimum side effect profile.

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