Human Microbiome and Allergic Diseases in Children: Pathogenetic Role and Therapeutic Options

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Abstract: The recent and extensive study of the microbiome has provided an enormous amount of data concerning the type and possible functions of microorganisms present in the gut, airways, genital tract, and skin. These data showed interpersonal differences in the composition of the microbiome and these differences suggest a link between the microbiome, the immune modulation, and the pathogenesis of allergic diseases.

This research is particularly relevant in paediatrics, since allergic diseases are constantly increasing and there is evidence in the paediatric age that shows that the composition of the microbiome in the foetal and neonatal period plays a key role in the development of the immune system: vaginal delivery, breastfeeding, childhood spent in rural environments and/or in contact with animals result in a greater biodiversity of the microbiome with the presence of protective species that reduce the activation of Th2 lymphocytes, involved in allergic reactions.

Further studies are necessary to better understand the microbiota role in the pathogenesis of atopy in order to understand if specific probiotics and prebiotics, administered orally or topically, can affect the microbiota composition and modulate immune system functions, producing a therapeutic effect in the treatment of allergic diseases.

This narrative review analysed the available literature regarding the correlation between the microbiome and the development of allergic diseases and with special focus on paediatric studies. The skin, gut or lung dysbiosis can be a cofactor in the pathogenesis of allergies and the remodulation of the microbiome becomes an important therapeutic challenge.

Keywords: Allergy, asthma, atopic, children, dermatitis, dysbiosis, food allergy, infant, microbiome, microbiota, pediatric.

1. INTRODUCTION

In recent years, the potential role of human microbiome in immune modulation and in the pathogenesis of allergic diseases has been extensively studied.

The microbiome is defined as the set of microorganisms (bacteria, fungi, archaeabacteria and protozoa) and viruses that colonise a specific environment and that physiologically, or sometimes pathologically, co-exist in a symbiotic relationship with the human body [1].

The innovations in the molecular field, in particular the amplification and sequencing of the gene that encodes the 16S ribosomal RNA, allowed to obtain a large amount of data on the different bacterial species present in our body and map the biogeography of microorganisms that inhabit areas of the body such as the skin, intestines, the oral or vaginal mucosa [2].

The first studies aimed at finding links between the onset of allergic diseases and environmental factors using epidemiological data and developing the so-called hygiene hypothesis: it was found that respiratory allergies or atopic dermatitis were more prevalent in children from small families than those from large families. Further studies showed that children who had lived the first years of their lives in rural environments, in close contact with animals and had consumed unpasteurised milk had a lower incidence of allergies [3].

These studies led to the assumption of a role of the microbiome in these diseases, which allowed to identify some neonatal and maternal factors that can affect the development of the immune system in the first months of life and thus determine a key role in the potential to develop allergic diseases [4].
Indeed, in immunology, allergic patients show an increased Th2 lymphocytes (T helper 2 lymphocytes) response which secrete inflammatory cytokines such as interleukin 4, 5, 13 and which generate eosinophils and the IgE-mediated response. Tissues in asthma sufferers, during acute allergic reactions, show a high concentration of activated Th2 lymphocytes and mast cells. Atopic eczema, on the other hand, is mainly caused by Th1 lymphocytes (T helper 1 lymphocytes): these lymphocytes are naturally responsible for the eradication of intracellular microorganisms and this immune response, when over-expressed, causes eczematous lesions to become chronic. The action of interferon-γ and tumour necrosis factor-α, produced by the activation of Th1 lymphocytes, causes the activation and apoptosis of epithelial cells, which characterises eczematous skin lesions [5].

The gut microbiome is an important post-natal immune regulator that promotes the immune maturation of Th1 and Treg lymphocyte functions and suppresses the Th2 response, which is prevalent in the foetal period. A dysbiosis, intended as a dysregulation of the microbiome, especially if present in the neonatal period, can be a cofactor in the genesis of allergic diseases due to its role in the disruption of immune maturation [6].

Currently, factors that are known to lead to the establishment of a protective microbiome against allergic diseases include: absence of antibiotic therapies in the early years of life, exclusive breastfeeding for the first 4 months, vaginal delivery, presence of pets in the home during infancy or during pregnancy and absence of maternal antibiotic therapies during pregnancy. All these factors, indeed, are linked to lower rates of childhood allergies [3].

But changes in the gut, skin or oral microbiome have been found not only as a result of antibiotic therapies but also as a result of dietary changes, sleep deprivation, living with animals, work environment (rural areas or cities), thus broadening the potential applications of these microorganisms [2].

The role of the dysregulation of the gut microbiome, following antibiotic therapies, has been well described in the relevant literature through studies carried out on gut infections caused by C. difficile: the growth of this bacterium and the resulting stage of infection have been effectively inhibited through the use of faecal transplant in order to correct the gut dysbiosis. This therapeutic success support the finding of microorganisms (also called probiotics) or substances that can be metabolised and contribute to the growth of some bacterial species (prebiotics) used to treat other states of dysbiosis that cause allergic diseases [1].

A potential link between the microbiome and the genesis of allergic diseases has been investigated by a number of studies in recent years, confirming the role of these microorganisms (especially those present in the neonatal microbiome) in either contributing to or maintaining immune-mediated inflammatory processes [7].

The presence of the microbiome in the skin and gut has been known for some time now. As for its presence in the lungs, the notion that the lower airways were a sterile site was dispelled in 2010 by the discovery of microbial species through DNA sequencing techniques, which allowed the isolation of microorganisms that could not be detected by the culture method. This finding fostered research on a potential link between lung diseases and lower airway dysbiosis leading to the detection of different patterns of microorganisms in different diseases, including allergic diseases such as asthma [8].

2. METHODS

This literature review focuses on major studies concerning the interactions between microbiome and allergic diseases, specifically addressing respiratory, food and skin allergies.

The research was conducted using PubMed, searching for articles in English, published in the last 10 years (2009-2019), using relevant keywords such as eczema, dermatitis, atopic, asthma, allergy, microbiome, microbiota, 16S rRNA, Lactobacillus, Bacteroides, Bifidobacterium, feces, faeces, stool, gut, skin, lung.

We also included articles published on RIAP, an important Italian journal of Pediatric Society allergology and immunology.

We included the following article types: reviews, systematic reviews and clinical trials concerned with the relation between microbiota and allergy diseases in pediatric population. No studies on adult patients were included in this paper. Relevance was given to studies focusing on the immunological aspects of this interaction such as the role of some microbiome’s species in the secretion pattern of cytokines with immunomodulation function on lung, skin and gut for the development of allergy disease. The studies that will be analysed have been realized on human or animal models (murine) (Algorithm 1).

3. FINDINGS

3.1. Respiratory Allergies

Among respiratory allergies, asthma has been the main focus of scientific works in the study of the microbiome.

This chronic disease is on the rise and affects 10% of children worldwide [9]. It is characterised by a bronchial obstruction that, in most cases, regresses spontaneously or after therapy, causes bronchial hyperreactivity and a rapid drop in lung capacity, which may result in an irreversible airway obstruction. A number of factors are involved in the pathogenesis of this disorder, in particular infiltration of inflammatory cells, release of mediators and airways remodeling [10].

Research into the potential link between the microbiome and this disease was prompted by the discovery of the lung microbiome, i.e. the presence of microorganisms (detected by DNA amplification using 16S RNA) that led to a revision of the theory on the sterility of the lower airways [8].

Indeed, in 2010 Hilt M. et al. tested, using bronchial brushing or nasal and pharyngeal swabs, 20 children including 17 with asthma and 7 healthy subjects: the findings revealed that asthma patients had more germs belonging to the phylum of Proteobacteria, which includes the genera Haemophilus, Moraxella and Neisseria, as opposed to healthy subjects in which there was a prevalence of phylum Bacteroidetes of the genus Prevotella, make up the normal oral and vaginal flora [11].
Subsequently, Dickson’s study showed that phylum *Firmicutes* is also widespread in healthy individuals, although it generally colonizes upper airways [12].

In 2007 a prospective study recruited 411 newborns of asthmatic mothers, therefore considered at risk for developing asthma. The microbiota collected from hypopharynx aspirates at 1 month of age was analysed and children were monitored up to 5 years old, recording recurrent wheezing episodes. It was found that children colonized in the hypopharyngeal region with *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, or with a combination of these organisms, were at higher risk for recurrent wheeze and asthma early in life [13].

Some studies have reported a lower incidence of asthma in children living in a rural area compared to those living in urban areas: exposure to animals, above all, plays a protective role against bronchial hyperreactivity, not only in children but this also occurs in the foetal period; mothers who spent their pregnancies in rural areas have less chance of having children with asthma suggesting a modulation of the foetal immune system by the microbiome [14].

The finding in recent years of a gut-lung axis in which the gut microbiome can affect the pathogenesis of respiratory allergies is significant: studies have shown that children grew up with animals were less likely to develop allergic diseases. An experimental study was carried out on mice fed with domestic dust from houses in which a dog was present; following the administration of an allergen or the inoculation of respiratory syncytial virus, mice fed with this dust showed reduced lung inflammation compared to those who had not ingested this dust. A higher concentration of *Lactobacillus johnsonii* was found in the intestines of inoculated mice demonstrating the effect of the gut microbiome on lung allergic reactions [15].

Immunology provides an explanation for this evidence, an inadequate presence of induced Treg lymphocytes in the intestine (in the mesenteric lymph nodes), lead to increased growth of Th2 lymphocytes with the consequent production of pro-inflammatory cytokines (IL4 and IL 6) which cause inflammation as a result of a lung allergic reaction [8].

Further evidences have been provided by recent studies on mice, which have shown that a diet rich in fermented fibres containing *Firmicutes and Bacteroidetes* increases the short-chain fatty acids inhibiting dendritic cells Th2-response, thereby providing protection against allergic lung inflammation [16].

Studies conducted on newborns delivered vaginally or by caesarean section have shown that the colonisation of various areas (skin, mouth, intestines) consists of species such as *Sneathia and Lactobacillus spp.* (bacteria present in the maternal genital tract), while children delivered by caesarean section show a prevalence of *Staphylococcus and Streptococcus spp.*. These findings support previous epidemiological studies showing a reduced risk of developing allergic diseases in children delivered vaginally [8].

### 3.2. Food Allergies

Food allergies are the result of an altered immune response to some foods. It is not clear why some people develop sensitivity to some foods, which most people tolerate, but scientific evidences suggest that the environment plays a role in these processes [17].
In young children, allergies to milk and egg proteins are the most common, but as they grow, allergies to peanuts, nuts, fish or shellfish become prevalent [18]. It is now clear how food affects the microbiome, but studies shown that colonisation by different microorganisms (and the consequent interaction with the immune system) occurs mainly over a 1000-day period between the prenatal period and the neonatal period. Within this time frame, indeed, the microbiome develops individual-specific features, acquiring some species that will characterise it throughout its life [19].

Indeed, through vaginal delivery, the newborn comes into contact with its mother’s gut and genital tract microbiome, thus promoting colonisation by Bifidobacterium, Lactobacillus, Bacteroides and Clostridium [20]. In caesarean section colonisation occurs mainly by maternal skin microorganisms [21].

Studies about such differences in individuals are helping in identifying a potential link between the microbiome and food allergies. Most of the studies focused on a potential link between the prevalence of some gut bacterial species and the onset of allergy to cow’s milk proteins [23, 24, 26].

Breast milk is naturally colonised by Bifidobacteria and Lactobacilli, especially Bifidobacterium breve, Lactobacillus salivarius and Lactobacillus fermentum, but above all it contains oligosaccharides resistant to digestive enzymes promoting the growth of Bifidobacteria with an increase in short-chain fatty acids whose immunomodulatory effect has already been addressed in relation to asthma. Formula milk, on the other hand, shows greater diversification (Enterobacteriaceae, Enterococcus species and Bacteroides species) and does not contain Bifidobacterium resulting in lower production of short-chain fatty acids and thus greater inflammatory response of Th2 lymphocytes [17].

These findings were used to compare the microbiomes of healthy children with those suffering from food allergies. It has been demonstrated that a decrease in Bifidobacterium and Lactobacillus species at 1-2 months can be associated with the onset of allergies at 5 years of age, thus demonstrating the protective effect of breast milk against food allergies [22].

On the other hand, a Spanish study compared the microbiome of 46 children allergic to cow’s milk proteins and 46 children with no allergies by analysing their gut microbiome: allergic subjects showed a greater variety of bacterial species compared to healthy subjects and at the end of a 6-month hydrolysed milk diet the microbiome of allergic subjects showed changes such as a reduction in bifidobacteria and an increase in lactobacilli compared to healthy subjects [23].

An increase in bifidobacteria in with cow’s milk allergy (CMA) subjects is also supported by another study examining the microbiome of infants with CMA at 3 and 6 months of age. These findings were then reviewed at 8 years of age to see if these children outgrew CMA. A microbiome rich in Clostridia and Firmicutes is common in children who at 8 years of age shown an acquired tolerance to milk proteins, suggesting a potential role played by these microorganisms in the treatment of CMA [24].

Mouse models whose intestines were selectively colonised with only one bacterial species led to the identification of the genus Clostridia (thus supporting the previous findings) as a microorganism capable of protecting against the onset of allergies through its immunomodulatory effect on the intestines by interacting with lymphatic cells and modulating intestinal permeability [25].

In a randomized control 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’s milk allergy. The acquisition of tolerance at 36 months was greater for infants fed with formula + probiotic in comparison with formula-only group [26].

This result is a demonstration as that probiotics could be represent important therapeutic option in food allergy disease, but further studies needed.

### 3.3. Skin Allergies

Skin microbiome, as already mentioned, is strongly influenced during childbirth by delivery method.

If children with normal delivery are colonised by bacteria belonging to the maternal gastrointestinal and vaginal microbiota, those born by caesarean section shall be colonised by bacteria present on the skin [8].

This was demonstrated by a study published in 2010 that analysed the skin microbiome of 4 children born by vaginal delivery and 6 by caesarean section, confirming that Lactobacillus, Prevotella and Sneathia species were prevalent in children delivered naturally while Staphylococcus, Corynebacterium, and Propionibacterium were prevalent on the skin of children delivered by caesarean section [27].

Atopic dermatitis (AD) is a disease characterised by an impaired skin barrier that leads to an increase in permeability, higher pH, greater risk of allergic sensitisation and lower protection against resident microbes. This disease is on the rise in children and is therefore a subject of study, as little is known about the specific pathogenesis that makes the skin susceptible to chronic relapsing inflammation [28].

The global estimate of the prevalence of this disease is 20% in children and 2-5% in adults [29].

The skin microbiome was therefore studied to determine potential differences between the skin of healthy subjects and the skin of subjects suffering from this disease: the findings showed that individuals suffering from AD had a greater concentration of S. aureus compared to healthy subjects and that the composition of the skin microbiota underwent drastic changes as a result of corticosteroid therapy. During exacerbation, indeed, the skin showed reduced bacterial biodiversity that was gradually repopulated as a result of corticosteroid therapy. Conversely, the most prevalent staphylococcal species on the skin of healthy controls belonged to the genus epidermids that, in association with other CoNS (Coagulase negative staphylococci), can secrete antimicrobials that limit the overgrowth and biofilm formation of S. aureus [30].

The prevalence of S. aureus compared to S. epidermidis in patients suffering from AD provides a useful starting hint for further scientific research aiming primarily to define its
potential role in the disease and its link with caesarean sec-
tion, related to greater susceptibility to *S. aureus* colonisa-
tion.

This assumption is supported by a German study pub-
ished in 2018, which found a closer link between children
diagnosed with AD and delivery by caesarean section [6].

The presence of skin barrier impairments can also lead
to a loss of lipids such as sphingosines and ceramides that
act as substrates for the growth of some bacterial species
and prevent the growth of others: for instance, sphingosines
act as powerful antimicrobials against *S. aureus* and their
loss in the AD acute phase could favor the growth of *S. au-
reus* [31].

Not only bacterial species of the microbiota are impli-
cated in the development of atopic dermatitis: as described in
a review of Lujiani *et al.*, fungal Malassezia DNA has been
detected in 90% of atopic dermatitis skin lesions and could
contribute at the inflammatory process pathogenesis by se-
creting immunogenic proteins that induce the production of
proinflammatory cytokines on keratinocytes [32].

Several studies have instead focused on the analysis of the
faecal microbiome and its role in the development of
atopy: a systematic review published in 2018 and covering
44 studies analysing a potential link between the gut micro-
biome and AD found that currently the role of the gut micro-
biome in this disease is still disputed and therefore further
research is needed to determine a potential use of oral pro-
biotics in modulating the gut microbiome and thus reduce the
severity of dermatitis exacerbations [30].

Probiotics or topical skin prebiotics, which could modu-
late the growth of protective species and controlling the
overgrowth of *S. aureus*, could be more widely used. Indeed,
atopic skin shows a reduced diversity of bacterial species
and this difference with healthy skin can in part contribute to
this inflammation [7].

4. FINDINGS AND DISCUSSION

The examined studies show that, currently, the prelimi-
nary links between the immunomodulatory role of the mi-
crobiome and allergic diseases are gradually being disclosed.
It is possible to map the microorganisms involved in the
pathogenesis of respiratory, skin and food allergies by identi-
\-fying species that could act as either pathogens or protective
agents against these diseases.

Current studies agree on the key role played by the mi-
icrobiome at childbirth or in the first month of life and how it
is later less likely to lead to a greater susceptibility to allergic
diseases [7, 19].

The study of the human microbiome is paving the way
for new scientific findings. There is currently a wide range
of data available: a huge database of bacterial species isolat-
ed from different body areas is now available to scientists,
whose challenge is to identify any potential dysbiosis re-
sponsible for allergic diseases [1].

The human microbiota, however, in addition to bacterial
species, also consists of viruses and fungi that currently do
not yet command the same standards of decoding and analy-
sis as those that have been carried out for bacteria and there-
fore are additional information to date still under-researched
[2]. The potential inflammatory role played by fungi on the
pathogenesis of asthma is indeed little discussed in the litera-
ture, as the analysis of the approximately 1200 viral species
that are present in our body [7].

Summarizing the results illustrated in this review, we
observe that in the literature a list of bacteria that are fre-
cently present in pediatric patients with respiratory, food or
skin allergies begins to emerge. In particular we have seen
the presence of germs belonging to the phylum of Proteo-
bacteria, which includes the genera *Haemophilus, Morax-
ella* and *Neisseria* [11] and *S. pneumonia* [13] in the pediat-
ric population diagnosed with asthma. In relation to allergy
to cow’s milk protein, it has been demonstrated that a de-
crease in *Bifidobacterium* and *Lactobacillus* species can be
associated with the onset of allergy [22], and that supplement-
ing with *L. rhamnosus GG* can accelerate the develop-
ment of tolerance to milk [26]. Finally, in atopic dermatitis,
*S. aureus* is the prevalent bacterium in the skin in affected
subjects, whereas in the skin of healthy controls the predom-
ant bacteria belong to the genus *epidermidis* [30].

Many evidences pointed out that children with allergic
sensitization, eczema or asthma have a lower diversity of the
intestinal microbiota, but recently it was denied by studies
with modern sequencing techniques proving that particular
microbes might have more influence than diversity on devel-
oping allergic diseases [7].

Probiotics are live microorganisms which, when adminis-
tered in adequate amounts, confer a health benefit to the
host. The definition of a probiotic however does not explain
what kinds of potential health benefits it confers, it is also
clear that not all probiotics will influence the immune system
in the same way [4]. In this review, we illustrated how some
bacterial strains are probably correlated with a protective
role in allergic disease (e.g. *L. rhamnosus GG* in infants with
cow’s milk allergy [26]). However, further research is need-
ed to better identify bacterial strains with a protective role in
allergic disease.

CONCLUSION

In conclusion, allergic disease are on the rise in children
and new disease modifying therapies are demanded. Probiot-
ics can reduce the risk of allergic sensitization and AD inci-
dence and severity, but not asthma incidence. Early-life mi-
crobiota influences the development of allergic diseases.

Further studies are needed to define gut microbial eco-
system associated with increased risk of allergic disease and
asthma in order to identify high-risk infants and new ap-
proach to allergic diseases, such as modifying gut
microbiota.

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CONFLICT OF INTEREST

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REFERENCES