

Allergies

THE ROLE OF MICROBIOTA



BIOCODEX 
Microbiota Institute

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1 ● ASTHMA



Asthma is considered a major public health issue. Since the end of the 20th century, there has been an increase in the prevalence of this condition which affects all generations, and particularly children. The disease causes varying degrees of respiratory difficulties depending on severity. Fortunately, there are treatments for acute attacks and disease-modifying treatments, which provide asthma patients with a better quality of life. This chronic inflammation of the bronchi has a multifactorial etiology: genetic, immunological and environmental. There are more and more breakthroughs in this growing research area, in particular with regard to the role of the different microbiota that are potentially involved—pulmonary, nasal, intestinal—in the onset and course of the disease. The first results allow new preventive and therapeutic avenues to be considered in the coming years.

Asthma and the respiratory microbiota: a close relationship

The respiratory system of healthy individuals has been disregarded for a long time as it was assumed to be sterile. However the lungs in particular have proven to contain a wealth of bacteria. Studies increasingly implicate these bacteria in the development of asthma. Their objective is to identify new therapeutic avenues.

Although the intestinal microbiota has been the subject of many studies in recent years, the other microbial communities (respiratory, among others) have not attracted the curiosity of researchers to the same degree. And for good reason! Thinking it to be sterile in healthy individuals, scientists excluded it from their field of study. This preconception was shattered in 2010 through the use of new cutting-edge

genetic analysis techniques¹: since then it has been established that bronchi and lungs of healthy individuals naturally host a true bacterial flora (mainly composed of *Proteobacteria*, *Firmicutes* and *Bacteroidetes*) which protects them against infections and allergies. Like the intestinal microbiota, this flora interacts continuously with the immune system. This discovery is an important step towards understanding the respiratory microbiota, whose role in pulmonary immunity is still poorly known and which is complex to study since its composition can vary according to sample quality and type (sputum, aspirate, bronchioloalveolar lavage...).



¹ Madan JC, Koestler DC, Stanton BA, et al. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. mBio 2012;3

Many factors involved

From birth², the respiratory microbiota is shaped by multiple factors: climate, geographical area, habitat, cohabitation with farm or domestic animals, mode of delivery, antibiotic intake before the age of two years. To this list is added any intake of antibiotics by the mother up to twenty-one months before the birth, and diet... Overall, the improvement in living conditions in developed countries saves lives but on another note contributes to the progression of asthma³. The development of hygiene in particular disrupts maturation of the immune system due to restricted contact with microbes. To understand the impact of the respiratory microbiota on the immune system and the occurrence of asthma in greater detail, researchers triggered a microbial imbalance –otherwise known as dysbiosis–in newborn mice, by exposing them to allergens at a very early age⁴ (in this case dust mites). As a result, the development of their immune system was disrupted and its functions were impaired, two factors that favour the occurrence of more or less long-term allergic asthma.

Microbiota and disease severity

Although this does not transfer accurately to humans, it is however known that colonization by certain bacteria before the age of one year can cause infantile asthma, and at least, persistent respiratory wheezing⁵. According to other studies⁶, the respiratory microbiota of healthy individuals is different from that of asthmatics, both in terms of abundance and diversity (particularly a decrease in *Bacteroidetes* and an increase in *Proteobacteria* in asthmatics). This difference depends on the severity of asthma, which may be mild, moderate, or severe. Unexpectedly, contrary to observations made in other diseases, the respiratory microbiota is more abundant and diversified in a case of moderately severe asthma than in a healthy individual. The flora of an individual with severe asthma, suffering additionally from severe bronchial obstruction is less diversified than that of an individual also with severe asthma but who does not have any obstruction. The relationships between microbiota and asthma exacerbation (prolonged attack), often associated with viral respiratory infections, remain to be identified.



KEY FIGURES

KEY FIGURES ON ASTHMA

235 million

cases across the world
(WHO estimate)

383,000

deaths across the world in 2015
(WHO estimate)

4 million

cases in France
(Source: Santé Publique France)

Almost 63,000

hospitalizations in France
taking all ages together,
2/3 of which involve children
under 15 years old
(Source: Santé Publique France)



2 Gilliland 3rd MG, Erb-Downward JR, Bassis CM, et al. Ecological succession of bacterial communities during conventionalization of germ-free mice. *Appl Environ Microbiol* 2012;78:2359–66

3 Barnig C, Martin C. *Asthma and the microbiome*. *Revue des Maladies Respiratoires* (2018) 35, 103–115

4 Gollwitzer ES, Saglani S, Trompette A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat Med* 2014;20:642–7

5 Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487–95

6 Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS One* 2010;5:e8578



New therapeutic avenues

The causal relationship that is now accepted between an imbalance of the respiratory microbiota and the occurrence of asthma—even if it is not yet known whether the dysbiosis is the cause or the consequence—opens new avenues for reflection. These could lead to new strategies for the prevention and management of this disorder, which according to the WHO, is underdiagnosed and undertreated⁷. This is particularly the case in the elderly if the onset of the disease occurs after the age of sixty: the concomitant presence of other age-linked disorders makes

diagnosis difficult⁸. Prebiotics and probiotics, which interact with the intestinal flora, could help regulate the immune response even if the relationship between these two microbiota themselves still needs to be better understood. An antibacterial treatment—or even a transplant of respiratory microbiota as is done successfully with intestinal microbiota—could help preserve and/or restore the respiratory flora and oppose or restrict the development of asthma. These hypotheses will require confirmation by future studies but the numerous possibilities are promising.

Secrets of the nasal flora

It seems logical that the respiratory microbiota should be implicated in a respiratory disease such as asthma... but this still requires proof! Some studies of the nasal microbiota are working to show its influence on the development of asthma and have just added a piece to the jigsaw.

While the microbiota of the lower respiratory tract (bronchi and lungs) has already been linked to the development of asthma⁹, the nasal microbiota has been little investigated from this perspective up until now. To remedy this, a team of American researchers focused on this topic. Their aim was to identify the composition of the nasal microbiota of asthma patients and compare it with that of healthy individuals in order to identify signs of its role in the disease. Samples were taken from the nasopharynx (at the end of the nasal cavity) over a one-year period, from individuals aged between 10 to 73 years old, some of whom presented with asthma exacerbation, other with a stable form, and finally others who were healthy controls¹⁰.

Distinct microbiota

The initial hypothesis was confirmed: the nasal microbiota of each group had significantly different composi-

tions. Asthmatic individuals has a microbiota richer in two families of bacteria (*Bacteroidetes* and *Proteobacteria*) compared to the control group. Four species were generally observed to a greater extent in asthmatic individuals: some already associated with localized inflammatory diseases (but never with asthma), others already reported in other respiratory disorders; finally others better known for their role in vaginosis. The microbes detected also differed depending on whether the form of the disease was exacerbated or stable.

Biomarkers within reach of a swab

This study is one of the largest conducted to date on the correlation between the nasal microbiota and asthma. Its results—to be consolidated by future analyses—represent an important step forward for research and are consistent with data collected for the lower airway study. On this basis it may be suggested that nasal microbiota, samples of which can be obtained much more easily and less invasively than by an internal bronchial examination (bronchoscopy), can be used to understand the role of the respiratory microbiota in the pathogenesis of asthma and its course. The bacteria identified could be subject



to more detailed analysis to clarify their respective roles and be considered a simple tool to detect and monitor asthma. These are numerous avenues to be explored in the future from a scientific perspective.

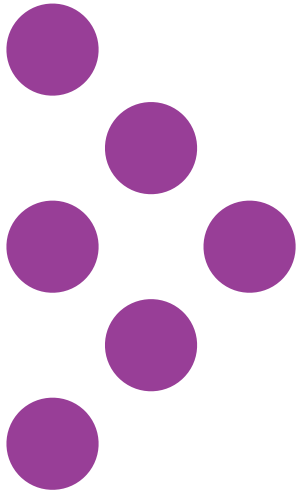
7 <http://www.who.int/mediacentre/factsheets/fs307/fr/>

8 Di Lorenzo G., Di Bon D. et al. *Immunological and non-immunological mechanisms of allergic diseases in the elderly: biological and clinical characteristics*. *Immunity & Ageing* (2017) 14:23 DOI 10.1186/s12979-017-0105-4

9 Gilstrap DL, Kraft M. *Asthma and the host-microbe interaction*. *Journal of Allergy and Clinical Immunology* 2013; 131:1449-50.e3

10 Fazlollahi M, Lee TD, Andrade J et al. *The Nasal Microbiome in Asthma*. *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.02.020

2 • FOOD ALLERGIES



According to the World Health Organization, food allergies are the fourth most common chronic disease worldwide. In France, they currently affect 3.5% of the general population, and almost 10% of children. In the same way as asthma, atopic dermatitis or rhinitis –other allergies which have multiplied in recent decades– they originate in a combination of environmental, societal and medical factors. Since the spectrum of allergic reactions ranges from simple redness (or erythema) to death, we need to take them very seriously. As a result of its link with the immune system, the study of the intestinal microbiota could provide insights and lead to preventive measures other than the exclusion of the allergen responsible or desensitization, when the latter approach is possible.

Egg allergy: involvement of the early intestinal microbiota

Egg allergy is the second most common food allergy in children, just behind cow's milk allergy. It affects almost 10% of children with an allergy under three years old. Although variations in the early intestinal microbiota have already been associated with sensitization or the onset of allergies to certain foods in the scientific literature, what is the situation for egg allergies?

The very rapid rise in the incidence of food allergies, incompatible with the time-line of genetic evolution, forces researchers to broaden their thinking to better understand the processes at work. While allergies, as other diseases, have been re-examined in the light of the growing understanding of the microbiota in recent years, some studies have taken a new



approach by focusing specifically on the link between the early intestinal microbiota and egg allergy¹¹. The study of children aged between three and sixteen months at enrolment showed that 46% of them were allergic to eggs exclusively and 71% were sensitized to eggs. Their intestinal microbiota was characterized by stool sample analysis, supplemented

by blood and skin tests to monitor the progression of their sensitization/ allergy. They were followed up regularly until the age of eight: most allergic children were no longer ill a few years later.

¹¹ Fazlollahi M., Chun Y., Griechin A. & al. *Early-life gut microbiome and egg allergy*. Allergy. 2018;1–10

Intestinal microbiota of allergic children: surprising results... actually not so surprising

This long-term follow-up shows that the early intestinal microbiota of children with egg allergy is more diversified than that of the control group. At first sight, this is an astonishing discovery in view of what is usually said in the literature, in which an opposite theory is sometimes advanced with respect to other pathologies such as obesity. But comparable results have previously been documented for asthma and the respiratory microbiota¹². Enough to alert the researchers, who know the common origins of the two allergies and who did not settle for microbial diversity alone to explain the role of the microbiota in the disease. The intestinal microbiota of children with egg allergy is moreover composed of families of bacteria distinct from that of healthy children, some (*Lachnospiraceae* and *Streptococcaceae*) being more abundant in the first group. Others, like lactic acid bac-

teria whose protective effects against allergy have already been observed in animals, were more abundant in the second group. Detailed genetic analyses also allowed researchers to note that certain bacteria present in the intestinal microbiota of the children with egg allergy modified the metabolism of purines—molecules present in the organism which are strongly involved in some biological reactions and already associated with peanut allergy in children¹³.

The role of resident bacteria still needs to be clarified

At the end of the eight-year study, the egg allergy had disappeared in 60% of the children initially affected. However the researchers did not note a significant difference in the early intestinal microbiota according to whether the allergy has ceased or persisted. This result remains to be confirmed by larger studies, which should also explore the role of resident bacteria. In any event, the discoveries already made open

the way to a preventive or therapeutic strategy for this widespread allergy.



12 Huang YJ, Nelson CE, Brodie EL, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol.* 2011;127:372-381. & Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD. Asthma associated differences in microbial composition of induced sputum. *J Allergy Clin Immunol.* 2013;131:346-352

13 Kong J, Chalcraft K, Mandur TS, et al. Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy. *Allergy.* 2015;70:495-505

Is there a microbial signature for our food allergies?

The first months of life are essential for the proper development of the immune system of newborns. This is closely linked to the composition of the intestinal microbiota, itself dependent on factors such as mode of delivery, digestive tract secretions, environment, diet... While food allergies are increasingly correlated with imbalances of the intestinal microbiota, the researchers hope to understand their onset by studying the first moments of life.

In the wake of the hygiene hypothesis which consists in believing that excessive hygiene in recent years is partly responsible for the explosion of allergies in the industrialized world, it is now accepted that an immune system less exposed to microorganisms in very early childhood can lead to allergies later in

THE KEY POINTS

HYGIENE HYPOTHESIS

Theory which supports a causal relationship between excessive hygiene and the development of allergies.

This excess is thought to cause:

- ❖ defective balance of Th1/Th2 cells, two immune cell lines (lymphocytes)
- ❖ domination by Th2 cells which triggers an allergic response of the immune system

The supporters of this theory consider that:

- ❖ populations with a high level of parasitic infection display a very low frequency of asthma and allergic rhinitis
- ❖ children contaminated by infectious agents early in childhood develop fewer allergies subsequently
- ❖ children living in the country and in constant contact with farm animals have fewer allergies than those living in towns.

(Source: ANSES)

life¹⁴. This problem, which is very acute in industrialized countries, finds a rather worrying echo in Japan where the incidence of allergies in three-year-old children in Tokyo rose from 8.5% in 2004 to 16.7% in 2014¹⁵. Faced with this public health issue, the local scientists are taking action.

In Japan, they know that everything (or almost everything) plays out before the age of one year

Some of their scientists examined the first year of life with the aim of finding a sort of “microbial signature” in infants who developed food allergies before they were two years old¹⁶. In their hypotheses, these researchers paid great attention to the two key moments in the development of the intestinal microbiota: the lactation period (usually up to nine months after birth), during which the flora is dominated by lactic

acid bacteria (*Bifidobacterium*); and the weaning period, during which the microbiota diversifies, becomes more abundant, and becomes closer to that of an adult¹⁷. During these periods, tolerance to food antigens—food-derived macromolecules external to the organism capable of triggering an immune response via the production of antibodies—develops. Any impairment of this process can then create allergies in children¹⁸. For this prospective study in 56 babies, the Japanese team analyzed stools at one month, two months, six months and one year: fourteen developed food allergies before they were two years old—ten of them even before they were one year old. By correlating these results with analyses of the microbiota, the researchers noticed that during the lactation period, and from the age of two months, certain lactic-acid producing bacteria were

already present in markedly smaller numbers in children who developed food allergies before the age of two years. This was enough to assign a protective role to certain species, present particularly in the mother’s milk and derived from the mother’s intestinal microbiota, according to some studies¹⁹. The researchers also noted considerable differences in bacteria which assimilate lactate (*Veillonella*), under-represented in the allergic children.

14 Feehley T, Stefka AT, Cao S et al. *Microbial regulation of allergic responses to food*. *Semin Immunopathol* 2012;34: 671–88

15 <http://www.fukushihoken.metro.tokyo.jp/allergy>

16 Tanaka M., Yuki Korenori Y., Washio M. et al. *Signatures in the gut microbiota of Japanese infants who developed food allergies in early childhood*, *FEMS Microbiology Ecology*, 93, 2017

17 Bergström A, Skov TH, Bahl MI et al. *Establishment of intestinal microbiota during early life: A longitudinal, explorative study of a large cohort of Danish infants*. *Appl Environ Microb* 2014;80:2889–900

18 Ling Z, Li Z, Liu X et al. *Altered fecal microbiota composition associated with food allergy in infants*. *Appl Environ Microb* 2014;80:2546–54

19 Jost T, Lacroix C, Braegger CP et al. *Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding*. *Environ Microbiol* 2014;16:2891–904



Lactation and weaning at the heart of the allergic process

During weaning, the intestinal microbiota of children with allergies (food and other allergies) was characterized by a greater abundance of some types of enterobacteria—one of the largest bacterial families—and that of children with food allergies was characterized by two species of *Clostridium* whose mode of action should be the subject of more detailed research. Moreover, the bacterial diversity of allergic children was significantly lower than that of healthy children. This is interpreted by

the researchers as a sign of the imminent onset of the allergy. Finally, these results—showing that very early impairment of the intestinal microbiota during lactation and weaning can cause food allergies—are globally consistent with those of previous studies conducted in other countries. Whether it is the dysbiosis that generates the disease (the allergy) or the other way around, remains to be determined. Thanks to the results of this research, the mother’s intestinal microbiota, a source of protective lactic acid bacteria, represents a means of preventing food allergies in infants.

KEY FIGURES

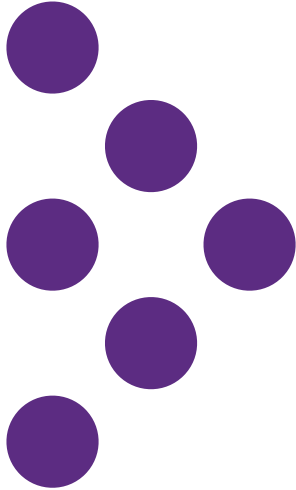
KEY FIGURES ON FOOD ALLERGIES

1 to 3%
of adults and 4 to 6%
of children across the world
are affected by food allergies
(WHO data)

75%
of infantile food allergies
are caused by a small number
of foods: eggs, peanuts,
milk, fish, walnuts, hazelnuts
and almonds
(WHO data)

More than 30%
of children affected also have
an allergy or intolerance
to cow’s milk in France
(Source: ANSES)

3. ALLERGIC RHINITIS



Even more than asthma, with which it is strongly associated, allergic rhinitis is the most common and consistent manifestation of respiratory allergy. Repeated sneezing, continuously runny or blocked nose, irritated eyes: these are the main symptoms of this pathology which affects 25% of the general population in France and half a billion people worldwide²⁰. As with any allergy, it has a significant hereditary component and the symptoms it triggers are caused by an excessive immune system response to allergens (mites, pollens, cat hairs). Moreover, dysbiosis of the intestinal and ENT microbiota have already been associated with these respiratory disorders. There still needs to be a better understanding of the onset period and which bacteria generate them. It is a necessary step to designing treatments that are an alternative and/or an addition to current standard management methods (elimination of the allergen, drugs and desensitization), such as a rebalancing of the microbiota...

Ruminococcus gnavus, an unfriendly bacterium



There is much research that aims to identify the bacteria responsible for dysbioses, which themselves generate diseases. A new step forward has just been made in this field: the bacterium *Ruminococcus gnavus*, already implicated in intestinal dysbiosis, has just been linked to allergic—in particular respiratory—diseases in infants. An important discovery at a time when the WHO is predicting new increases in the prevalence of some respiratory allergies in the coming years²¹.

Although research into microbiota and the impact of their imbalance on health is progressing through the use of cutting-edge genetic analysis technologies, much remains to be discovered and understood. And although dysbiosis of the intestinal microbiota is considered a determining factor in the development of allergies in very young children²², the bacteria specifically involved in this process are still unknown. Studies in this subject area are difficult to perform as the microbial communities interact constantly with the organism—and are therefore sen-

sitive to multiple parameters. This is why research into the same topics sometimes produces divergent, even contradictory results—and does not allow a single factor responsible for certain diseases to be isolated. In other words, the experimental protocol and the methodology adopted are essential to the pertinence of the conclusions.



²⁰ Ozdoganoglu T, Songu M. *The burden of allergic rhinitis and asthma*. Ther Adv Respir Dis. 2012;6(1):11-23

²¹ Pawankar R. *Allergic diseases and asthma: a global public health concern and a call to action*. World Allergy Organ J 2014;7:12

²² Fujimura KE, Lynch SV. *Microbiota in allergy and asthma and the emerging relationship with the gut microbiome*. Cell Host Microbe 2015;17:592–602

An original experimental protocol

Aware of these pitfalls and to maximally limit bias—especially genetic and environmental—in the identification of the microbes involved in allergic diseases, scientists chose to run a study in twins from birth²³. Through this prospective approach the onset of the disease can be observed and changes in the microbiota which may have caused the disease can be detected. To do so, the intestinal microbiota of newborns was characterized based on the analysis of stool samples taken at birth then over a one year period. The children were monitored until they were three years old, the stage at which the intestinal microbiota of infants usually achieves a balance similar to that observed in adults²⁴. This time period was therefore sufficient to detect any correlations between allergic symptoms and microbial variations.

A culprit was identified

At the end of the study, 45.5% of the children monitored had developed respiratory and cutaneous allergic diseases. From the age of two months and up to the end of the first year of life, the allergic children had a much higher proportion of *Ruminococcus gnavus* in their stools than healthy children. This excess is thought to increase levels of *Lachnospiraceae* in the intestinal microbiota and the occurrence of allergic symptoms, especially respiratory symptoms (rhinitis, asthma). More precisely, *Ruminococcus gnavus* triggers inflammation of the digestive tract, itself responsible for a localized allergic response in the respiratory tract. This new finding highlights the major role of microbes in the gut-lung axis and could lead to targeted and effective treatments to be considered.



23 Chua H.-H., Chou H.-C., Tung Y.-L., et al. *Intestinal Dysbiosis Featuring Abundance of Ruminococcus gnavus Associates With Allergic Diseases in Infants*; *Gastroenterology* 2018;154:154–167

24 Yatsunenkeno T, Rey FE, Manary MJ, et al. *Human gut microbiome viewed across age and geography*. *Nature* 2012;486:222–227

Allergic rhinitis: the first months of life are key



The first months of life are decisive for microbial colonization, including that of the nose. While early dysbiosis has been associated with the onset of some allergic diseases from early childhood, what is really known about the impact of the nasal microbiota on the development of early rhinitis and wheezing?

Rhinitis, and more generally respiratory disorders accompanied by a whistling sound (wheezing, asthma²⁵), are common in babies and children²⁶. Several studies have addressed the link between respiratory microbiota and onset of these disorders and have demonstrated the direct influence of the environment and lifestyle²⁷.

However, the precise link between the nasal microbiota of infants and allergic diseases such as rhinitis and wheezing—which is expressed by the emission of a whistling sound on expiration and/or inspiration—had never been studied up until now. This has now been done by an international comparative study whose results were published recently²⁸.

25 Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N, et al. *The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development*. *Cell Host Microbe* 2015;17:704-15

26 Tan TN, Lim DL, Lee BW, Van Bever HP. *Prevalence of allergy-related symptoms in Singaporean children in the second year of life*. *Pediatr Allergy Immunol* 2005;16:151-6

27 Schuijs MJ, Willart MA, Vergote K, Gras D, Deswarte K, Ege MJ, et al. *Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells*. *Science* 2015;349:1106-10

28 Ta L. D., Yap G. C. & al, *Establishment of the nasal microbiota in the first 18 months of life: Correlation with early-onset rhinitis and wheezing*. *J Allergy Clin Immunol* 2018

Very distinct microbial diversity

The development of the nasal microbiota of three groups of children was analyzed and compared very regularly over the first eighteen months of life: 23% of subjects were affected by rhinitis alone, 28% by rhinitis associated with wheezing and the others were in good health. Result: their nasal microbiota developed differently depending on whether or not they had rhinitis, particularly when it was accompanied by wheezing. As these differences were observed very early (sometimes at three weeks old), and most importantly before the onset of clinical symptoms, the researchers deduced that the nasal microbiota played a crucial role in the development of this respiratory disease. More specifically, according to the literature, the nasal microbiota of infants is dominated by three large bacterial groups (Actinobacteria, Proteobacteria and Firmicutes²⁹). This was confirmed by the study. Another result that agrees with the literature³⁰ is that the bacterial diversity in the nasal microbiota of the infants with rhinitis (with or without concomitant wheezing) was reduced. The increased abundance of certain bacteria was thus associated either to allergic rhinitis and concomitant wheezing (*Oxalobacteraceae*, *Aerococcaceae*), or to the composition of the healthy flora of unaffected children (*Corynebacteriaceae* and early nasal colonization by bacteria belonging to the *Staphylococcus* family).



In other words, the nasal microbiota incorporates a “microbial signature” that reflects the respiratory pathology and its severity level.

A predictive factor for rhinitis

These data show that some of the bacteria involved protect against respiratory diseases in infants³¹. They also confirm that specific nasal microbiota profiles before the age of eighteen months may favor the early development of allergic rhinitis and wheezing. Another observation: one child out of five suffering from rhinitis in the study was still affected at the age of five, while those without rhinitis throughout their very early childhood did not develop it subsequently. Therefore whether or not a child suffers from

rhinitis at eighteen months old has a predictive value in this matter. In addition to the potential development of new therapeutic strategies, these results could allow for a better understanding of the role of the nasal microbiota in asthma, a disease which is closely associated with rhinitis.

29 Bosch AA, de Steenhuisen Piters WA, van Houten MA, Chu M, Biesbroek G, Kool J, et al. *Maturation of the infant respiratory microbiota, environmental drivers and health consequences: a prospective cohort study.* Am J Respir Crit Care Med 2017;196:1582-90

30 Sakwinska O, Bastic Schmid V, Berger B, Bruttin A, Keitel K, Lepage M, et al. *Nasopharyngeal microbiota in healthy children and pneumonia patients.* J Clin Microbiol 2014;52:1590-4

31 Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N, et al. *The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development.* Cell Host Microbe 2015;17:704-15

INTERMITTENT RHINITIS

Symptoms present
< 4 days a week or < 4 weeks a year

MILD RHINITIS

(All the following symptoms)

- Normal sleep,
- Normal daily activities,
- Normal professional and/or academic activities,
- No troublesome symptoms.

PERSISTENT RHINITIS

Symptoms present
≥ 4 days a week or ≥ 4 weeks a year

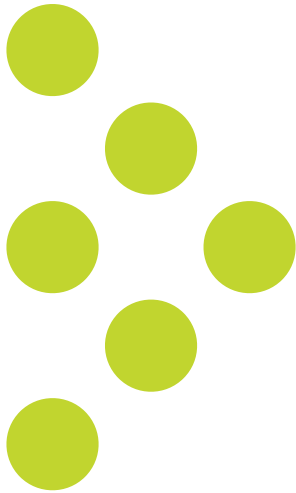
MODERATE TO SEVERE RHINITIS

(One or more of the following symptoms)

- Disturbed sleep,
- Disrupted daily activities,
- Disrupted professional and/or academic activities,
- Presence of troublesome symptoms.

Classification of allergic rhinitis based on its symptoms ARIA Classification (Allergic Rhinitis and its Impact on Asthma – OMS)

4 • ATOPIC DERMATITIS



Atopic dermatitis, sometimes called “atopic eczema”, is a chronic, allergic, inflammatory disease of the skin. Its prevalence is increasing and it is the most common skin disease. Although it affects 15 to 20% of babies and usually disappears before the age of four years, it can persist to adolescence, even to adult age in one out of ten cases. The skin is very dry and sensitive, with red plaques, lesions, itching: these are the main symptoms of this disease which manifests in flares. Available treatments aim to eliminate the symptoms and to improve quality of life for those affected. Its cause has not been fully identified but there is a significant genetic component which incorporates other allergies such as asthma and allergic rhinitis. As with asthma and food allergies, excessive hygiene –and therefore reduced exposure of the organism to bacteria–could contribute to an overreaction by the immune system. The first element to be affected is the cutaneous microbiota, whose diversity is impoverished by the disease and whose balance must be restored to optimize treatment.

Emollients and atopic dermatitis: proven benefits

Emollients (substances that promote relaxation and softening of the skin) are already one of the essential treatments for atopic dermatitis. Employed to restore the cutaneous barrier of the skin, they are also used as a preventive measure in infants at risk. It is not known precisely how they act on the cutaneous microbiota and it needs to be clarified to improve the therapeutic solutions.

Atopic dermatitis is a chronic, allergic, inflammatory skin disease which manifests mainly during the first six months of life³². Not all newborns are equally susceptible: babies whose parents themselves suffer from atopic dermatitis or an allergic disease such as asthma or allergic rhinitis have in fact a two–even three–times greater risk

of developing the disease than babies with no family history³³.

***Staphylococcus aureus*: an identified enemy**

The skin is permanently colonized by the microorganisms which form the cutaneous microbiota. But in atopic dermatitis, its barrier function is impaired and no longer provides the same level of protection against external attacks. This condition weakens the skin, makes it more vulnerable and subject to colonization by undesirable microbes³⁴. In this case, *Staphylococcus aureus* plays a crucial role: analyses have shown that, on the skin of infants suffering from atopic dermatitis with lesions, the bacterium was present in more than 90% of cases.



Staphylococcus aureus

32 Bieber T. *Atopic dermatitis*. N Engl J Med. 2008; 358(14):1483±94

33 Bohme M, Wickman M, Lennart Nordvall S, Svartengren M, Wahlgren CF. *Family history and risk of atopic dermatitis in children up to 4 years*. Clin Exp Allergy. 2003; 33(9):1226±31

34 Ong PY, Leung DY. *The infectious aspects of atopic dermatitis*. Immunol Allergy Clin North Am. 2010; 30(3):309±21

This figure falls to 78% for dermatitis without lesions, but drops to 10% if the skin is healthy³⁵. The density of *Staphylococcus aureus* is therefore thought to be correlated with disease severity. Since this is not however systematic, the researchers consider that the disease displays heterogeneity between individuals. Other methods of analysis have enabled better characterization of the bacterial communities of skin affected by this disease: of course the proportion of *Staphylococcus aureus* increases during the flare phases, but significant changes have been observed in other bacteria (classic or more rare) during and after treatment³⁶.

The role of emollients better understood

Effective and widely approved in the treatment of atopic dermatitis in infants, emollient creams rehydrate and repair the damaged skin by regenerating injured tissue, and reduce disease severity³⁷. Their regular application over the first six months of life to the skin of babies with a high probability of developing the disease might prevent its manifestations, although results diverge from one individual to the next³⁸. Some researchers have studied these emollients, in order to better understand their preventive action on the cutaneous flora. They compared different skin parameters, monitored the development of atopic dermatitis and genetically analyzed skin samples taken from different places on the body, depending on whether the infants (all at risk) had received emollient treatment or not³⁹.

Result: the skin of infants treated beforehand had a lower pH than that of the control group (healthy skin has a slightly acidic pH, which helps it to function correctly, while that of skin with atopic dermatitis is higher⁴⁰, favouring colonization by *Staphylococcus aureus*). Modulation of pH might then be a means of rebalancing the cutaneous microbiota. Generally, the *Streptococcus* genus also clearly contributed to the differences observed in the samples from the two



groups. The skin of the infants who received treatment contained a richer and more diverse bacterial population, which resembled a restoration of the balance of the cutaneous microbiota. In particular, *Streptococcus salivarius* bacteria were present in greater numbers. The latter colonize the organism (especially the mouth) from the first moments of life and seem to have a protective role: they are present in higher levels in infants who do not have atopic dermatitis than in those who have the condition. This result is consistent with those of other studies conducted in older children which showed that the higher the proportion of *Streptococcus salivarius*, the milder the atopic dermatitis.

These data confirm the benefits of the long-term use of emollients as a preventive measure in infants at high risk. They provide new information on the way in which emollients act, although

the mechanisms involved and the impact of localized changes in the cutaneous microbiota on the development of the disease in these infants remain to be identified.

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War has been declared against *Staphylococcus aureus*

Microbial imbalance—or dysbiosis—of the skin, caused particularly by *Staphylococcus aureus*, and atopic dermatitis, are closely linked. We now know that the imbalance precedes the onset of the disease in some individuals. An important step forward for basic research, and even more for the development of new therapeutic strategies.

It has been established since the mid-1970s that the skin of individuals suffering from atopic dermatitis is generally colonized by *Staphylococcus aureus*. Highly pathogenic, its name often refers to tricky infections⁴¹. It is in particular responsible for the largest number of nosocomial infections (in hospitals), as well as skin or food-borne infections of variable severity. It was also recently discovered that the infection is more severe where these bacteria are present in greater numbers and belong to certain specific strains⁴². Meanwhile, technical progress made in the field of genetics has allowed the composition of the cutaneous flora of affected individuals to be better described, and has revealed a reduced bacterial diversity⁴³. But up

until now, it was not known whether the abundance of *Staphylococcus aureus* was the cause of atopic dermatitis or an observed consequence of the dysbiosis of the cutaneous microbiota.

Staphylococcus aureus, herald of the disease

A recent prospective clinical trial has just shown that colonization by *Staphylococcus aureus* precedes the onset of the disease in children⁴⁴. This led to imparting a causal role to the bacterium in the onset of the disease, which partly contradicts the conclusion of another recent study⁴⁵ (which however also incriminates *Staphylococcus* strains). It required two years of study to arrive at this conclusion: the researchers regularly analyzed skin samples taken from the elbow creases and the axillae, areas that are classically affected. First result: one child in four developed atopic dermatitis. Secondly: the proportion of *Staphylococcus aureus* increases markedly from the age of three months in infants who were subsequently reported to have atopic dermatitis. On the contrary, other bacteria, less abundant in infants suffering from atopic dermatitis, seem to have a potentially protective role⁴⁶.

Other methods of eradication

Non-clinical and *in vitro* experiments have shown that *Staphylococcus aureus* could promote the disease in individuals with a genetic predisposition by triggering toxic and/or inflammatory reactions in skin cells exposed directly to the microbes⁴⁷. By extrapolation, researchers thought that elimination of this bacterium could be beneficial in the treatment of atopic dermatitis. Relatively effective treatments already exist—topical antimicrobials, antibiotics, dilute bleach baths—but paradoxically, it is not known whether they really eliminate *Staphylococcus aureus* colonization nor what is their impact on the cutaneous microbiota. For this reason, similar to transplants of

fecal flora, transplants of cutaneous microbiota⁴⁸ containing bacteria known to act against *Staphylococcus aureus* is an emerging protocol. The first trials conducted on affected individuals led to a considerable reduction in colonization by *Staphylococcus aureus*. We wager that the next trials will validate this promising start and will be accompanied by improvements in treatment.



Staphylococcus aureus in culture

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What do we know about the links between atopic dermatitis and the microbiota?

We recently learned that the bacteria which make up the cutaneous flora do not just exploit their host to acquire nutrients: our body also uses them to protect itself against invasion by pathogenic microbes in order to avoid skin infections. Some bacteria which do not cause diseases—called commensal—actually have an antimicrobial effect which allows them to destroy pathogenic bacteria.

under-investigated. For the moment, we know that the intestinal bacterial community can also influence the immune response and could therefore play a role in the onset of cutaneous inflammation. But before considering the restoral of intestinal balance as a treatment, fundamental research work still has to be carried out.

Is the hygiene hypothesis the only one to explain the disease, which affects increasing numbers of individuals?

Genetics plays an essential role in the predisposition to atopic dermatitis, but environmental factors are important triggering elements. In fact, the hygiene hypothesis proposes that individuals who have had little exposure to external microbes have an increased risk of developing the disease. One of the reasons is thought to be that these individuals do not acquire the microbiota necessary to protect themselves against invasion by *Staphylococcus*



cutaneous microbiota of babies born by cesarean section. This is promising research which is however, still in its infancy, as we do not yet know if this solution is effective. The application of probiotics directly on the skin as a preventive measure is also being explored. Creams, already available on the market, have proved to be protective as they contain “good” bacteria. Now that we have clearly identified those implicated in atopic dermatitis, we could design products that are more targeted, therefore potentially more effective. Many trials are currently underway...

Between now and 2050, the WHO estimates that one person in two will suffer from one or more allergic diseases. How can we act effectively?

Recent technological advances have opened up new prospects and avenues of research. They allow us to go as far as identifying bacterial subspecies and clarifying their respective roles more precisely. These advances have shown the great bacterial diversity of the skin, as well as the variations observed between individuals. Atopic dermatitis is the disease in which most progress has been made, particularly with regard to the specific role of *Staphylococcus aureus*. But skin dysbiosis is thought to play a very important role in other cutaneous inflammatory diseases: it is also linked to acne vulgaris and hidradenitis suppurativa, a chronic painful disease which progresses in flares. Since there is now a good understanding of dysbiosis in atopic dermatitis, efforts are focusing on the development of preventive strategies. Finding the means to act early to eliminate the disease and promote the well-being of the individuals affected is the strategic challenge of the next few years. We still have 30 years to make progress!

“SUCCEEDING IN RESTORING THE MICROBIAL BALANCE VERY EARLY IN LIFE IS BECOMING ONE OF THE FAVOURED AVENUES OF INTERVENTION.”

In atopic dermatitis, an imbalance of the skin microbiota (or dysbiosis) is observed, which causes a reduction in certain protective bacteria and a rise in infections, in particular by *Staphylococcus aureus*. There are two causative mechanisms: firstly a deficiency in the cutaneous barrier, which allows penetration by pathogenic bacteria; secondly, a deficiency in the immune response, which triggers production of IgE (immunoglobulin E) antibodies and impairs the antimicrobial response. *Staphylococcus aureus* then invade the skin, take over and trigger cutaneous inflammation. This is how atopic dermatitis appears. While the link between cutaneous dysbiosis and atopic dermatitis is well established, the role of the intestinal microbiota in this disease remains

aureus. For this reason, delivery by caesarean section is considered a predisposing factor: by not being exposed to the mother’s vaginal microbes, the baby’s skin is thought to present a dysbiosis from the first months of life, and therefore more vulnerable to *Staphylococcus aureus*—which might increase the risk of atopy. Studies in babies moreover have shown that cutaneous dysbiosis could occur early and that it preceded the development of atopic dermatitis and respiratory allergies. Succeeding in restoring the microbial balance very early in life is becoming one of the favoured avenues of intervention. By putting babies in contact with their mother’s vaginal flora after birth, we are already succeeding in re-establishing the balance of the

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