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Il ruolo dei miRNA in allergologia
The role of miRNAs in allergology
El papel de los miRNA en alergología

L'allergia al veleno di imenotteri
Allergy to Hymenoptera venom
Alergia al veneno de himenópteros

Il polline oltre gli allergeni
Pollen beyond allergens
El polen mas allá de los alérgenos

SCOPRI GLI IMENOTTERI: RISCHI E ALLERGIE
DISCOVER HYMENOPTERA: RISKS AND ALLERGIES
DESCUBRE HYMENOPTERA: RIESGOS Y ALERGIAS

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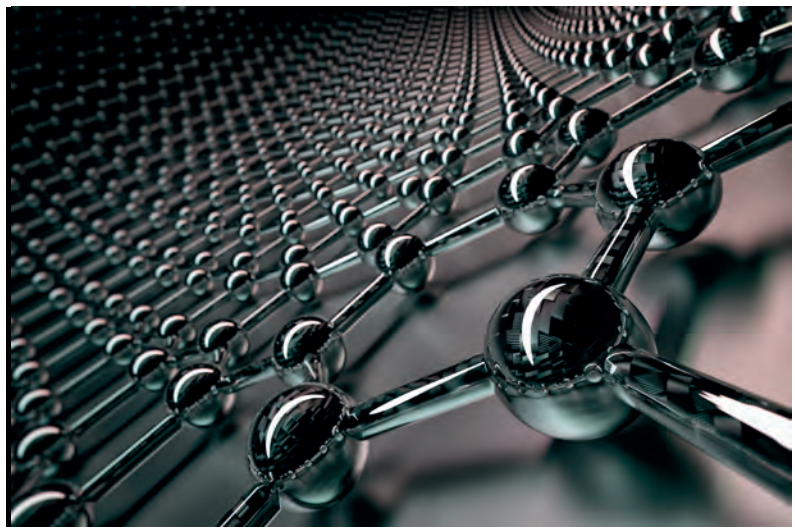

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SUMMARY

Notiziario Allergologico, *2025 Vol. 43, n. 1*

Notiziario Allergologico

PDF VERSION

Notiziario Allergologico has been alive and well for over forty years. Today, it becomes international with a new layout that includes the translation of all content into three languages. The purpose remains unchanged if not implemented: to promote allergology culture by offering readers the possibility of an in-depth study and update on various allergology topics, also with a view to the future, thanks to the competence and authority of the authors of the articles published. The popular character of the articles contributes to making them accessible to a vast number of specialists, not only allergologists but also pulmonologists, paediatricians, dermatologists, etc.

ENGLISH

EDITORIAL

edited by **Gianni Mistrello**

Stem cells would not exist, brains and muscles would not develop, cells would not divide and so much more... in other words, chaos, if microRNAs (miRNAs) did not exist. They are small RNAs (21-25 nucleotides) not coding for proteins, but which play a key role in the regulation of gene expression and consequently in the control of a large number of cellular mechanisms. Their discovery 'earned' the two researchers (Victor Ambros and Gary Ruvkun) the 2024 Nobel Prize in Medicine. The following article is an in-depth look at the role of these molecules, particularly in allergic diseases. The author (Dr. Borrelli, University of Turin) begins his article with a welcome reminder of basic genetics, with particular reference to the production pattern of miRNAs. He then focuses on the possible mechanisms underlying the inflammatory response generated by an allergen and the ways in which miRNAs are able to regulate both the initial inflammation and its persistence over time. Among the numerous miRNAs studied, some emerge as key players in the regulation of allergic-type immune responses such as bronchial asthma, allergic oculorhinitis and atopic dermatitis. More recently, several studies have highlighted a correlation between specific expression levels of certain miRNAs and the development and severity of allergic diseases. Therefore, they could, in the not too distant future, be used not only as diagnostic biomarkers, but also as possible targets in the development of revolutionary therapeutic strategies.

As we will see later, pollens are not the only potential cause of allergies. With the approach of fine weather, people tend to spend more time outdoors and may therefore happen to be stung by stinging insects. In particular, Apidae (bees) and Vespidae (wasps, hornets), known as Hymenoptera, are the insects with the greatest allergenic impact. It should be emphasised that in most cases the reactions caused by their stings remain localised, and are due to a toxic effect of the venom injected through their stingers. In a certain percentage of cases, however, these reactions can take on a systemic character and be the cause of specific allergies to the components present in the venom and, for sensitised individuals, result in fatal outcomes. An exhaustive update on the problem is the subject of the article by Dr. Boni (Ospedale Maggiore, Bologna); the author begins her contribution by pointing out that the clinical manifestations resulting from hymenoptera stings can be of varying severity, and for this reason they have been classified according to their level of severity. After a series of insights into the taxonomy, morphology and entomology of the various hymenoptera (in particular, wasps can be distinguished into two types: vespula and polistes), Dr. Boni emphasises the importance

of undergoing a thorough specialist examination when a subject develops an extensive and long-lasting local reaction following a sting. The allergy specialist, once he has collected information from the 'victim' to identify the stinging insect, will proceed to perform specific skin allergy tests (prick tests), or laboratory tests (there are several), thus arriving at a correct diagnosis. When taking the anamnesis, it is equally important for the specialist to also assess any risk factors (e.g. subjects suffering from systemic mastocytosis, or exposed to the risk of numerous stings such as beekeepers). The resulting information is fundamental for the specialist, who can then assess the degree of risk and set up an appropriate therapeutic strategy based on the use of drugs such as antihistamines, anti-inflammatory drugs or adrenaline (emergency therapy) or specific anti-allergy vaccines (specific immunotherapy, AIT), which is the only form of effective prevention that can protect the patient in the event of new stings. The article concludes with a few hints on the mechanism of action of AIT and the indications to follow when performing it.

The authors of the concluding article (Dr. Biagioni and Dr. Cecchi, San Giovanni di Dio Hospital, Florence) chose a title that in itself is very appealing, a sort of invitation to read its contents. In fact, the article is full of very interesting insights, some of them quite original (at least for me). Generally, when it comes to pollen, one refers (as the authors themselves initially do) to the different plant species that produce it, their structure and size, the range of allergens present in it and the role of the latter in first sensitising and then eliciting, in subjects thus sensitised, specific immunological reactions associated with the development of respiratory allergies. In addition, the aspect of the importance of multiple environmental factors influencing atmospheric concentration and pollen allergenicity is also among the topics covered in the article. Among these factors, climate change and air pollution are mentioned, and the ways in which they would increase both the risk of increased rates of sensitisation of allergic diseases in the population, and the exacerbation of symptoms in sensitised patients are discussed. Particularly interesting is the authors' in-depth study of the mechanisms by which pollen interacts with the respiratory epithelium, highlighting the key role played by the Ripoptosome, a protein complex that, when activated, induces apoptosis of epithelial cells and stimulates the secretion of particular immune mediators (alarmins) that amplify the allergic response. These recent insights into the effects of pollen on immunity are improving our knowledge of the causes behind the development of allergic diseases, paving the way for innovative therapeutic strategies.

The role of miRNAs in allergology: present and future

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1. Introduction

DNA, Deoxyribonucleic Acid, is the fundamental element that contains genetic information and is found in condensed form inside the cell nucleus. It consists of nucleotides (A-T-C-G), which in sequence produce both coding portions (i.e. directly involved in coding for protein transcription) and non-coding portions, with a regulatory function on transcription (1). DNA transcription is in fact the first step in the process that leads to protein synthesis; it takes place in the nucleus, where a segment of DNA, the gene, is copied into a molecule of RiboNucleic Acid (otherwise known as RNA), in a specific type called messenger (mRNA) thanks to an enzyme called RNA polymerase; this element recognises a specific sequence, the promoter, and initiates the synthesis of mRNA complementary to the mould strand of DNA; the mRNA thus formed contains the genetic information necessary for protein synthesis (2). In fact, the mRNA thus formed leaves the nucleus to reach the ribosomes in the cytoplasm, where it binds to read codons, i.e. sequences of three nucleotides; each codon encodes for a specific amino acid, which is transported to the ribosome by the transport RNA (tRNA). The tRNA has an anticodon that is complementary to the one on the mRNA (which was itself complementary to the DNA), thus ensuring that the correct amino acid is incorporated into the protein chain being formed. The amino acids thus selected are bound together by peptide bonds, forming a polypeptide chain. This process continues until a stop codon is reached, marking the end of protein synthesis and the production of the final product: the protein (3).

However, there are numerous other types of RNAs that, although not directly involved in transcription, play a fundamental role in the regulation of this activity; these include miRNAs, the role of which we will explore in more detail later in this article.

miRNAs (short for microRNA) are single-stranded RNA molecules, approximately 22 nucleotides long, that modulate gene expression at the post-transcriptional level through pairing with complementary mRNA sequences (4). They were discovered in 1993 in a nematode, a vermiform organism called *Caenorhabditis elegans* (5), and have since been explored in numerous other organisms and species, including humans. Within eukaryotic organisms, miRNAs have shown numerous roles in the regulation of various physiological and pathological processes, including development, growth, differentiation, immune response and stress adaptation (6).

The mechanism of miRNA production and function begins in the cell nucleus, where miRNA-specific genes are transcribed into long precursor molecules called pri-miRNAs (Figure 1) (7).

These precursors, characterised by a typical hairpin structure, are then recognised by a protein complex that cuts them to produce shorter molecules called pre-miRNAs, which are transported from the nucleus to the cytoplasm (8).

Here, the pre-miRNAs undergo a further maturation process by an enzyme (called Dicer), which cuts them again to obtain their final form: a mature

SUMMARY

Keywords

• miRNA • gene expression • inflammation • immune response • allergy • Th2 • biomarkers

Acronyms

• **mRNA** messenger RNA
• **tRNA** transport RNA
• **miRNA** microRNA
• **pri-miRNA** primary precursor RNA
• **pre-miRNA** mature precursor RNA
• **RISC** RNA-Induced Silencing Complex
• **NF-κB** Nuclear Factor kappa-light-chain-enhancer of activated B cells
• **NLRP3** NOD-like receptor family pyrin domain containing 3
• **TLR4** Toll-like receptor 4
• **TRAF6** TNF Receptor Associated Factor 6
• **LTB4** Leukotriene B4
• **LTC4** Leukotriene C4
• **LTD4** Leukotriene D4

miRNAs are small non-coding RNA molecules that regulate gene expression, profoundly influencing the immune response in allergic reactions; they are able to modulate T-lymphocyte differentiation, cytokine production and activation of effector cells such as mast cells and eosinophils. MiR-155 and miR-21, for example, which are increased in patients with allergies, promote the Th2 response, thereby favouring allergic inflammation, while miR-146a and miR-223 play an anti-inflammatory role and are shown to be reduced in pathological pictures. Their altered expression in allergic patients offers new diagnostic opportunities and paves the way for targeted therapies; innovative strategies such as the use of antagomiRs to block pro-inflammatory miRNAs or mimics to restore regulatory miRNAs, together with nanotechnology-based delivery systems, are indeed emerging as potential treatments. Thus, miRNAs represent a promising frontier for personalised allergy diagnosis and therapy.

double-stranded miRNA. At this point, one of the two strands, called the guide strand, is incorporated into a multiprotein complex known as RISC, while the other strand, considered non-functional, is eliminated; it is here that the RISC complex, guided by the miRNA, becomes operational and ready to perform its regulatory task (9).

The guide miRNA identifies specific target mRNAs based on complementarity between its sequence and that of the messenger; once this connection is established, the miRNA can lead to two different results. If the complementarity between miRNA and mRNA is almost perfect, the messenger RNA is degraded, completely preventing the synthesis of the corresponding protein; if, on the other hand, the complementarity is partial, the mRNA is not degraded, but its translation is blocked, leaving the messenger inactive in the cytoplasm. In both cases, however, the result will be a reduction in gene expression and thus inhibition of its target (8, 9).

This system allows miRNAs to regulate cellular activity in a precise and targeted manner, simultaneously influencing a large number of genes; a single miRNA can bind to several mRNAs, just as one mRNA can be the target of several miRNAs. This complex network of interactions enables highly sophisticated control of cellular processes.

2. Immune system and allergies

In order to better understand the role of miRNAs in allergopathies, it is first necessary to introduce its possible target mechanisms, i.e. all those processes that induce an inflammatory response originating from an allergen, as well as the mechanisms produced by it.

An allergen is defined as a substance that is normally harmless to most people, but in predisposed individuals is capable of triggering an excessive and exacerbated response of the immune system, known as an allergic reaction. Allergens can be proteins, glycoproteins

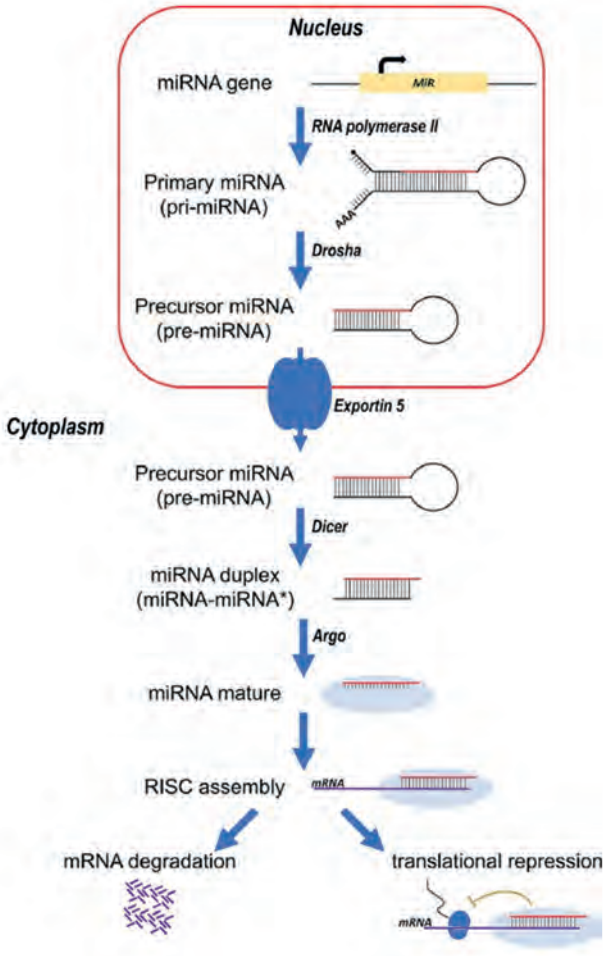
or, in some cases, carbohydrates present in a wide range of environmental materials, including pollen, dust mites, pet dander or scales, certain foods, insect bites or even certain drugs (10). Although these substances are harmless to most individuals, in allergic individuals they are recognised as potentially dangerous by the immune system, which mistakenly treats them as pathogens. This leads to a disproportionate immune response, resulting in the characteristic symptoms of allergies, such as itching, swelling, sneezing, breathing difficulties and, in severe cases, anaphylaxis.

The allergic response is an extremely complex process, involving several cells of the immune system, inflammatory molecules and chemical mediators. It is a series of closely co-ordinated events, consisting of two main phases: the sensitisation phase and the actual response phase (11).

The sensitisation phase represents the first encounter between the immune system and the allergen. This phase does not yet cause obvious clinical symptoms, but creates the conditions for allergic reactions to develop in the future. It occurs when a person who is predisposed, genetically or due to environmental factors, comes into contact with an allergen through the skin, respiratory tract or gastrointestinal tract; allergens are able to penetrate the body by overcoming epithelial barriers, which should normally act as the first line of defence against foreign substances.

Once through these barriers, allergens are immediately recognised and captured by a type of highly specialised cells of the immune system called dendritic cells. Dendritic cells play a crucial role in the initial phase of the immune response, as they act by identifying the allergen, processing it internally and then exposing it on their surface by means of a system of molecules called the major histocompatibility complex (MHC class II). This process, known as antigen presentation, is essential to activate the effector cells of the immune system; in fact, once the antigen (in this case the allergen) has been processed, the dendritic cells migrate to the nearest lymph nodes, where the

Figure 1 Schematic of miRNA production



(7)

interaction with naive T lymphocytes, i.e. lymphocytes that are not yet specialised, takes place; these naive T lymphocytes, once exposed to the allergen presented by the dendritic cells, differentiate into a specialised subpopulation of lymphocytes called Th2 (T helper type 2), from which the T2 phlogosis takes its name (Figure 2) (12). Th2 lymphocytes are central to the allergic response, as they are responsible for most of the processes that lead to allergy-associated inflammation; this is achieved because Th2s release several interleukins (ILs), signal molecules with inflammatory activity (13). Among the interleukins produced by Th2 lymphocytes, the most important are:

- Interleukin-4 (IL-4), a key cytokine in the regulation of the Th2-type immune response that plays a crucial role in the activation of B lymphocytes. In the presence of IL-4, B lymphocytes are induced to differentiate into plasma cells, effector cells specialised in the synthesis and secretion of immunoglobulin E (IgE). IgE, once produced, binds to high-affinity receptors on the surface of mast cells and basophils. Subsequently, upon contact with the specific allergen, IgE mediates the activation of mast cells and basophils, triggering degranulation and the release of inflammatory mediators responsible for allergic symptoms (14).

- Interleukin-5 (IL-5), promotor of the maturation and recruitment of eosinophils, a cell type involved in the chronic inflammatory response. Eosinophils release toxic proteins that have the ability to amplify tissue damage and aggravate the inflammation produced (15).
- Interleukin-13 (IL-13), which acts in synergy with IL-4 to increase IgE production and exacerbate mucus production in the airways (16).

Figures 3 and 4 show the mechanisms mediated by these interleukins (17, 18). This binding between IgE and FcεRI leads to the stabilisation of the receptor on the cell membrane, predisposing (i.e. 'sensitising') mast cells and basophils to rapidly and specifically recognise the allergen; the mast cells and basophils thus sensitised act as the primary effectors of the immediate allergic response. These cells contain cytoplasmic granules rich in pre-formed mediators, including histamine, tryptase, kinase and other proteolytic enzymes; they are also capable of synthesising *ex novo* lipid mediators, such as leukotrienes (LTB4, LTC4, LTD4) and prostaglandins (PGD2), as well as pro-inflammatory cytokines and chemokines (19).
During the sensitisation phase, the binding of IgE to the FcεRI receptor induces a functional stabilisation of the receptor-ligand complex, making mast cells and basophils ready to respond in an exacerbated and rapid manner to future contact with the same allergen. At the next encounter, in fact, the allergen will bind to more IgE already associated with the FcεRI receptor, and this phenomenon will act as a critical signal to initiate a cascade of intracellular events that will culminate in the degranulation of mast cells and basophils, with the immediate release of pre-formed mediators from the granules and the *de novo* synthesis of lipid mediators and cytokines. These released mediators, such as histamine, cause vasodilation, increased vascular permeability and smooth muscle contraction, which translate clinically into itching, oedema, bronchoconstriction and the other typical manifestations of the allergic response. At the same time, cytokines and leukotrienes attract other inflammatory cells, such as eosinophils and neutrophils, perpetuating the inflammation and aggravating the symptoms (20).

3. The role of miRNAs in allergic diseases

To understand the role of miRNAs in allergies, it is necessary to analyse how they interact with the main players in the allergy-associated immune response. Allergic diseases, including allergic oculorhinitis, are characterised by the Th2 response described above, as well as by the activation of mast cells, eosinophils and other inflammatory cells leading to both the production of IgE and the release of mediators such as histamine.
miRNAs are involved in every step of this process, regulating both inflammation in its initial phase and its persistence over time. Indeed, recalling what was described in the introduction to this article on the mechanism of miRNA functioning, three different scenarios are possible in the pathological context of allergies. In the first scenario there may be a decrease in miRNAs related to control mechanisms; this condition produ-

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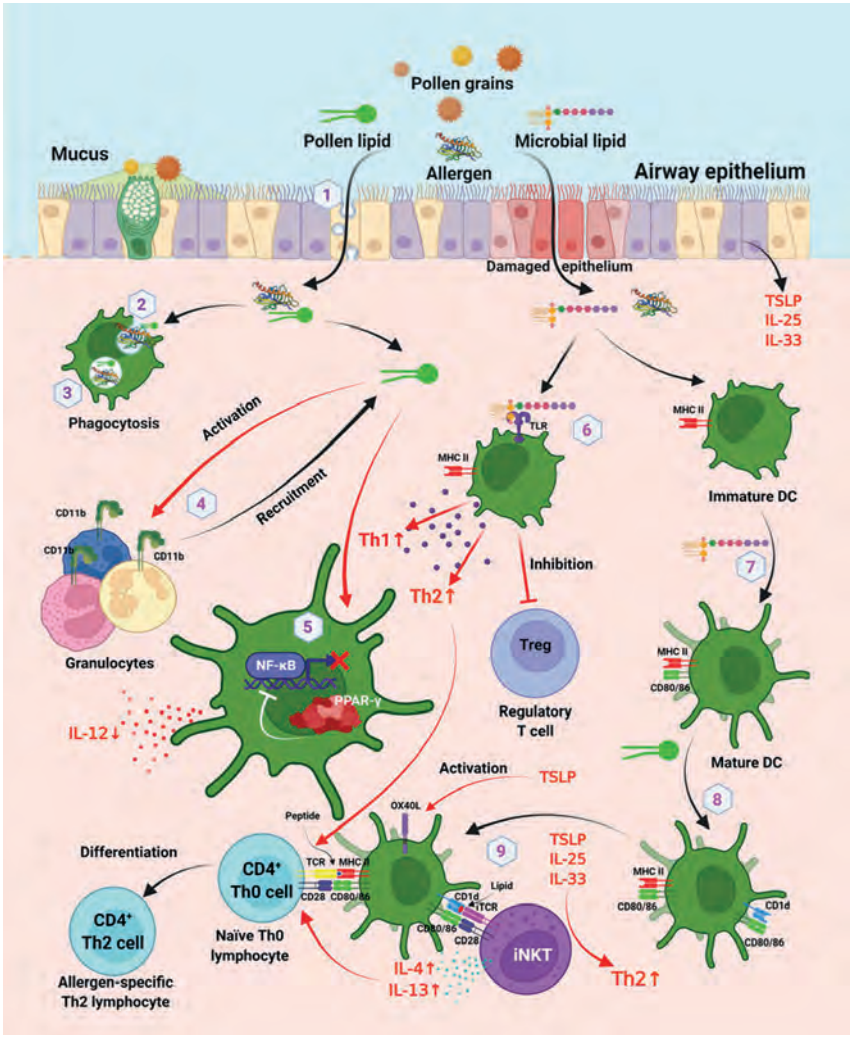
ces a decrease in the ability to block or inhibit the production of pro-inflammatory elements, leading to an exacerbation of their levels. In the second scenario, we can find an increase in miRNAs related to mechanisms suppressing the inflammatory response; in this context, increased microRNA values produce a reduced ability of anti-inflammatory systems to exert their control function on the genesis and development of the inflammatory framework, thus leading to an environment prone to the perpetuation of inflammation. Lastly, there is the scenario in which miRNAs affect differentiation mechanisms; in this context, there is a change in the ability of cells to differentiate into responses other than T2, thus favouring the development of this pathway and favouring the activity of its effector mechanisms (21)

Studies conducted in recent years have identified a number of miRNAs closely linked to allergic diseases, emphasising their crucial role both in determining the allergic response and in providing potential targets for diagnosis and treatment. miRNAs are not simply secondary regulators, but actively participate in all phases of the immune response, influencing multiple aspects of allergic pathology.

Among the numerous miRNAs studied, some emerge as key players in the regulation of allergic-type immune responses.

MiR-155 is one of the most studied miRNAs in the context of allergy; it directly regulates the differentiation of naive T lymphocytes towards Th2 subpopulations. Overexpression of miR-155 has been associated with an increase in the production of cytokines such as IL-4, IL-5 and IL-13, which, as previously indicated, are related to the genesis and maintenance of the allergic response (22). Studies conducted in animal models have shown that the reduction of miR-155 expression leads to a significant reduction in the ability of lymphocytes to differentiate towards Th2

Figure 2 Mechanism of allergen recognition and production of T2 phlogosis



(12)

populations, as well as to produce an effective allergic response; this condition suggests that blocking miR-155 may be considered a useful therapeutic target to modulate T2 inflammation (23, 24). In addition, several studies have shown that topical corticosteroid therapy administered nasally not only exerts its already known anti-inflammatory effects, but actually contributes to significantly reducing miR-155 levels, empirically confirming this hypothesis (25).

MiR-21 is another of the most relevant miRNAs in the pathogenesis of allergic diseases. In fact, miR-21 has been verified in several studies to be increased in the dendritic cells, T lymphocytes and mast cells of patients with allergic diseases. Its role is twofold: on the one hand it promotes the shift of the inflammatory response towards a T2 phenotype, while on the other hand it is able to inhibit anti-inflammatory mechanisms, such as the differentiation of lymphocytes towards a regulatory phenotype (regulatory T, or Treg, 22). It has also been shown that in patients in whom there was anaphylaxis the levels of this miRNA were increased, while in animal model studies with guinea pigs deficient for miR-21 there was a reduced likelihood of developing eosinophilia or allergic reactions (27). These functions make miR-21 a crucial element in maintaining and amplifying allergy-associated inflammation and a possible biomarker to be evaluated in the field of allergy research.

Like its predecessors, miR-146a is a miRNA that is strongly implicated in the regulation of the inflammatory response; it exerts this action through the inhibition of molecules with pro-inflammatory activity (such as NF-κB and pro-inflammatory cytokines). In studies on animal models, a reduction in its levels has been observed, a factor that compromises the control mechanisms that act to reduce inflammation; furthermore, this mechanism has been verified in humans both in allergies mediated by inhalant allergens (such as pollen or dust mites) and in patients suffering from atopic dermatitis (28).

Finally, the importance of miRNA 223 (miR-223) should be described, as it acts across multiple cellular targets, including macrophages and epithelial cells. Physiologically, it acts as an inhibitor of the expression of pro-inflammatory genes, such as NLRP3 and NF-κB, attenuating the release of cytokines and chemokines involved in allergies, including IL-1β, IL-6 and IL-8 (29). Recent studies, however, link a reduction in its levels to allergic conditions such as asthma and allergic rhinitis and also highlight its role in regulating bronchial hyperreactivity and mucus production (30). In addition to these products, reduced levels of miR-223 have also been correlated with the development of more severe forms of atopic dermatitis (31). These findings suggest its use as a biomarker and potential therapeutic target in allergic diseases, as demonstrated by several ongoing studies.

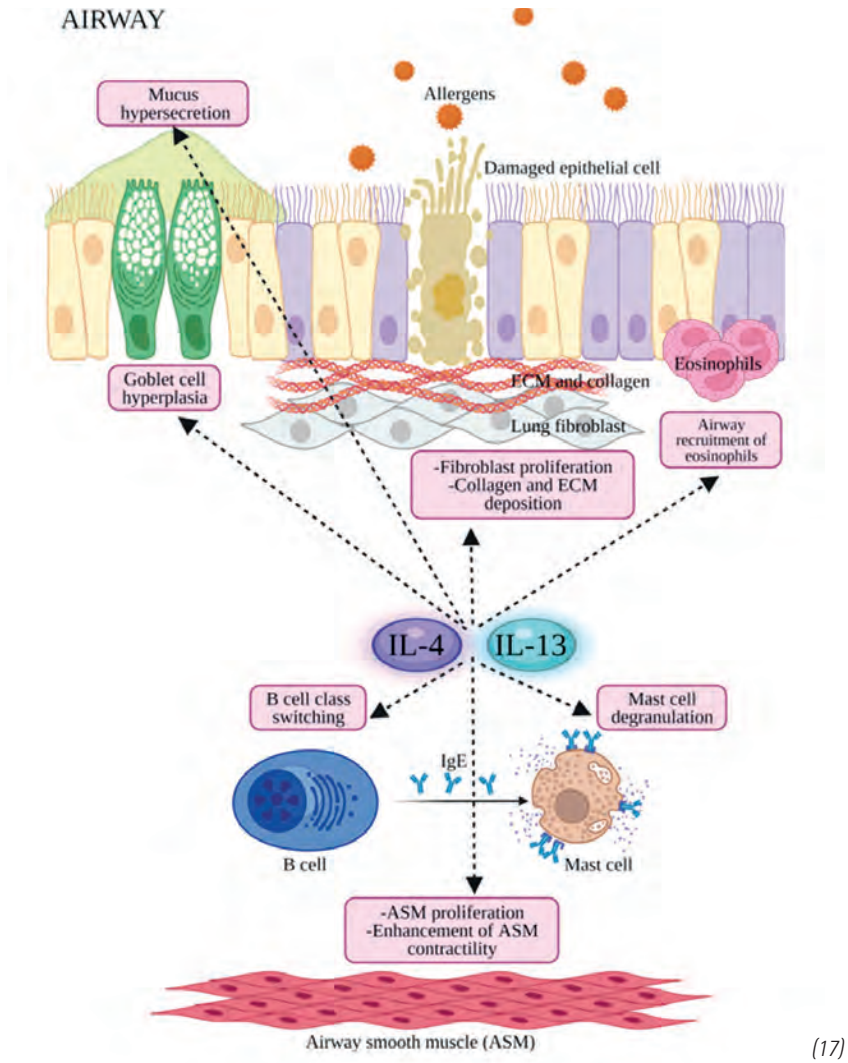
4. miRNAs as diagnostic biomarkers

One of the most promising aspects of miRNAs is their use as diagnostic biomarkers. Their stability in body fluids, such as plasma, serum and saliva, makes them ideal tools for non-invasive diagnosis. Several recent studies have related the specific expression profiles of miRNAs to the development and severity of allergic diseases:

- **T2-high bronchial asthma and allergic oculorhinitis**:
  MiR-21 and miR-155 are consistently overexpressed in patients with allergic oculorhinitis and/or T2-high bronchial asthma, conditions characterised by inflammation mediated by the type 2 (T2) immune response. These microRNAs are closely associated with the activation of inflammatory pathways linked to key cytokines such as IL-4, IL-5 and IL-13, which are typical of allergic diseases. Their quantification in blood samples could represent a non-invasive method to assess not only the level of systemic inflammation, but also the severity of the disease, offering a complementary tool for patient stratification and monitoring of therapeutic response.

- **Atopic dermatitis:**
  MiR-223 and miR-146a have been identified as markers of chronic inflammation in atopic dermatitis (AD) patients. MiR-223, known for its role in the regulation of the innate immune response, can modulate the activation of neutrophils and macrophages and influence the expression of inflammatory mediators such as NLRP3 and NF-κB, both implicated in the pathogenesis of AD. MiR-146a, on the other hand, acts as a

Figure 3 Role of IL-4 and IL-13



(17)

negative modulator of proinflammatory pathways, suppressing NF-κB-mediated signals and reducing the production of cytokines such as IL-6 and TNF-α. The alteration of these miRNAs in AD patients highlights their potential not only as biomarkers of the disease, but also as therapeutic targets to attenuate the associated chronic inflammation.

5. The therapeutic potential of miRNAs

Alongside their diagnostic role, miRNAs represent an interesting therapeutic frontier. Strategies currently under development include:

- **Inhibition of miRNAs (antagomiR)**
  AntagomiRs are synthetic molecules designed to specifically bind to a miRNA and block its activity. For instance, inhibition of miR-21 in animal models of asthma has been shown to significantly reduce airway inflammation and Th2 cytokine production (32). Similarly, antagomiRs directed against miR-155 are being studied for the treatment of chronic allergic diseases (33).

- **miRNA mimics**
  miRNA mimics are molecules that mimic the function of reduced or absent miRNAs in patients. In the case of allergies, the use of miR-146a mimics could restore immunological tolerance and reduce chronic inflammation (34).

- **Targeted delivery by nanoparticles**
  One of the main challenges in the therapeutic use of miRNAs is their specific delivery to target tissues. Recent advances in nanotechnology have enabled the development of nanoparticles (i.e. particles with a size in the nanometre range, or 10^{-9} metres) capable of delivering miRNAs or antagomiRs directly to immune cells involved in the allergic response, reducing systemic side effects (35).

6. Conclusions

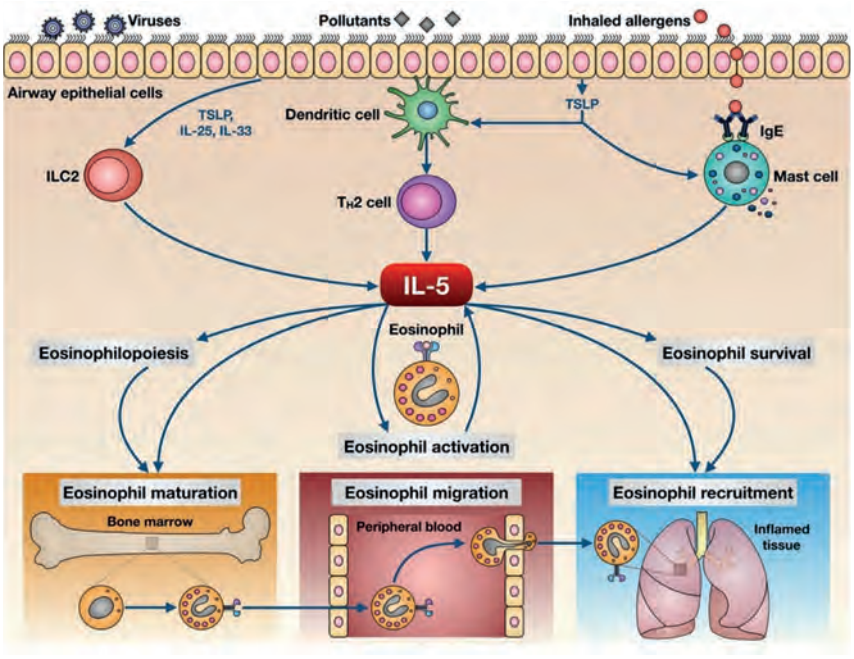
miRNAs represent an emerging and promising frontier in the understanding, diagnosis and treatment of allergic diseases. As discussed in this article, their role is not limited to that of passive regulators, but extends to the active modulation of inflammatory and immunological processes underlying allergic diseases. Their ability to regulate gene expression in specific and versatile ways makes them valuable tools for understanding the pathogenetic mechanisms of complex conditions, including bronchial asthma, allergic oculorhinitis and atopic dermatitis. From a diagnostic point of view, miRNAs offer significant advantages over traditional biomarkers. Their stability in body fluids, combined with the possibility to measure specific expression profiles non-invasively, opens the way for new methods for patient stratification and disease monitoring. For instance, as mentioned in the previous section, miR-21 and miR-155 emerge as key biomarkers for T2 inflammation, while miR-223 and miR-146a highlight their diagnostic potential in diseases characterised by chronic inflammation, such as atopic dermatitis. These expression profiles not only make it possible to detect the presence of a disease, but can also provide information on its severity and the effectiveness of current treatments.

The therapeutic aspect of miRNAs represents another particularly interesting field of research. Strategies such as the use of miRNA antagomiRs and miRNA mimics demonstrate the possibility of directly intervening in the pathogenetic mechanisms of allergies by modulating the expression of key genes involved in inflammatory processes. Inhibition of miR-21 or miR-155 in preclinical models, for instance, showed promising results in reducing inflammation and modulating the Th2-type immune response. At the same time, restoring the expression of miRNAs with regulatory function, such as miR-146a, could help to restore immunological tolerance and reduce chronic inflammation.

However, one of the main obstacles remains the targeted delivery of miRNAs to target tissues. Recent innovations in nanotechnology, such as the use of nanoparticles, have opened up new possibilities to overcome this challenge. These advanced delivery systems promise to increase the therapeutic efficacy of miRNAs while minimising systemic side effects, thus improving the clinical applicability of these approaches.

Despite significant progress, many questions remain open. There is a need to further deepen our understanding of the complex interactions between miRNAs and their molecular targets, and to develop more precise and standardised diagnostic and therapeutic tools. Moreover, the transition from preclinical research

Figure 4 Role of IL-5



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to clinical application requires rigorous clinical trials to assess the safety, efficacy and economic feasibility of these interventions.

In conclusion, miRNAs represent a milestone in precision medicine, offering unprecedented potential to transform the landscape of allergic disease management. As research advances and new technologies are integrated, miRNAs could become essential tools in personalised diagnosis and treatment, significantly improving the quality of life of patients suffering from allergies and other immunological conditions.

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Allergy to Hymenoptera venom

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1. Introduction

Allergy to Hymenoptera venom is among the leading causes of fatal allergic reactions in the European adult population (1).

Systemic reactions are characterised by the immediate onset of clinical manifestations that may involve one or more of the following systems: mucocutaneous, respiratory, gastrointestinal and cardio-circulatory.

Based on the severity of the reactions, they are divided into four grades according to Mueller's classification (table 1) (2).

Extensive local reactions (ELRs) also show an IgE-mediated mechanism with peculiar late-phase inflammatory reaction characteristics, and consist of an erythematous oedema at the sting site with a diameter of more than 10 cm that persists for at least 24 hours (3).

Hymenoptera venom allergy has a highly effective therapeutic weapon in the form of venom-specific immunotherapy (VIT), the only treatment capable of preventing even fatal systemic reactions in individuals with a history of previous generalised reaction to a sting with proven IgE-mediated sensitisation. It is therefore to be considered a life-saving therapy to be provided to any individual at risk of potentially fatal systemic reactions (4).

The prevalence of systemic reactions is between 0.3-8.9%, highest among beekeepers (14-32%). ELRs affect 2.4-26% of the adult population and 38% of beekeepers (5, 6).

The incidence of mortality is between 0.03 and 0.48 cases per million inhabitants per year, which means that about 15 deaths per year occur in Italy. These figures are underestimates due to the difficulty of correlating some sudden deaths with Hymenoptera stings.

The prevalence of asymptomatic sensitisation to venom in the general population is 9.3-40.7% (4, 5). Sensitisation alone, i.e. in the absence of a clinical history of allergic reaction to the sting, is associated with an increased risk of developing ELRs, but is not associated with an increased risk for systemic reaction (7). This is why diagnostic tests should only be performed in children or adults with a history of allergic manifestations to the sting.

The natural history assesses the risk of local or systemic reactions following the previous reaction presented by the patient: a person with an ELR following a sting has a limited risk of presenting a systemic reaction to re-sting. In case of a systemic reaction, the risk of re-presenting a systemic reaction increases based on the severity of the previous reaction (table 2). In children, the prognosis is usually better than in adults (8-11).

2. Taxonomy, morphology and entomology of Hymenoptera of allergological interest

Aculeate Hymenoptera of allergological interest in Italy are divided into Apidae and Vespidae.

The family Vespidae comprises the subfamilies Vespinae and Polistinae; they differ morphologically by the segment joining the thorax and abdomen, which is truncated in the Vespinae and

SUMMARY

Keywords
• allergy to Hymenoptera venom • venom-specific immunotherapy
• venom allergy testing

Acronyms
• **VIT** venom immunotherapy
• **SM** systemic mastocytosis
• **ELRs** extended local reactions
• **BAT** basophil activation test
• **CCDs** cross-reactive carbohydrate determinants
• **REMA** Spanish Network on Mastocytosis

Allergy to Hymenoptera venom is among the leading causes of fatal allergic reactions in the adult European population.
Venom-specific immunotherapy is the only effective and safe treatment that can prevent potentially fatal systemic reactions in allergic individuals. It is therefore imperative that all adults and children with a history of systemic reaction to a sting be referred to a specialised allergy centre and undergo allergy testing.
Once the venom responsible for the reaction has been identified, specific immunotherapy can be started, which in most patients should be undertaken for five years based on validated induction and maintenance protocols.
Various risk factors, such as cardiovascular diseases and mast cell clonal disorders, which influence the severity of sting reactions, the possibility of adverse reactions to specific immunotherapy, or the risk of therapeutic failure of desensitising therapy, if discontinued, must also be taken into account.

tapered in the Polistinae (figure 1).
Within the subfamily Polistinae, which is widespread worldwide, the most common species in Europe is *P. dominula*, followed by *P. gallicus* and *P. nimpha*. The tapered body is yellow-black in colour and 10-17 mm in size, and is characterised by its long legs that are typically outstretched during flight. Their honeycomb has no casing, is less than 10 cm in diameter, attached to a surface by means of a peduncle, and contains a maximum of a hundred individuals; they are usually found in enclosed spaces that are well exposed to the sun, e.g. under roof tiles or under metal sheets.
The family Vespinae includes the genus *Vespa*, *Vespula* and *Dolichovespula.* The most important species in Europe of the genus *Vespula* are *V. germanica*, *V. vulgaris* and *V. rufa.* They range in size from 11 to 19 mm and are yellow-black in colour. The nests consist of several honeycombs stacked on top of each other and protected by a casing. They nest in the ground or in attics. Colonies can reach a size of up to 10,000 individuals.
The genus *Vespa* is represented in Italy by two native species, *Vespa crabro* (European hornet) and *Vespa orientalis*. For the past 20 years, *Vespa velutina* (Asian hornet) has also been present in Europe. It has reached France from south-east Asia and from there north-west Italy (figure 2) (12).
Vespa crabro is characterised by a squat, massive body of a typical yellow and rust-red colour, which can be up to 35 mm long. It frequently nests in hollow trees or in enclosed spaces. Colonies can number up to 1,000 individuals.
Vespa orientalis is a species found in the southern regions of Italy, although for years its presence has been documented in regions further north such as Latium and even Friuli-Venezia Giulia. It is similar in shape and size to the European hornet, but its colour is almost completely rust-red with the end of the abdomen characterised by a bright yellow band.
Vespa velutina is similar to our hornet, but smaller. It is darker, has a yellow-orange band near the sting and a narrow lighter yellow line near the waist. The ends of the legs are yellow. They build small spherical primary nests in spring, attached by a stalk and with a hole in the lower part. Subsequently, they rebuild secondary nests of up to 90 cm in size in a pyriform shape with

Table 1 Classification of the degree of systemic reaction according to Mueller

Grade	Symptoms
I	urticaria, malaise, anxiety
II	previous symptoms plus angioedema, nausea, vomiting, diarrhoea
III	previous symptoms plus dyspnoea (laryngeal oedema)
IV	previous symptoms plus hypotension or shock with loss of consciousness

Adapted from: J Asthma Res. 1966;3(4):331-3 (2)

a side entrance, even at considerable heights such as in tree tops. They can contain between 6,000 and 12,000 individuals.

Among the Apidae, *Apis mellifera* is considered the insect most useful to humans for its products and indispensable for the pollination of numerous plants.

Bees use their sting to defend the honeycomb; being serrated, unlike the sting of vespids and bumblebees, which is smooth, it remains in the enemy's skin, eviscerating and resulting in the bee's death. The venom continues to be poured into the skin, pumped from the reservoir of the venom glands.

Bumblebee bites are extremely rare; in fact, sensitisation and allergy to its venom can be considered a consequence of occupational exposure of greenhouse workers, or of people who work in the farms of these pollinators.

3. Diagnosis of Hymenoptera venom allergy

The diagnostic tools at our disposal are medical history, skin tests, specific IgE assay, molecular diagnostics, basophil activation test (BAT) and CAP-inhibition test.

The *sting challenge* with the insect believed to be responsible for the reaction has no indication in the diagnostic pathway but can be a valuable tool to verify the effectiveness of VIT, once it has been undertaken.

The medical history provides useful data to define the type of reaction, the number of stings and the time of onset of symptoms. This also makes it possible to distinguish IgE-mediated reactions from toxic reactions.

Information about the circumstances of the sting makes it easier to recognise the insect: the morphological description, if possible, the nocturnal flight, which is characteristic of *Vespa crabro*. The position and description of the nest give important clues: having stepped on the entrance hole of an underground nest allows the suspicion of *Vespula* in case of a nest in a hollow tree, it is very likely to be *V. crabro*; being stung while eating or drinking sugary drinks can be referred to *Vespula*; while inside a fruit picked from a tree there will be a *Vespula* or a hornet.

The medical history also identifies risk factors represented by frequent exposure to stings (occupation, hobby) and comorbidities. Among the latter, systemic mastocytosis (SM) is closely related to Hymenoptera venom allergy: it affects 1 to 8% of patients with venom allergy, and Hymenoptera sting is the main trigger of anaphylaxis in SM patients (13, 14). For this reason, the basal tryptase serum value must be assessed for each patient. However, bone marrow mastocytosis, which is the most frequently diagnosed form in patients with a history of anaphylaxis to stings, is characterised by basal tryptase values in the normal range and no cutaneous mastocytosis. In the case of a Hymenoptera sting, it is instead characterised by a typical anaphylactic reaction: involvement of the cardiovascular system alone, without skin symptoms (15). For this reason, even in the event of basal tryptase in the normal range, the REMA score (table 3) must be evaluated, which, if greater than or equal to 2, identifies patients worthy of haematological evaluation (16). Some data would suggest that the coexistence of mastocytosis and hereditary alpha-tryptasemia is associated with an increased risk of severe anaphylactic reactions, but more recent studies do not seem to confirm this. Further studies are therefore needed (17).

3.1 Allergy tests

Diagnostics for Hymenoptera venom have a dual purpose: to confirm the

IgE-mediated mechanism underlying the reaction and to identify the venom involved. Bee, bumblebee, *Polistes*, *Vespula* and *V. crabro* venoms are available for skin tests and specific IgE assays.

Skin tests involve prick tests with aqueous purified venom at a concentration of 100 mcg/ml followed by intradermal testing at a concentration of 0.1 mcg/ml and then at a concentration of 1 mcg/ml (9, 18).

Positive skin tests and/or specific IgE assays for multiple venoms are found in about one in two patients and do not always reflect genuine sensitisation, but are often caused by cross-reactive components (19).

Cross-reactivity between venoms depends on:

- sequence homology between major allergens expressed in the different venoms (e.g. Ves v 5 and Pol d 5 are major allergens of *Vespula spp* and *Polistes* dominula respectively, and have a sequence homology of about 60 per cent; the same can be said for Ves v 1 and Pol d 1, with a sequence homology of 30-55 per cent) (20-22);
- sequence homology between allergens expressed in different venoms that are generally not clinically relevant;
- Presence of IgE against CCDs responsible for cross-reactivity in vitro.

CCDs are ubiquitous carbohydrate components and are the result of post-translational modification of proteins (glycosylation). In a 2013 study, Ebo highlighted that 20 per cent of pollen allergy patients and 20 per cent of patients with genuine allergy to venom, particularly *A. mellifera* venom, are sensitised to CCDs. In bee venom, several allergens are glycosylated, whereas in *Vespula* venom the phenomenon is present to a lesser extent and is absent in *Polistes* venom (23, 24).

The use of species-specific CCD-free allergens (such as the recombinant allergens expressed in *E. coli*) and the availability of CCD markers (e.g. MUXF) increases the accuracy of the test by allowing real sensitisations to be distinguished from sensitisations due to cross-reactive molecules (22), thus bringing additional elements for a correct diagnosis in the case of positivity to different venoms (figure 3) (25).

It has also been shown that in the case of dual positivity of Ves v 5 and Pol d 5, when the value of one is twice as high as the other, the one with the higher positivity can be considered as the allergen responsible for the reaction, allowing the correct choice of extract for VIT (26).

The allergenic molecules of Hymenoptera of allergological interest are described in table 4 (25).

Among patients allergic to bee venom, the most frequently detected major allergens are phospholipase A2 (Api m 1) and icarapin (Api m 10).

The major allergens of *Vespula vulgaris* and *Polistes dominula* are phospholipase A1 (Ves v 1 and Pol d 1) and antigen 5 (Ves v 5 and Pol d 5). Within the genus *Vespula*, the sequence homology between phospholipase and antigen 5 is very high, therefore the species is of little relevance both diagnostically and therapeutically. On the other hand, with regard to *Polistes*, the sequence homology between the major allergens of *Polistes* present in the United States and those of *Polistes* present in Europe is rather low. It is therefore important to use a product prepared from the venom of *Polistes* for both diagnosis and VIT (27).

While the diagnosis of a specific allergy has become fairly straightforward for bee venom, thanks also to

Table 2 The clinical presentation of the sting reaction indicates the risk of reaction to re-sting

Grade	Risk of systemic reaction to re-sting
Extended local reaction	5-24%
Grade I systematic reaction	10-20%
Grade II systemic reaction	20-40%
Grade III-IV systemic reaction	40-79%

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Table 3 REMA score

Variabile		Score
Sex	Male	+1
	Female	-1
Clinical symptoms	Absence of urticaria and angioedema	+1
	Urticaria and/or angioedema	-2
	Presyncope and/or syncope	+3
Basal tryptase	<15 ng/ml	-1
	>25 ng/ml	+2

(16) adapted from: Int Arch Allergy Immunol. 2012;157(3):275-80

the availability of various recombinant allergens, the diagnosis of allergy to vespids is more complicated.

In fact, in spite of numerous insects of allergological interest from the Vespidae family present in Italy (*Polistes spp, Vespula spp, Vespa crabro, Vespa velutina, Vespa orientalis*), and the possible cross-reactivity of the allergens present in them, which is responsible for the frequent occurrence of multiple positives, only the allergenic molecules Pol d 5, Ves v 5 and Ves v 1 are available for diagnosis. The possibility of also being able to assay specific IgEs to Pol d 1 would increase diagnostic accuracy in the case of *Vespula-Polistes* double positivity (28, 29).

Where anamnesis, traditional diagnostics and molecular diagnostics are inconclusive, further tests such as CAP-inhibition and possibly BAT are recommended, but these tests are performed in only a few laboratories. CAP-inhibition consists of assessing the ability of a specific venom to bind the specific IgEs that the first-level diagnostics indicated as binding another venom. In concrete terms, it consists of assaying specific IgEs after incubating the serum with the venoms concerned. If the specific IgEs bind to the venom, i.e. they are inhibited, they will not bind to the CAP solid phase antigen. Based on the heterologous inhibition of specific IgEs by the different venoms, it is possible to distinguish cross-reactivity from genuine allergy (20). On the other hand, BAT is based on the cytofluorimetric demonstration of a surface marker of basophil activation, after incubating the serum with the venom. It is useful in cases of multiple sensitisations or in patients in whom the IgE-mediated mechanism could not be demonstrated (i.e. skin- and serum-negative patients) despite a positive history of systemic reaction to a sting (30).

If it is not possible to identify the poison responsible for the allergic reaction, it is recommended that the patient undergo immunotherapy with several venoms (18).

4. Treatment of allergic reactions to Hymenoptera venom

Therapy includes measures to prevent further allergic reactions, represented by venom-specific allergen immunotherapy (VIT) and emergency symptomatic drug therapy including self-injected adrenaline.

VIT results in a considerable level of protection in preventing new systemic reactions in the event of Hymenoptera sting; in the case of vespid venom, the protection is about 95%, while it is slightly lower (85%) in the case of bee venom (4).

4.1 Mechanism of action of VIT

The therapeutic efficacy of VIT correlates with a number of immunological events. The most relevant are: (a) significant reduction in the expression of interleukins IL-4 and IL-5 by CD4+ T lymphocytes and at the same time an increase in the production of IFN-γ; this results in a rapid shift of the immunological response from Th2 to Th1, and thus inhibition of the development of allergic symptoms; b) a significant increase in the production of interleukin 10 (IL-10) by FOXP3+ regulatory lymphocytes with a consequent reduction in basophil and mast cell activation and the induction of

Table 4 Allergenic proteins expressed in venoms

Insect	Allergen	Biochemical name	M.W. (kDa)	
Apis mellifera	Api m 1	Phospholipase A_2	16	Major allergen
	Api m 2	Hyaluronidase	39	Major allergen Cross-reactive molecule
	Api m 3	Acid phosphatase	43	Major allergen
	Api m 4	Mellitine	3	Major allergen
	Api m 5	Dipeptidyl-peptidase IV	100	Cross-reactive molecule
	Api m 6	Protease inhibitor	8	
	Api m 7	CUB-serine protease	39	
	Api m 8	Carboxylesterase	70	
	Api m 9	Serine-carboxypeptidase	60	
	Api m 10	Icarapin	50-55	Major allergen
	Api m 11	Major royal jelly protein	50	
	Api m 12	Vitellogenin	200	Cross-reactive molecule
Polistes spp.	Pol a 1, Pol d 1	Phospholipase A_1	34	Major allergen Cross-reactive molecule
	Pol d 2	Hyaluronidase	50	Cross-reactive molecule
	Pol d 3	Dipeptidyl-peptidase IV	100	Major allergen Cross-reactive molecule
	Pol d 4	Serine protease	33	Major allergen
	Pol a 5, Pol d 5	Antigen 5	23	Major allergen Cross-reactive molecule
Vespula spp.	Ves v 1	Phospholipase A_1	35	Major allergen Cross-reactive molecule
	Ves v 2	Hyaluronidase	45	Cross-reactive molecule
	Ves v 3	Dipeptidyl-peptidase IV	100	Cross-reactive molecule
	Ves v 5, Ves g 5	Antigen 5	25	Major allergen Cross-reactive molecule
	Ves v 6	Vitellogenin	200	Cross-reactive molecule
Vespa crabro	Vesp c 1	Phospholipase A_1	34	Major allergen Cross-reactive molecule
	Vesp c 2	Hyaluronidase	35	Cross-reactive molecule
	Vesp c 5	Antigen 5	23	Major allergen
Vespa velutina	Vesp v 1	Phospholipase A_1	34	Major allergen Cross-reactive molecule
	Vesp v 2	Hyaluronidase	35	Cross-reactive molecule
	Vesp v 5	Antigen 5	23	Major allergen Cross-reactive molecule

Adapted from:(12, 25) Pediatr Allergy Immunol. 2023;34 Suppl 28:e13854 Allergy. 2023;78(8):2089-108

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an immunological tolerance phenomenon; c) a significant increase in the levels of specific IgG1 and IgG4 antibodies and a simultaneous reduction in the production of specific IgE antibodies (4, 18).

4.2 VIT indications

Candidate patients for VIT are adults and children who, after a sting, manifest (4):

- respiratory and cardiovascular symptoms for which IgE-type sensitisation to the venom is documented (grade II, III and IV reactions according to Mueller);
- urticaria, accompanied by risk factors or deterioration of quality of life (grade I reaction according to Mueller);

According to the guidelines, specific immunotherapy is not indicated either in subjects in whom IgE sensitisation to the venom is not documented, even if clinical manifestations are present, or in those who present atypical reactions, e.g. of the toxic type. VIT for patients with a history of previous ELR is currently a matter of debate among experts and to date the guidelines exclude it as an indication (31). However, the guidelines do call for VIT to be considered in patients in whom IgE-type sensitisation is detected and who, exposed to numerous stings over the course of a year (e.g. beekeepers), show extensive local reactions. Indeed, VIT has been shown to be effective in reducing the frequency and intensity of ELR (32), improving patients' quality of life and preventing them from taking specific drugs frequently.

In subjects who only show cutaneous reactions following Hymenoptera stings, however, the impairment of quality of life and risk factors such as comorbidities (bronchial asthma, cardiovascular diseases, mast cell activation disorders) and exposure to frequent stings must be assessed. With regard to ongoing treatments, a recent large multicentre study showed that the use of beta-blockers and ACE inhibitors is not a risk factor in causing increased severity of sting reactions, i.e. adverse reactions to VIT. Therefore, there is no need to discontinue these drugs (33).

Systemic mastocytosis (SM) is associated with an increased risk of severe or fatal systemic reactions to stings; for this reason, VIT, whose efficacy and safety has also been amply demonstrated in individuals with SM, is absolutely recommended. However, it must be borne in mind that SM is associated with an increased risk of adverse reactions, particularly during the VIT boost phase, and should therefore be given in specialised allergy centres. Since SM is also associated with a lack of protection against re-sting after discontinuation of VIT, it is prudent to continue VIT indefinitely in patients with SM (4).

Immunotherapy is not contraindicated in pregnancy if already underway, while the start of treatment should be postponed (4).

4.3 VIT protocols and duration

Extracts for VIT can be divided into purified or unpurified, and into aqueous or depot formulations (adsorbed on aluminium hydroxide or tyrosine). There are various induction protocols that involve gradually increasing the dose (*build-up*) of venom over a few hours or at weekly intervals. While in the past the build-up was carried out only with aqueous products, induction schemes with depot products have been adopted in numerous studies for years now, which have demonstrated their safety and efficacy (18, 34, 35, 40-42). The risk of systemic reactions to VIT has slight differences between the various types of build-up schemes, while more significant differences have been found in relation to the type of venom; the aforementioned risk is certainly greater in the case of bee venom than vespid venom.

The optimal maintenance dose guaranteeing a high degree of protection corresponds to 100 µg of venom, which can be doubled in subjects who are not fully protected. The intervals between administrations in the maintenance phase are four weeks for the first year, six weeks for the second and third years, and eight weeks for the fourth and fifth years of therapy.

The optimal duration of treatment is five years, after which discontinuation is possible. Long-term efficacy after discontinuation of VIT has been demonstrated in various studies, stating that only 9-14% of patients who had completed the VIT cycle and were subsequently re-stung experienced

systemic reactions (36).
More recently, a study by an Italian team showed that the level of protection induced following a more prolonged period of VIT (average period of 10 years), discontinued afterwards, was better as systemic reactions to post-VIT re-sting were reduced to 3.4% (37).

5. Conclusions

Allergy to Hymenoptera venom is one of the most frequent causes of anaphylaxis in the adult population.
It is important that any adult or child with a history of systemic reaction to a Hymenoptera sting be evaluated in a specialised allergy centre in order to receive an accurate allergological diagnosis, identify any comorbidities such as SM, and start VIT, the only treatment that can prevent further systemic re-sting reactions.
VIT should be prescribed and administered by experienced allergists, with experience in interpreting diagnostic tests that are not always easy to interpret, who are able to manage the dosage of VIT in the boost and maintenance phases and to intervene in the event of any systemic reaction to therapy. Discontinuation of VIT, which may be considered after 5 years of treatment, must be carefully evaluated by the allergist, who must rule out risk factors associated with *relapse*. Patients at high risk of systemic re-sting reaction after discontinuation of VIT are those with a history of very severe reaction prior to VIT, systemic reaction to VIT or to stinging during VIT, with allergy to bee venom, exposed to frequent stings due to their work, with high basal tryptase levels or suffering from systemic mastocytosis, cardiovascular or respiratory disease. Even patients who, after discontinuing VIT, presented a systemic re-sting reaction, should resume treatment and continue it for their whole life (4, 38). In these cases, the allergist will recommend continuing VIT for a prolonged time or indefinitely with intervals between injections of up to 12 weeks, improving treatment compliance while maintaining efficacy (39).

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ENG

Pollen beyond allergens: environmental interactions and effects on the respiratory epithelium

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1. Pollen and pollinosis

Pollen is one of the most relevant airborne allergens to human health, being a major contributor to respiratory allergies, a cluster of allergic manifestations that affect millions of people worldwide. Pollinosis encompasses symptoms ranging from seasonal allergic rhinitis to pollen-induced asthma, a form of allergic asthma that occurs or worsens in conjunction with the pollen season. Allergenic pollens are derived from various plant species, including tree and shrub species. Their seasonality and peak concentrations vary depending on the geographical region considered and the concomitant environmental conditions.

Epidemiologically, pollinosis is constantly increasing globally, with a variable prevalence, depending on the geographical region. It is estimated that over 20% of the world's population suffers from seasonal allergic rhinitis (1), with an alarming growth in urban areas, where air pollution can enhance the allergenic effect of pollen, altering its protein structure and increasing its ability to induce an inflammatory response. Pollen-induced asthma is also on the rise, representing one of the main causes of access to emergency services during the pollen season. Allergic rhinitis is the most common initial manifestation, but over time the inflammatory process can spread to the lower airways, contributing to the development of asthma. The association between allergic rhinitis and asthma is well documented and based on the concept of 'one airway, one disease', according to which allergic inflammation simultaneously involves the upper and lower airways (1).

The discovery of pollen as an aetiological agent of allergic reactions dates back to the 19th century, when Charles Blackley experimentally demonstrated its ability to induce respiratory symptoms in sensitised individuals. Since then, research has made enormous progress in understanding the immunological mechanisms underlying pollinosis and its impact on public health. Pollen exposure not only induces allergic sensitisation, but also plays a key role in the progression of respiratory disease. In susceptible individuals, contact with pollen triggers an immune reaction involving both innate and adaptive immunity, as described below. In sensitised individuals, each new seasonal exposure to pollen reinforces this immune response, promoting chronic allergic inflammation. The effect of pollen on respiratory disease is not limited to the induction and progression of allergy, but can obviously cause exacerbation of pre-existing conditions. In asthmatic subjects, exposure to high pollen concentrations may in fact trigger more frequent and severe asthma attacks, sometimes resistant to standard therapy.

Insight into the mechanisms by which pollen affects immunity and determines the development of respiratory allergy is essential to improve prevention and treatment strategies.

2. Pollen as a source of allergens

To date, 1,126 different allergens have been officially described, of which 572 are aeroallergens and among these 252 are of plant origin (2).

SUMMARY

Keywords
• pollen • allergens • environment • exposome • innate immunity

Acronyms
- **DCs** dendritic cells
- **GINA** Global Initiative for Asthma
- **IL** interleukin
- **ILC2** type 2 innate lymphoid cells
- **MHC** major histocompatibility complex
- **PM** atmospheric particulate matter
- **PRRs** Pattern Recognition Receptors
- **ROS** reactive oxygen species
- **SPP** Sub-pollen particle
- **TA** Thunderstorm Asthma
- **TLRs** Toll-like receptors
- **TSLP** Thymic Stromal Lymphopoietin

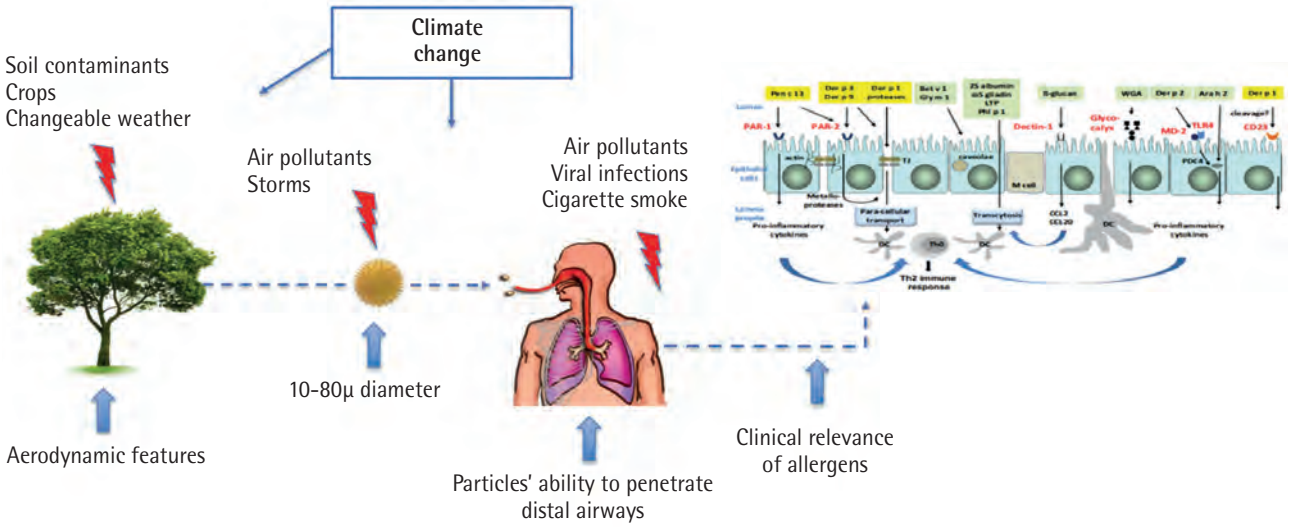
Pollen is recognised as a major cause of respiratory allergic diseases, as it contains a wide range of allergens that trigger immunological reactions in sensitised individuals. Its atmospheric concentration and allergenicity are influenced by multiple environmental factors, including in particular climate change and air pollution.
Pollen's role in respiratory allergy is not limited to the sole source of allergens, as the numerous bioactive molecules in the pollen matrix interact with the respiratory epithelium, stimulating innate as well as adaptive immunity.
Pollenosis has a significant impact on allergic asthma, being a factor in its development, progression and exacerbation, and pollen asthma is a specific phenotype that deserves targeted management.
This article will explore the interplay between external environmental factors, pollen, immunity and pathogenetic mechanisms of allergic asthma, providing an integrated view of the biological and environmental factors involved.

The factors influencing the pollen concentration in the atmosphere, its allergenicity, its ability to penetrate through the airways and trigger respiratory symptoms, are multiple and intertwined.
These factors act at several levels throughout the pollen's life, modifying the timing and duration of flowering of the various plant species, influencing the morpho-structural characteristics of pollen grains and modulating the immunostimulating capacity of allergenic proteins, as depicted in figure 1.

2.1 Factors influencing pollen concentration and allergenicity

Pollen concentrations can be significantly influenced by multiple factors, thus increasing the risk of respiratory symptoms and exacerbations in sensitised patients.
Considering the important role of pollen in respiratory symptoms, in observational or clinical studies it is of paramount importance to minimise the risk of, or mitigate the effect of, confounding factors in pollen concentration analysis. These factors include geographical differences in environmental and meteorological variables, sampling year or data collection methods. For example, there is evidence of considerable heterogeneity in pollen levels on an urban scale, suggesting that the use of a single monitoring site is not representative of pollen exposure across an entire urban area and may lead to significant measurement errors in epidemiological studies. Such errors could be reduced by using predictive daily pollen levels based on models combining vegetation maps, pollen production estimates, phenological patterns and dispersal processes.
The factors influencing pollen concentration are many and difficult to identify, quantify and predict in terms of the type of effect on pollen. According to current knowledge, atmospheric

Figure 1 Determinants of pollen allergenicity



factors (pollution, humidity, temperature, precipitation, UV radiation, wind speed, etc.) and environmental factors (pollution, type of plants, airborne transport of pollen, land use, etc.) interact to determine pollen concentration in the environment and its allergenicity, with direct and indirect consequences on respiratory health (3).

2.1.1 Climate change and pollen

One of the emerging environmental factors responsible for increasing rates of sensitisation and allergic diseases is climate change.

In the United States, for example, rising temperatures in recent decades have been associated an earlier pollen season of 3 to 22 days for spring-flowering species (e.g. *Betula, Quercus* and *Acer*), while late-flowering species (e.g. Artemisia and grasses) have delayed the start of the pollen season by up to 27 days. Extended pollen seasons were also recorded for both tree species and weeds, including *Quercus, Cupressaceae, Oleaceae, Urticaceae* and *Asteraceae* (4). Precipitation and humidity levels also influence pollen emissions: while intense, short-term precipitation significantly reduces pollen concentration in the atmosphere, long-term precipitation can both favour and hinder plant growth, thus altering overall pollen production (5). Furthermore, there is evidence that high humidity or abundant precipitation can cause pollen grains to hydrate, sometimes leading to their osmotic break-up and thus generating submicron (0.5-2.5 µm) pollen fragments, known as sub-pollen particles (SPPs). These fragments, containing allergens, can be carried by the wind in the atmosphere and reach the more distal airways causing acute symptoms, as occurs in Thunderstorm Asthma (TA) (6).

According to current climate change scenarios, episodes of intense precipitation, as well as thunderstorms, cyclones and hurricanes, are set to increase in intensity and frequency. Climate scientists also predict a steady increase in temperatures as well as precipitation, meteorological variables that directly influence pollen emission patterns. In addition, urbanisation will continue to

increase, changing the distribution and composition of vegetation and, consequently, pollen emissions.

2.1.2 Air pollution and pollen

In Europe, air pollution levels exceed the thresholds set by the World Health Organisation (WHO) for 96% of the urban population (3) and it is now known that air pollution can aggravate respiratory allergies both through direct effects on the bronchial epithelium and by increasing the allergenicity of pollen. Specifically, air pollutants increase the allergenic content of pollen and damage its surface, favouring the release of more allergens. They also alter the chemical composition of pollen, causing the release of more SPPs and increasing the total pollen concentration in the air. Ozone (O_3) and atmospheric particulate matter (PM) are the main pollutants linked to the triggering of asthmatic symptoms; PM in particular, can penetrate deep into the respiratory tract and cause direct damage to the respiratory epithelium through the destruction of *tight junctions* and the reduction of muco-ciliary activity. The emission of carbon dioxide (CO_2) due to human activity and global warming can favour the growth of vegetation, thus enhancing the photosynthetic capacity of plants and prolonging the duration of the pollen season, resulting in a higher pollen concentration during peak periods (7). Furthermore, it has been demonstrated that air pollutants such as nitrogen dioxide (NO_2) increase the allergenicity of birch, ragweed, oak and sycamore pollen (7, 8).

The main changes caused by environmental factors in terms of allergenicity and flowering times of the different pollen species are shown in table 1.

2.2 Pollen concentration threshold required to trigger symptoms

In recent decades, atmospheric pollen concentrations have increased, and the duration of exposure has lengthened due to climate change, so knowing the atmospheric concentration of allergenic pollen is crucial for prevention measures. Pollen threshold values used in public warning systems serve to inform the population about the risk of developing allergic symptoms; however, there is no unanimous consensus on which pollen concentrations trigger allergic symptoms. A recent systematic review of 22 studies investigated the relationship between alder, ash, birch, hazel, mugwort and ragweed pollen concentrations and their effects on respiratory health, in terms of doctor visits, medication consumption and allergic symptoms (9). The strongest evidence was reported for ash pollen, where a significant increase in the number of medical visits was observed for concentrations of 18-28 grains/m³ in three studies, while five studies on birch pollen showed a threshold value of 45 grains/m³ for a significant increase in drug consumption.

Evidence of a clear threshold is currently more limited for the other pollen species under study, and factors such as age, gender, pollen allergen content, individual sensitivity and predisposition could explain the differences in the observed results and should be further investigated in future research.

2.3 Factors influencing pollen deposition in the airways

Experimental models aimed at predicting the relationship between the size of aerosol particles and their penetration into the airways show that large particles, with aerodynamic diameters of more than 6 µm, are mainly deposited in the oropharynx, while smaller particles penetrate into the bronchial tree up to the bronchiolar level (10). Intact pollen grains are generally between 22 µm (birch) and 100 µm (maize) in size, so they are too large to reach the lower airways, where the reactions leading to the asthmatic attack are triggered. While pollen viability is related to its biological function, the ability to trigger symptoms is more closely linked to the presence and release of allergenic proteins, which can occur from both viable and non-viable pollen grains.

2.3.1 Pollen size

Different pollen particles are heterogeneous in size, with possible different impacts on the airways. For example, grass pollen allergens are known to be present in the atmosphere in a range of sizes from whole pollen grains (approximately 20-55 µm in diameter) to fractions smaller than 2.5 µm. Pollens of trees belonging to the Cupressaceae family have spheroid-shaped granules, ranging in size from 20 to 38 µm, while the size of *Ambrosia* pollen has been re-

Table 1 Influence on allergenicity and flowering in different pollen species by main environmental factors

	POLLEN-PRODUCING SPECIES	CO_2	O_3	NO_2	TEMPERATURE
Allergenicity	Birch		▲	▲	
	Ragweed	▲		▲	
	Hornbeam			▲	
	Oak			▲	
	Plane			▲	
Early blooming	Gramineae	▲			▲
	Birch				▲
	Oak				▲
	Olive				▲

(8)

ported to be between 16 and 27 µm in diameter and that of *Parietaria* pollen around 16-18 µm (11). The granules of *Artemisia* species, one of the most frequent and severe causes of pollinosis in many parts of the world, are ellipsoidal, with an average polar axis length of 20.6 µm and an average equatorial axis length of 22.1 µm (12).

As already reported, during heavy rainfall or periods of high humidity, pollen grains become hydrated and can undergo osmotic breakdown, releasing allergen-containing SPPs that can penetrate deeper into the lungs. The highest concentrations of SPPs occur during convective thunderstorms, characterised by strong downdrafts, high rainfall, electric ions and lightning (13).

The allergenic properties of SPPs depend both on their small size, which allows them to penetrate deeper into the airways, and on their allergenic content. Studies on SPP obtained from pollen after osmotic shock show that these particles retain their allergenicity (14). The major allergens of *Phleum pratense* Phl p 2 and Phl p 5, for example, are the most concentrated allergens in the SPPs of grasses and are closely associated with allergic respiratory symptoms and, therefore, possibly useful for identifying patients at increased risk (14).

Due to the specific structure of the pollen wall and the lipophilicity of the exine, the outer portion of the pollen, different types of pollutants, including gaseous compounds and PM fractions, can adhere to the pollen surface, inducing physical and chemical changes, altering its allergenic potential and causing it to break down into granules resulting in the release of SPPs (15). Overall, mounting evidence suggests that a large proportion of aeroallergens are associated with respirable particles small enough to settle in the peripheral airways and induce respiratory symptoms in predisposed individuals. These particles could be pollen fragments, soluble allergens adsorbed to air pollutants of various origins, or parts of the dehiscent pollen sac (anther), released upon pollen dispersal.

2.3.2 Pollen morphology

Few studies have investigated the impact of pollen morphology on its

deposition in the airways. Some high-resolution imaging techniques have revealed that pollen grains can be round, ellipsoidal, triangular, disc or bean-shaped, with a smooth or spiny surface. Wind-pollinated plants produce large amounts of light, smooth pollen, whereas pollen from insect-pollinated plants is heavier and sticky.

Experimental studies have shown that pollen particles exhibit greater resistance in a fluid environment (air or water) and lower particle density than aerodynamically equivalent spheres, suggesting that pollen has greater mobility in its aerodynamic flight and greater potential for penetration into the nasal cavity (16). Whether the adhesiveness and aggregation of pollen is influenced by morphology and may influence its deposition in the airways has not yet been demonstrated.

2.3.3 Site of deposition of inhaled pollen particles in the airways

Since SPPs are several times smaller than intact pollen grains, they can evade the filtration of the nasopharynx and penetrate deeper into the airways, causing respiratory symptoms.

Evidence of an association between exposure to grass pollen and early signs of asthma exacerbation, such as changes in lung function and increased airway inflammation, is fairly limited. However, the results of available studies (17) suggest that there is a correlation; in particular, in a cohort study of 936 adult participants, increased grass pollen concentrations correlated significantly with changes in FEF 25-75% and FEV1/FVC ratio, measured 2-3 days after exposure, although there was no correlation with changes in FEV1. This suggests that the greatest impact occurs on medium and small airways. Changes in lung function parameters (FEV1 and FVC) after pollen exposure have also been reported in pollen-infected children and adolescents (17).

3. Innate immune response to pollen

The molecular bases of allergenicity, i.e. the ability of certain molecules to induce type 2 inflammation and the production of specific IgE antibodies, are not yet fully understood. However, the results of epidemiological and experimental studies support the idea that allergic sensitisation depends not only on host genetics and environmental factors, but also on the intrinsic characteristics of the allergenic source itself (7). Pollen exposure, influenced by specific and non-specific external triggers (*pollen exposome*), can damage the airway epithelial barrier through complex interactions, which underlie the onset, development and exacerbations of respiratory allergic diseases.

3.1 The pollen matrix

Pollen allergens are embedded in a complex and heterogeneous matrix, composed of various bioactive molecules, whose composition is in turn influenced by climatic factors and air pollutants (18, 19).

As shown in figure 2, the pollen matrix can be divided into two compartments:

- Intrinsic matrix, consisting of pollen's own compounds (proteins, lipids, carbohydrates, metabolites such as PALMS, adenosine and flavonoids);
- Extrinsic matrix, which includes viruses, aerosols, particulate pollutants and the pollen-associated microbiome.

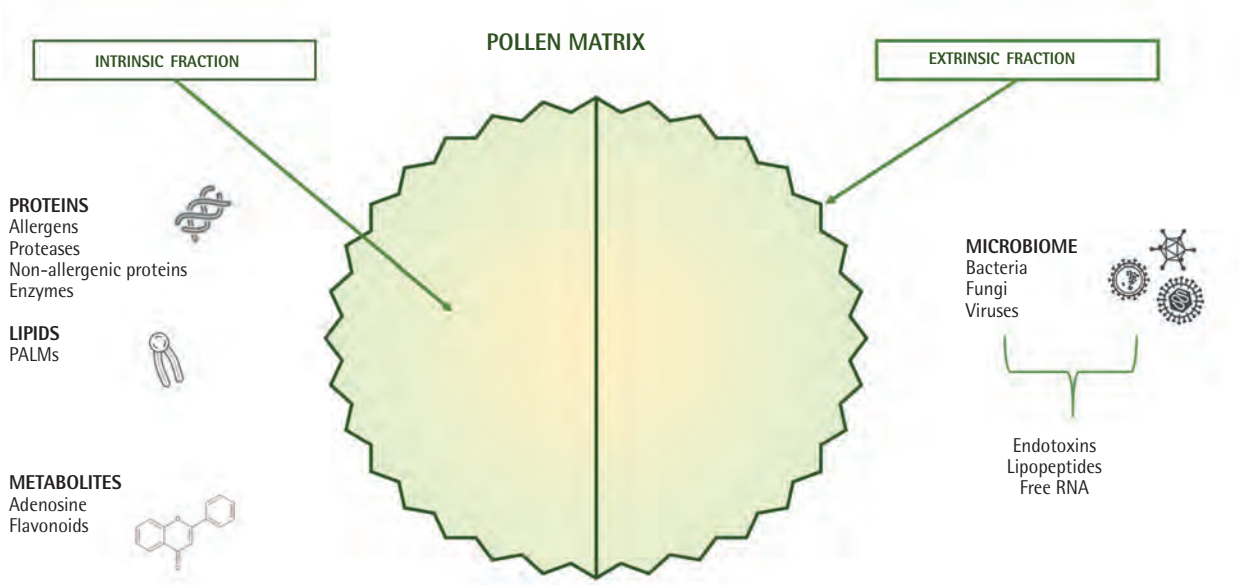
Both components provide an allergen-specific context and are crucial in the sensitisation process. The initiation of allergic sensitisation to various pollen types appears to occur through distinct molecular mechanisms, involving species-specific immune adjuvants for the different pollens, which may contribute to a pro-inflammatory microenvironment and promote Th2 polarisation. Experimental studies have shown that several purified allergens have no intrinsic sensitising potential, supporting the role of other pollen-derived components as key players in the initiation of the inflammatory allergic response in predisposed individuals (18, 19).

3.1.1 Intrinsic compartment of the pollen matrix

Among the non-allergenic proteins in the pollen matrix, proteases play a crucial role in determining the onset and severity of respiratory allergies.

Acting directly on the respiratory epithelium, pollen matrix proteases alter the epithelial barrier, activate inflammation and promote allergic sensitisation. In addition, increased air pollution and climate change amplify the effect of pollen proteases (20-22).

Figure 2 Intrinsic and extrinsic pollen matrix and its main components



Barrier damage by pollen matrix proteases occurs through:

- Direct damage to epithelial junctions: proteases degrade proteins that maintain the integrity of tight junctions between epithelial cells, such as occludin, claudin and ZO-1;
- Increased permeability: the destruction of junctions facilitates the penetration of allergens and pathogens, exposing dendritic cells and activating the immune system;
- Activation of *Pattern Recognition Receptors* (PRRs): proteases interact with receptors such as PAR-2 (*Protease-Activated Receptor* 2), which promotes inflammation through the release of alarmins such as IL-25, IL-33 and TSLP

Pollen grains are also rich in lipids that have immunomodulatory effects. For example, it has been shown that in sensitised subjects, unlike in healthy controls, cypress pollen-derived phospholipids are presented to T lymphocytes via the MHC molecules of dendritic cells, causing T cell proliferation and the release of bioactive mediators such as interferon INF-γ and interleukin IL-4, which are essential for the initiation of sensitisation. Subsequent studies in humans with regard to olive pollen, and in vitro in mouse models with regard to birch pollen, have shown that lipids in these pollens activate invariant natural killer T cells (iNKT) through upregulation of CD1d on dendritic cells.

Besides lipids, low molecular weight non-protein compounds also appear to play a role in activating innate immunity and promoting a Th2 response (18, 19)

3.1.2 Extrinsic compartment of the pollen matrix

The pollen microbiota, the composition of which varies between different pollen species, seems to have an influence on allergenic inflammation, for in addition to the intrinsic lipids of pollen, microbial

lipids also act as potent sensitising adjuvants. The influence of viral infections of plants on the sensitising potential of pollen, on the other hand, is still poorly understood and under investigation.
Air pollutants (irritant gases, diesel particulates, ozone, CO_2, nitrogen oxides) can also change the composition of the pollen matrix and pollen microbiota, facilitating allergic inflammation. Indeed, a correlation has been observed between exposure to pollutants and the content of allergens and immunostimulating compounds in pollen (23).

3.2 The role of the respiratory epithelium

Once considered only a passive barrier against allergen penetration, today the respiratory epithelium is recognised as an active element of the inflammatory response. The initiation of allergic sensitisation is a complex process involving several immune cells, such as dendritic cells (DCs), innate lymphoid type 2 cells (ILC2s) and neutrophils.
The facilitated access of allergens to underlying tissues is not the only result of epithelial damage, as the epithelium itself triggers a cascade of effects on both the innate and adaptive immune systems. When pollen comes into contact with the epithelium, it hydrates and releases its contents, which include allergens and various other bioactive matrix molecules, which break the epithelium's tight junctions, allowing allergens and other molecules to cross the membrane (24, 25).
This activates PRRs such as *Toll-like receptors* (TLRs) and *Protease-Activated Receptors* (PARs), which trigger the release of alarmins (TSLP, IL-25, IL-33) and pro-inflammatory cytokines (IL-8, IL-1, IL-6, TNFα), activating DCs and other innate immune cells. As a result, activated DCs migrate to the lymph nodes, where they present processed antigens to naïve T lymphocytes via MHC-II triggering the adaptive response (24, 25).
Thymic stromal lymphopoietin, TSLP, is a key mediator in the development of asthma and allergic inflammation. Pollen induces the release of TSLP through a TLR4- and MyD88-dependent mechanism, probably in turn dependent on oxidative stress. It has also been shown that epithelial activation by pollen extracts of ragweed, birch, grass and cedar causes an increase in reactive oxygen species (ROS) (26).
DAMPs (*damage-associated molecular patterns*) released by airway epithelial cells, epithelial cytokines, ROS and other inflammatory mediators that act as danger signals, promote the early recruitment of innate immune cells such as ILC2s, basophils, macrophages and dendritic cells, contributing to the Th2 bias of the adaptive immune system. These molecules are also responsible for morphological and functional changes in the airways, as they have the ability to induce metaplasia of goblet cells and alterations in mucus characteristics, with negative effects on anatomical barriers (24-26).
Once the Th2 immune response is initiated, there is a class change of B cells, which become antigen-specific IgE-producing plasma cells, thus leading to sensitisation of susceptible individuals to pollen allergens.
Among the key mechanisms involved in the epithelial response to pollens, the role of the Ripoptosome, a protein complex that modulates the epithelial response to pollens and whose activation can lead to two main outcomes, namely regulated cell death, through apoptosis or necroptosis, and inflammatory activation, with the release of immune mediators that amplify the type 2 allergic response, has recently been highlighted (27). The Ripoptosome is a multi-protein complex consisting of several molecules that regulate cell death and inflammation, including RIPK1 and RIPK3 (*Receptor-Interacting Protein Kinases*), FADD (*Fas-Associated Death Domain*), TRADD (TNF *Receptor-Associated Death Domain*), Caspase-8 and Caspase-3/7. Pollen entry into the respiratory tract activates several signalling pathways including the Ripoptosome, which induces apoptosis of epithelial cells and stimulates the secretion of alarmins (IL-33, IL-25 and TSLP).
In particular, caspase-8, activated by the Ripoptosome, induces the cleavage of pro-IL-33 into its mature, biologically active form (mIL-33). IL-33 interacts with the ST2 receptor, expressed on ILC2, basophils and mast cells, stimulating the release of IL-4, IL-5 and IL-13, mediators of eosinophilic inflammation. This inflammatory cascade is responsible for the bronchial hyperreactivity and mucus production typical of allergic asthma. Ripoptosome activation is not limited

to the inflammatory response, but also contributes to the impairment of the epithelial barrier by inducing apoptosis and necroptosis, with the loss of respiratory epithelium integrity and disruption of *tight junctions*, thus facilitating the penetration of other allergens and air pollutants, and further increasing the production of inflammatory cytokines, which perpetuate tissue damage. These mechanisms favour exposure to allergens and the worsening of allergic disease over time.

Ripoptosome activation can be influenced by environmental factors such as air pollution and climate change. Air pollutants such as PM10 and NO_2 increase epithelial damage and amplify the inflammatory response, favouring ripoptosome activation. Climate change influences the production and biochemical composition of pollens, increasing the concentration of allergens and proteases, with a consequent increase in their ability to stimulate inflammation (27).

In summary, the mechanisms involved in pollen-induced innate immune system activation and Th2 polarisation are complex and not yet fully understood. It appears that different sources of allergenic pollen interact with distinct innate receptors and signalling pathways, influenced by genetic polymorphisms that regulate epithelial pattern recognition, barrier function and cytokine production. Taken together, the data suggest that allergic sensitisation to pollen probably results from specific combinations of pollen-related signals, rather than from a common determinant of allergenicity. Taken together, the recent findings on the effects of pollen on innate immunity have opened new perspectives in the understanding of allergic asthma. These findings could lead to innovative strategies for the prevention and treatment of pollen-induced asthma, such as targeted therapies to modulate early inflammation.

4. Pollen asthma

Asthma is a heterogeneous chronic respiratory disease characterised by airway inflammation, bronchial hyperresponsiveness and variable expiratory flow limitation. According to the GINA 2024 definition (28), it affects more than 300 million people worldwide, representing one of the leading causes of morbidity and mortality among chronic respiratory diseases. Among the different phenotypes of asthma, allergic asthma and, in particular, pollen asthma, constitute a significant portion of cases, with important implications for clinical management and prevention of flare-ups.

Allergic asthma falls into the T2-high group, characterised by a type 2 immune response mediated by eosinophils, mast cells and Th2 lymphocytes. It is estimated that up to 80% of childhood asthma cases and over 50% of adult asthma cases have an allergic component. The average age of onset of allergic asthma is earlier than that of non-allergic asthma and allergic rhinitis is frequently associated, contributing to worsening of symptoms and increased risk of flare-ups. Sensitisation to aeroallergens is a major factor in the development and progression of allergic asthma. Epidemiological studies have shown that sensitisation to perennial allergens, such as dust mites and animal epithelia, is associated with a higher risk of asthma than seasonal allergens. However, sensitisation to pollens is also a significant risk factor, especially in patients with polysensitisation.

Allergic asthma accounts for more than 60 per cent of mild-to-moderate forms and pollen asthma accounts for a significant proportion of these. Pollen allergens contribute to the development, progression and exacerbation of allergic asthma. Exposure to these aeroallergens can be chronic, seasonal or acute, and acute exposure episodes include extreme exposure episodes, in which subjects are suddenly exposed to massive amounts of aeroallergens, concentrated in a short interval of time, as occurs during TA episodes, and other exceptional meteorological events (figure 3).

Pollen exposure is closely linked to asthma exacerbations, especially in younger patients. However, the correlation between pollen concentrations and exacerbations has limitations due to geographical, meteorological and methodological variations. A recent meta-analysis of 73 studies showed a strong link between high levels of grass pollen and severe asthma exacerbations in subjects under 18 years of age, with a significant, if less precise, association in adults as well (29). Increased weed pollen was also correlated with severe asthma attacks in subjects under 60

years of age, while tree species were associated with increased hospitalisations up to seven days after exposure, especially in younger people. Despite this evidence, data on other pollen types and age groups remain uncertain and inconsistent, requiring further research to better understand the impact of pollen exposure on asthma flare-ups and improve prevention in at-risk individuals (29).

An important association between pollen allergen concentrations and severe asthma flare-ups was recently demonstrated for the major grass pollen allergen in a study conducted in London, which found that Phl p 5 levels were more consistently associated with allergic respiratory symptoms and asthma hospital admissions than were levels of total grass pollen (30). These results suggest that measurement of airborne allergen levels could improve risk prediction and improve symptom control in sensitised patients.

The pollen asthma model finds its greatest expression in the phenomenon of thunderstorm asthma (TA). 'Thunderstorm asthma' is characterised by severe asthma attacks and asthma-related deaths in patients with respiratory allergy. First described almost 40 years ago, TA has been reported in North America, Europe, the Middle East and Australia. The most serious episode occurred in 2016 in Melbourne, when within a 30-hour period, ten people lost their lives and about 3,500 required medical attention, putting a strain on the city's healthcare system.

During these atmospheric events, clinical symptoms typically occur within the first 20-30 minutes after the onset of the storm in exposed individuals who are allergic to pollen and moulds. TA events result from a complex interaction between environmental factors and individual susceptibility. The most accredited hypothesis to explain this phenomenon is that during these events, aeroallergens concentrate at ground level and, as a result of the osmotic rupture of pollen granules and fungal spores, release inhalable allergenic particles less than 2.5 µm in size, capable of penetrating the airways in depth (6).

Major individual susceptibility factors to TA include:

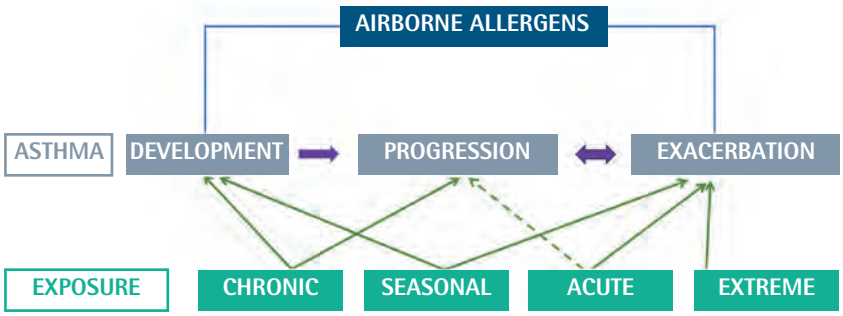
- Pre-existing sensitisation to seasonal aeroallergens;
- History of seasonal allergic rhinitis;
- Low rates of inhaled corticosteroid use in patients with allergic asthma.

In view of these data, patients with pollen-induced asthma should be carefully evaluated for background therapy, as under unfavourable conditions they may be prone to severe and even fatal flare-ups (31).

Despite these important aspects, pollen asthma has so far not been treated as a distinct phenotype in international guidelines, nor are specific therapy adherence data available for pollen asthma patients. However, the importance of careful phenotyping of mild-to-moderate asthma, as is already the case for severe asthma, is becoming increasingly apparent.

Allergy history and diagnostics, together with the measurement of functional parameters with correct timing, are essential in the assessment of pollen asthma, in order to adjust therapy according to the seasonality of exposure, to prevent flare-ups with management and therapeutic measures, and to implement adherence as much as possible, in a patient population that is generally younger than that of other

Figure 3 Role of allergen exposure in development, progression and exacerbations of bronchial asthma



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asthma phenotypes, often less perceptive of symptoms, and less inclined to undertake chronic therapy.

5. Conclusions

Recent discoveries on the immunological effects of pollen on innate immunity are revolutionising our understanding of pollen-induced asthma and opening up new therapeutic perspectives. From a simple trigger of allergic reactions, pollen proves to be a much more complex player in the modulation of the immune system, influencing not only adaptive but also innate responses. This new vision allows us to consider innovative therapeutic approaches aimed not only at symptom control, but also at modulating the dysfunctional immune responses underlying the disease.

The possible practical applications of these discoveries are manifold. On the one hand, the possibility of developing new therapies based on pollen-derived molecules capable of modulating the innate immune response could revolutionise the treatment of allergic asthma. On the other, understanding the role of pollen in the activation of specific immune receptors could lead to more effective preventive strategies, such as targeted immunotherapies and new asthma prevention strategies in at-risk individuals.

These discoveries also stimulate new fields of research: one of the most promising areas concerns the interaction between pollen and the respiratory microbiome, with potential implications for the treatment not only of asthma, but also of other chronic diseases. Furthermore, the study of different types of pollen and their specific interactions with the immune system could lead to a more detailed classification of risk factors for allergic asthma, enabling personalised prevention strategies. Finally, the use of nanotechnology for the targeted delivery of pollen-derived active ingredients could represent a breakthrough in the treatment of airway inflammation.

Accurate phenotyping of mild-to-moderate asthma in general, and allergic asthma in particular, is crucial for understanding and targeting the treatment of pollen asthma. The identification of the various phenotypes and endotypes allows personalisation of treatment, with the aim of increasing therapeutic adherence, improving efficacy and reducing side effects of treatment. This approach is crucial for the proper management of patients who may be at risk of very severe exacerbations, as in cases of *near fatal asthma* following massive allergen exposure. Proper identification and appropriate, individualised management of pollen asthma, as well as the implementation of targeted preventive strategies, are goals to be pursued in order to significantly improve quality of life and minimise risks for these patients.

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Anaphylaxis after cutaneous application of argan oil

Anaphylaxis after cutaneous application of argan oil

by Las Marinas Alvarez M.D. et al.

Journal of Investigational Allergology & Clinical Immunology. Vol. 31,4 (2021): 332-334. doi:10.18176/jiaci.0596

The argan tree (*Argania spinosa L.*, Figure 1) is an endemic plant of south-western Morocco, well adapted to arid and semi-arid climates. It can reach 10 metres in height and live up to 200 years; its deep roots help to slow down soil erosion and desertification. Apart from the environmental point of view, these trees also play a fundamental role on a socio-economic level. In particular, a valuable oil known for its nutritional and health-promoting properties is extracted from their seeds and has many uses in cooking, medicine and cosmetics. Traditionally, the oil extraction process is carried out by women (Figure 2). UNESCO designated the vast area where the argan tree grows as a 'Biosphere Reserve' in 1988 and recognised the 'argan tree practices and knowledge' as an intangible cultural heritage of humanity in 2014 (1).

Argan oil is becoming increasingly popular on the global market and is widely used in the cosmetics industry. In this paper, published in the *Journal of Investigational Allergology & Clinical Immunology*, a case of contact anaphylaxis due to the use of argan oil is presented for the first time. A 29-year-old woman, polysensitised, turned to the allergy service of the General Hospital of Valencia (Spain) because of two anaphylactic episodes (in 4 years) triggered by the use of cosmetic products containing argan oil, in the first case a hair product applied on the scalp, and in the second a body oil used on the arm. In both cases the woman experienced diffuse itching, anaemia, angio-oedema and hypotension, with more rapid onset in the second episode, which required emergency treatment with epinephrine, later prescribed to the patient in a self-injectable form. The woman stated that she had previously used cosmetic products with argan oil without any problems and that she had never ingested argan oil as a food condiment.

A skin prick test (SPT) performed with a standard set of aeroallergens and different foods gave positive results (wheal ≥ 3 mm in diameter) for pollen (grass, Salsola, mugwort), dust mites, peanut, lipid transfer protein (LTP) from peach (Pru p 3), mustard, tarragon and caraway. Specific IgE in

Table 1

ImmunoCAP		ImmunoCAP ISAC		
	sIgE (kUA/L)			sIgE (ISU)
Mustard	0.12	Grasses (pollen)	rPhl p 1	9.9
Peanut	0.34		rPhl p 5	3.0
Fishing	0.75	Salsola (pollen)	nSal k 1	2.4
		Dust mites	nDer p 1	1.1
			nDer f 1	1.3
Walnut	0.54	Walnut	nJug r 3	0.7
Pru p 3	0.99	Fishing	rPru p 3	0.6
		Artemisia	nArt v 3	0.7

ISU *ISAC Standardised Units (ISU), cut-off 0.3 ISU.*

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Figure 1. Argan tree specimen (2).

Figure 2. Women at work extracting argan oil (3).

serum (sIgE) was determined by ImmunoCAP (Thermo Fisher Scientific) for mustard, peanut, peach, walnut and Pru p 3, and the ImmunoCAP ISAC (Immuno Solid-phase Allergen Chip; Thermo Fisher Scientific) test was also conducted, which allows 112 allergens to be tested simultaneously. The results showed sensitisation to specific grass pollen, Salsola pollen and dust mite allergens, as well as cross-reactivity markers (LTP) in walnut, peach, and mugwort, as shown in Table 1.

They then conducted skin tests with protein extracts prepared directly from berries harvested from the argan tree in Morocco, which gave clearly positive results even at a certain dilution. Given the severity of the symptoms, no oral provocation tests were conducted.

The protein extracts, together with peach and sesame extracts, were then used for immunoblotting with the patient's serum, detecting several IgE-reactive bands in the water-soluble fraction of argan extract (10, 14, 18, 20, 32-34 and 48 kDa) and some weakly reactive ones in the fat-soluble fraction, and in mustard (several bands between 10 and 34 kDa) and peach (13-15 kDa) extracts. Immunoblotting inhibition tests by pre-incubation with peach or mustard extracts revealed the persistence of serum-specific IgE against 18 kDa and 20 kDa proteins, which may therefore be responsible for the symptoms. The dot blot with argan oil (pure or diluted 1:2) gave positive results, showing that the proteins in the berries that caused sensitisation in the patient remain in the oil.

Given the increasingly widespread use of argan oil in both food and cosmetics, an increase in cases of allergy to this product is expected.

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DIY face masks: watch out for hidden allergens

Nickel allergy in an adolescent mimicking angioedema

Ertugrul A. et al.
Contact Dermatitis, 2021, 84(4):263-265. doi: 10.1111/cod.13720

Nickel is the most frequent cause of contact allergy globally (1) with a prevalence between 8% and 10% in children and adolescents (more common in girls). Increased exposure to nickel and mucosal barrier dysfunction are the main risk factors.

Sensitisation to nickel can occur through skin contact, oral contact or inhalation. Being widely used in a wide variety of products (such as jewellery, watches, zips, buttons, keys, household items, cosmetics), skin contact is difficult to avoid in daily life. Nickel is naturally present in soil and water and often contaminates food, especially of plant origin, an important route of systemic exposure. Foods rich in nickel include chocolate, legumes, shellfish, cereals, nuts and canned food.

In this case report, published in Contact Dermatitis, Ertugrul and colleagues describe an unusual case of allergic contact dermatitis (ACD) caused by nickel. This was a 17-year-old girl with severe facial oedema that appeared after repeated and prolonged application (every night for a week) of a homemade face mask made from chickpea boiling water (Figure 1). On medical examination, the patient presented with angioedema on the eyelids and in the space between the eyebrows (glabellar region), erythema on the eyelids, and acne on the chin, cheeks and eyebrows. She was not itchy and there was no evidence of acute eczematous dermatitis. The girl had no ophthalmic diseases, and her laboratory test results were normal. She had already had a similar reaction a year earlier after using a clay mask to treat acne, and had been diagnosed with facial cellulitis.

Skin prick tests with common aeroallergens and with foods such as chickpeas, lentils, beans, nuts and peanuts were negative. Patch tests with the chickpeas used by the girl to prepare the mask and with standard TRUE test series (thin-layer rapid use epicutaneous test with ready-to-use patch), with readings on the second and third day, had shown no reaction to chickpeas, but had given a positive reaction to cobalt and especially nickel (with intense erythema and infiltration on the third day). The patient was asked questions to assess her exposure to nickel-containing substances in her daily life. She rarely wore watches or jewellery; she consumed all legumes, including chickpeas, without any problems.

A repeated open application test (ROAT) was conducted with chickpea water boiled in a metal coffee pot, as done by the patient, then applied locally to her face. Angio-oedema was observed on the patient's face on day 7. The metal coffee pot used by the girl was examined with the dimethylglyoxime (DMG) test, which confirmed nickel release (shown in Figure 2 of the original work). Moderate angio-oedema was also observed by means of a ROAT with chickpea water cooked in a glass container. The authors believe that the repeated and prolonged application of substances containing high levels of nickel, such as clay

Figure 1. Preparation of face mask with chickpea boiling water.

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and chickpea water, to the face induced nickel sensitisation and allergy in the patient; in addition, the use of a metal pot that released nickel may have led to high doses of nickel penetration.
In conclusion, the authors emphasise the possibility of an increased risk of allergic reactions to nickel in food, not only through systemic exposure, but also through skin contact, given the tendency to use homemade face masks with plant products. Furthermore, physicians should be aware that ACD may present in forms similar to angioedema. The first nickel-related case is presented here, but cases of angioedema-like ACD caused by black henna, castor oil and p-phenylenediamine are reported in the literature. A correct diagnosis therefore requires a detailed history combined with a patch test.

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Food allergies: what role do skin barrier and environmental factors play?

Epithelial barrier regulation, antigen sampling, and food allergy

Cook-Mills, Joan M.
Journal of Allergy and Clinical Immunology, Volume 150, Issue 3, 493 – 502, 2022

In this paper, researchers from the Indiana University School of Medicine discuss the link between the development of food allergies and alterations in the skin barrier. Food allergies can occur by the age of 2 years and often in children with atopic dermatitis (AD, or eczema). AD is an inflammatory skin condition with a chronic-recurrent course, characterised by dysfunction of the skin barrier with increased transepidermal water loss (TEWL) and manifested by itching, redness, swelling and skin lesions. AD is associated with mutations in genes, expressed by keratinocytes, with key roles in the maintenance and function of the skin barrier, such as loss-of-function mutations in the FLG gene coding for filaggrin (filament aggregating protein), or mutations in the SPINK5 (Kazal type 5 serine protease inhibitor) and TMEM79 (transmembrane protein 79 or mattrin) genes. A reduction in filaggrin expression in keratinocytes is also observed during allergic inflammation mediated by the cytokines IL-4 and IL-13.
In children, an association was observed between eczema, dry skin and elevated TEWL at the age of 3 months and increased sensitisation to food or inhalant allergens at 6 months. Furthermore, 35% of children with moderate-severe AD have IgE-mediated reactivity to food allergens, suggesting a link between skin barrier alterations and sensitisation to food allergens.
In turn, dysfunction of the skin barrier can trigger inflammation through the release of thymic stromal lymphopoietin (TSLP) and other cytokines, such as IL-25 and IL-33, activating a type 2 inflammatory response. Alteration of the skin barrier, therefore, plays a crucial role in AD and food allergies, and may also be linked to environmental factors such as exposure to air pollutants and toxic substances, microplastics and detergents (which may also contain enzymes, such as protease, lipase, amylase) and changes in the skin microbiome (with reduced diversity and/or enrichment of proinflammatory microbial components) (1, 2). In particular, the *Staphylococcus aureus* bacterium produces toxins capable of disrupting the skin barrier and its presence on the skin has been associated with the production of TSLP, IL-4 and IL-33, the severity of eczema and an increased likelihood of developing food allergies.
AD and food allergies have also been associated with environmental exposure to allergens. For example, significant amounts of several allergens have been detected in house dust, from the most common house dust mite (HDM) and

fungal allergens such as *Alternaria alternata*, to food-borne allergens such as peanut allergens (Figure 1).
This may contribute to the exposure of children during the early stages of growth, when they begin to crawl. Environmental exposure to peanuts in the early years of life appears to be associated with an increased risk of peanut sensitisation and allergy in children with the FLG mutation. Children's skin may also be exposed to food allergens through topical products such as detergents, creams and other cosmetic products. For example, the use of soaps containing hydrolysed wheat proteins has been associated with the development of wheat allergy, and the application of lotions containing peanut oil on the inflamed skin of children increased the risk of peanut allergies by a factor of 7, supporting the idea that the skin is a potential route of sensitisation to food allergens. These, in contact with the skin with an altered barrier, can be picked up by antigen-presenting cells such as Langerhans cells.
To study food sensitisation through dermal exposure to food allergens in the presence of environmental allergens, mouse models are often used, to which a section of the paper is devoted. The authors particularly highlight tape-stripping skin exposure models (removal of more superficial skin layers using adhesive strips) and Flaky Tail (FT) models with FLG and TMEM79 mutations. In heterozygous FT+/- newborn mice, skin exposure to both food and house dust allergens caused intense skin inflammation and induction of allergen-specific IgE (whereas in homozygous mice exposure to food allergens alone was sufficient). Given that food sensitisation in FT+/- mice occurs in the very first weeks of life and well before the development of skin lesions (which in these models appear at several months of age), the authors hypothesise that skin sensitisation with food antigens may occur before the clinical signs of AD even in infants with skin barrier defects, especially for those also exposed to ubiquitous environmental allergens. However, other factors may also be involved. For example, it has also been observed that children of allergic mothers have a higher risk of developing food allergies, as do newborn mice of mothers with allergies, although the allergens triggering allergic reactions in mothers and pups may be different.

Figure 1. **One gram of house dust may contain 5 µg to 2.2 mg of peanuts and 0.1 to 100 µg of A. alternata. A value of approximately 12 µg of HDM allergens (Der p 1/Der p 2) per gram of dust was also estimated.**

On the contrary, the early introduction of oral foods can induce tolerance to food allergens (e.g. cow's milk, eggs, peanuts), as demonstrated by several clinical and preclinical studies. In this regard, the US prospective study LEAP (Learning Early about Peanut Allergy) and the subsequent LEAP-On showed that introducing peanuts into the diets of children between 4 and 11 months of age can reduce the risk of developing peanut allergy by the age of 5 years, and that the tolerance thus induced persists even after stopping peanut ingestion for one year. However, other studies on children with eczema have shown that introducing peanuts later may increase the risk of developing allergy to these foods. Furthermore, protection against peanut allergy through peanut ingestion was less pronounced in patients with *S. aureus* colonisation. In newborn FT+/- mice, induction of tolerance by oral administration of peanut extracts was blocked by simultaneous skin exposure to *A. alternata*.
The authors believe that further studies are needed to investigate the mechanisms of food allergy induction through co-exposure of the skin to food allergens, aeroallergens and other environmental factors. Furthermore, in future clinical trials on food allergy in infants, patients should be stratified according to loss of skin barrier function before the development of AD.

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The autoimmune component in heart failure could pave the way for preventive vaccine therapies

Autoimmune-Like Mechanism in Heart Failure Enables Preventive Vaccine Therapy

Martini E et al.
Circ Res. 2025 Jan 3;136(1):4-25. doi: 10.1161/CIRCRESAHA.124.324999.

Heart failure (HF), a condition in which the heart struggles to pump blood effectively, can have several causes, including heart attack, hypertension, valvular heart disease and dilated cardiomyopathy. HF affects more than 64 million people worldwide and is a major clinical problem. Recent data show that there is a strong association between HF and inflammation; it has also been observed that T-cells play a role in the progression of pressure overload-induced HF (PO-HF).

In this interesting work, a team of researchers from the Humanitas Research Hospital (Rozzano, Milan, Italy) hypothesises the existence of autoimmune mechanisms underlying PO-HF heart failure. In order to define a condition as autoimmune, specific experimental criteria must be fulfilled: i. presence of antigen-specific T cells, ii. serum (containing antibodies) capable of mediating the disease, iii. presence of tissue-specific self-antigens capable of triggering the disease in animal models, and iv. antigen-specific T cells and identifiable antibodies in patients.

The study was conducted in animal models of PO-HF (mice undergoing transverse aortic constriction surgery) and also involved patients with HF.

The authors demonstrated that the transfer of both T-cells taken from lymph nodes draining the heart and serum from PO-HF mice to healthy mice is sufficient to induce a significant reduction in systolic function in recipient animals. They therefore assessed the presence of potential cardiac self-antigens using an innovative screening process. In the case of PO, a Th1-type immune response may be induced and the antigenic reactivity of IgG antibodies may reflect the activity of CD4+ T cells, which are crucial for PO-HF progression. They therefore used a microarray containing 29240 oligopeptides (derived from known autoantigens and randomly sequenced peptides) to detect IgG-recognised antigens in sera from CT- or sham-operated mice (sham controls). Through a careful sequential selection process, they were able to identify 12 peptides from 8 different proteins (table 1), including 3 peptides from the β-adrenergic receptor (Adrb1), already identified as an auto-antigen in autoimmune myocarditis.

The researchers then assessed whether the identified peptides were capable of inducing the disease in vivo. To this end, they injected mixtures of three cardiac peptides (two doses, the second after 21 days) subcutaneously into healthy mice and monitored the animals by echocardiogram at 2, 5 and 9 weeks after the first dose. The results showed a significant reduction in systolic function in the mice that had received group 3 peptides (Adrb1 peptides) and group 4 peptides (YWHAZ, SNRPD1, ATP5O). The presence of high levels of IgG specific for group 3 and 4 peptides in the sera of the mice was confirmed by ELISA assays. The animals also showed T-cell infiltration in the myocardium and T-cell infiltration/expansion in the lymph nodes draining the heart. Only a moderate increase in the expression of cardiac stress markers was observed in the mice with reduced cardiac function, suggesting that the induced cardiac stress was less than PO-HF. They performed further assays, incubating T cells purified from lymph nodes of immunised mice with dendritic cells exposed to group 3

Table 1

Protein	Peptide	Group
Actc1	DLTDYLMKILTERGY	1
H2-Ab1	LSGIGGCVLGVIFLGLGL	
Hnrnpa2b1	LSFETTEESLRNYYEQWGKLTDCV	
Hspd1	IIEGMKFDRGYISPY	2
	TSKGQKCEFQDAYVL	
	LLKGKGDKAHIEKRI	
Adrb1	SAPLSQQWTAGMGLLLALIVLL	3
	KALKTLGIIMGVFTLCWL	
	HRDLVPDRLFVFFNWL	
Ywhaz	LGLALNFSVFYYEIL	4
Snrpd1	EPVQLETLSIRGNNIRYFILPDSLP	
Atp5o	LTANLMNLLAENGRL	

or 4 antigens. Only T cells from mice immunised with group 4 were able to drive a significant immune response against immunising antigens.

The researchers then analysed T-cell and antibody reactivity towards new cardiac self-antigens in patients with HF (N = 35; 69.2% male; 41-87 years). Orthopaedic patients without signs of cardiac disease were included as controls (N = 29; 27.5% male; 31-82 years). ELISA analysis showed similar levels of IgG against group 3 and 4 peptides in both groups of patients, but analysis of the results according to patient age showed a significant increase with age only in healthy controls, suggesting that HF patients might develop cardiac autoantibodies earlier than healthy controls of the same age. They also assessed whether HF patients possessed CD4+ T cells already activated by cardiac antigens, and thus potentially actively involved in the pathogenesis of ongoing heart disease. To this end, they used a highly sensitive fluorescence-activated cell sorting (FACS) analysis protocol of mononuclear cells from peripheral blood isolated from HF patients and stimulated in vitro for 16 hours with group 3 or 4 antigens to detect CD4+ T cells that produced IFN-γ and were positive for a particular surface marker (4-1BB) The combined use of the two activation markers also allows the reliable detection of small populations of memory CD4+ T cells. Such cells were detected after incubation with group 3 and 4 peptides in a subgroup of patients with HF (2/13) indicating that in these patients the identified antigens can be considered as mediators of memory T-cell responses.

Finally, Martini and colleagues used the three peptides from group 4 in an oral tolerance protocol to preventively protect mice from subsequently induced PO-HF. The mice received a mixture of the three peptides (YWHAZ, SNRPD1, ATP50; 8 mg/day) or control solution (PBS) by forced oral administration (oral gavage) for 4 consecutive days prior to CT or sham surgery (sham controls), 1 week after the first administration. In all CT animals, cardiac function at 12 weeks after surgery was reduced compared to the sham controls. However, functional and structural alterations of the heart and the expression of cardiac stress markers were less severe in mice that had received the peptides than those observed in animals treated with saline alone, indicating that induction of prior tolerance to the self-antigen peptides is able to reduce the severity of PO-HF induced later. The researchers then conducted further analyses to investigate the mechanisms underlying this protective effect with methods based on single-cell RNA sequencing on T-cells of the heart, heart-draining lymph nodes and spleen of treated mice, coupled with TCR (T-cell receptor) sequencing. The results showed that induction of tolerance to cardiac antigens in CT mice leads to a reduction in the frequency and clonal expansion of pro-inflammatory T cells in the lymph nodes draining the heart and a clonal expansion of regulatory T cells with a highly immunosuppressive phenotype in the heart.

In conclusion, the authors point out that PO-induced HF presents some characteristics of an autoimmune disease. The initiation of autoimmunity presumably occurs as a side effect of cardiac stress that releases self-antigens from cell populations in the heart. These antigens can be used to reduce the severity of a future disease through oral tolerance, effectively acting as a protective vaccine. These findings could pave the way for the development of new therapeutic solutions for heart failure.

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Provide information, create a profession

Edited by **Franco Frati**

Specialist in Paediatrics, Allergology and Clinical Immunology
Director of Lofarma Academy

Hereditary angioedema

Dr. Roberta Gatti
Specialist in Allergology and Clinical Immunology

During my specialisation at the Federico II school directed by Prof. Amato de Paulis, I had the opportunity to study some rare pathologies in depth; in particular, together with Dr. Angelica Petraroli, I followed patients diagnosed with hereditary angioedema, thanks to the reference centre headed by Prof. Giuseppe Spadaro in the ITACA network (*Italian Network for Hereditary and Acquired Angioedema*).

Hereditary angioedema is characterised by recurrent episodes of oedema localised to the skin, abdomen or first airways due to bradykinin accumulation. The disease has autosomal dominant transmission and can be caused by mutations in several genes (SERPING1, FXII, PLG, KNG, MYOF, etc.)

This disease has a strong impact on the quality of life of patients who often have very long diagnostic delays, do not receive adequate treatment (common antihistamines or corticosteroids are ineffective) or may sometimes undergo unnecessary surgery. It is therefore essential to suspect the disease early and refer the patient to the relevant centres. Given the unpredictability of attacks, patients often live with anxiety and must always be provided with *on-demand* therapies such as icatibant (a bradykinin receptor antagonist) administered subcutaneously, which unfortunately is not always effective, and plasma-derived C1-INH, which is, however, only effective in intravenous form for acute attacks. In this regard, it is important to teach self-administration of these drugs, as patients often encounter considerable difficulties when visiting emergency rooms due to a lack of knowledge about the rare disease. I have often heard the experiences of patients who neglected the treatment of 'less severe' (mainly peripheral) attacks precisely because of the difficulty in administering them; or who postponed dental procedures or elective operations because they were possible triggers for attacks despite the availability of short-term prophylaxis; or finally, patients who restricted their travel because of the difficulty in finding drugs in other countries. It is therefore imperative to offer all patients a shared long-term prophylaxis that can make them free of attacks and improve their quality of life: both drugs with subcutaneous administration such as lanadelumab (monoclonal anti-callicrein antibody) or C1-INH plasma-derived, and drugs for oral use such as berotralstat, which act on various mediators of the complement cascade, are currently available.

Dust mite allergy

Dr. D'Onofrio Mario
Specialist in Allergology and Clinical Immunology

As early as the early 1950s, a great deal of evidence showed that house dust contained a miscellany of allergens, and as early as the 1960s it was discovered that most of them were mites. In the 1970s and 1980s, it was shown that the allergenic part of the mite resides in a faecal protein (cysteine protease) called 'Der p 1', which is capable of hydrolysing the keratin and collagen of human epithelial cells (including bronchial epithelium). Today, we know that allergens are produced by the '*Dermatophagoides spp.*' family, the major exponents of which are *D. farinae* and *D. pteronyssinus*, and whose cross-reactivity is now well established. These allergens are divided into: proteins carried by particles of the mite's faecal material (e.g. Der p 1 and Der p 23), cellular debris from its muscle tissue (e.g. Der p 10 - tropomyosin) or others involved in intracellular processes (e.g. Der p 13, 14, 16 and 17). The allergenicity of these proteins depends mainly on the duration of environmental exposure (home, school, work) and their size. Allergens of a faecal nature are transported in particle form and come into contact with our organism via the airways, while other contact routes have yet to be demonstrated for smaller particles (< 15-20 µm) that may cross other types of barrier in our organism, such as the epidermis. Patients with positive skin prick tests for the most common dust mites (mentioned above), and increased levels of total and specific IgE towards *D. pteronyssinus* and *D. farinae*, have an increased risk of developing allergic rhinitis, bronchial asthma, atopic eczema and urticaria. In my experience, paediatric subjects (6-18 years) assessable with SPT for inhalants/food, who test positive for dust mites, almost always present allergic rhinitis with significant hypertrophy of the turbinates, bronchial asthma with productive cough (especially on awakening) and a form of urticaria (mainly localised to the trunk) that occurs after contact with bedclothes and/or blankets that are slept on for several hours. Adults present a similar but more moderate cluster of symptoms. Furthermore, in some cases, there may be cross-reactivity with crustaceans, which have tropomyosin as their main protein/allergen, which is also present in mites (Der p 10), so a crustacean/mite allergy may develop with both gastroenterological and respiratory symptoms. Finally, the assay of specific IgE for Der p 23 (faecal protein associated with the development of asthma) may be useful in predicting the efficacy of AIT (allergen-specific immunotherapy): a high serum titre may in fact anticipate poor therapeutic efficacy in patients undergoing AIT used to treat respiratory allergy to dust mites.

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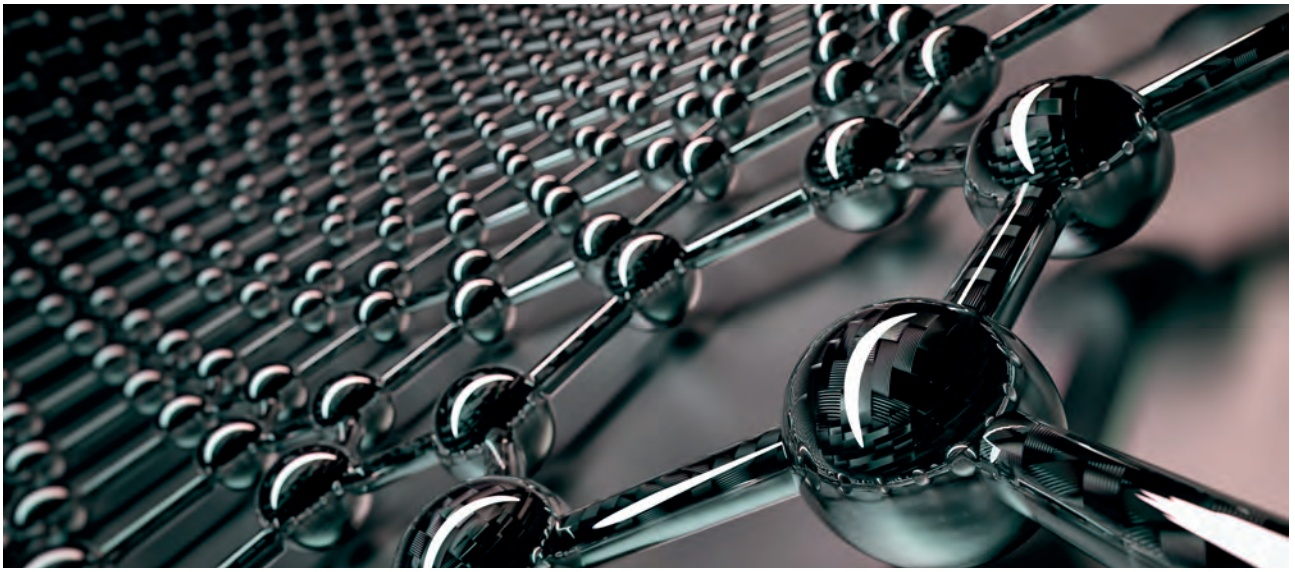


Il grafene è un materiale che si sviluppa su un piano, formato da un singolo strato di atomi di carbonio disposti su una griglia esagonale. Esso è costituito da un foglio sottilissimo che si estrae dalla grafite (il materiale che si utilizza per la mina delle matite), e in virtù della singolare combinazione di proprietà rappresenta un materiale estremamente innovativo. Il grafene, infatti, presenta un'elevata conducibilità elettrica e termica, ma anche un'elevata resistenza meccanica, superiore per esempio a quella dell'acciaio e, al contempo, è un materiale estremamente sottile, leggero e più elastico della gomma. In aggiunta, è dotato di un'elevata trasparenza alla luce (ne assorbe solo poco più del 2%). È evidente che la sua versatilità ha suscitato l'interesse di molti scienziati, aprendo la strada a una svariata gamma di applicazioni:

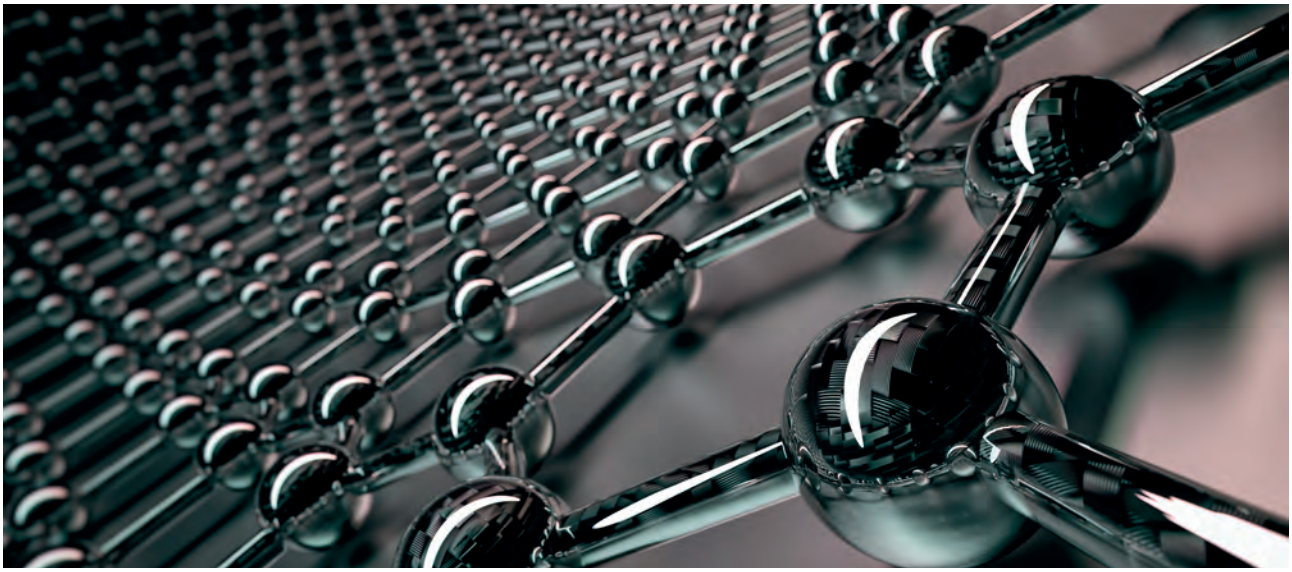
a. nel campo dell'elettronica e dell'energia, rappresentando il candidato numero uno per sostituire il silicio nella produzione di materiali elettronici di nuova generazione; inoltre, può essere utile per migliorare la sicurezza delle batterie al litio aumentandone la capacità di carica, nonché la durata;

b. grazie alla trasparenza, potrebbe essere utilizzato per la produzione di pannelli solari innovativi da usare in sostituzione ai vetri delle finestre;

c. in virtù della sua leggerezza potrebbe essere usato per rinforzare materiali compositi senza aumentarne il peso, ed essere quindi particolarmente utile in settori come l'aeronautica e l'aerospaziale;

d. in campo biomedico, il grafene potrebbe essere utilizzato nella progettazione di sensori ultrasensibili (nasi elettronici) capaci di rilevare quantità minime di sostanze chimiche o biomolecole, rendendoli per esempio utili per analizzare il respiro esalato dai pazienti e diagnosticare la presenza di una patologia respiratoria. Inoltre, questo materiale si potrebbe rilevare particolarmente interessante nella progettazione di dispositivi per impianti neurali per rilevare, trattare e monitorare (mediante stimolazione elettrica) una serie di malattie del sistema nervoso centrale e periferico.

Queste sono solo alcune delle possibili applicazioni del grafene, e la collaborazione continuata tra Accademia e Industria sarà cruciale per indirizzare gli sforzi della ricerca verso le applicazioni più promettenti.



Graphene is a material that develops in a plane, formed from a single layer of carbon atoms arranged on a hexagonal grid. It consists of a very thin sheet that is extracted from graphite (the material used for pencil leads), and by virtue of its unique combination of properties, is an extremely innovative material. In fact, graphene has high electrical and thermal conductivity, but also high mechanical strength, higher than, for example, that of steel. At the same time, it is extremely thin, light and more elastic than rubber. In addition, it has a high transparency to light (it absorbs only slightly more than 2%). Clearly, its versatility has attracted the interest of many scientists, opening the way to a wide range of applications:

a. in the field of electronics and energy, representing the number one candidate to replace silicon in the production of next-generation electronic materials; moreover, it may be useful to improve the safety of lithium batteries by increasing their charging capacity as well as their lifetime;

b. thanks to its transparency, it could be used for the production of innovative solar panels or to replace windowpanes;

c. by virtue of its lightness, it could be used to reinforce composite materials without increasing their weight, and thus be particularly useful in sectors such as aerospace;

d. in the biomedical field graphene could be used in the design of ultra-sensitive sensors (electronic noses) capable of detecting minute quantities of chemicals or biomolecules, making them useful, for example, for analysing the breath exhaled by patients and diagnosing the presence of a respiratory disease. Furthermore, this material could be of particular interest in the design of neural implant devices to detect, treat and monitor (by means of electrical stimulation) a range of diseases of the central and peripheral nervous system.

These are just some of the possible applications of graphene and uninterrupted collaboration between academia and industry will be crucial to direct research efforts towards the most promising applications.

El grafeno es un material que se despliega en un plano, formado por una sola capa de átomos de carbono ordenados en una rejilla hexagonal. Consiste en una lámina sumamente fina que se extrae del grafito (el material utilizado para la mina de los lápices), y en virtud de su combinación única de propiedades es un material extremadamente innovador. Efectivamente, el grafeno tiene una gran conductividad eléctrica y térmica, pero también una gran resistencia mecánica, superior, por ejemplo, a la del acero, y, al mismo tiempo, es un material extremadamente fino, ligero y más elástico que el caucho. Además, tiene una gran transparencia a la luz (sólo absorbe algo más del 2% de ésta). Es evidente que su versatilidad ha suscitado el interés de muchos científicos, abriendo el camino a una amplia gama de aplicaciones:

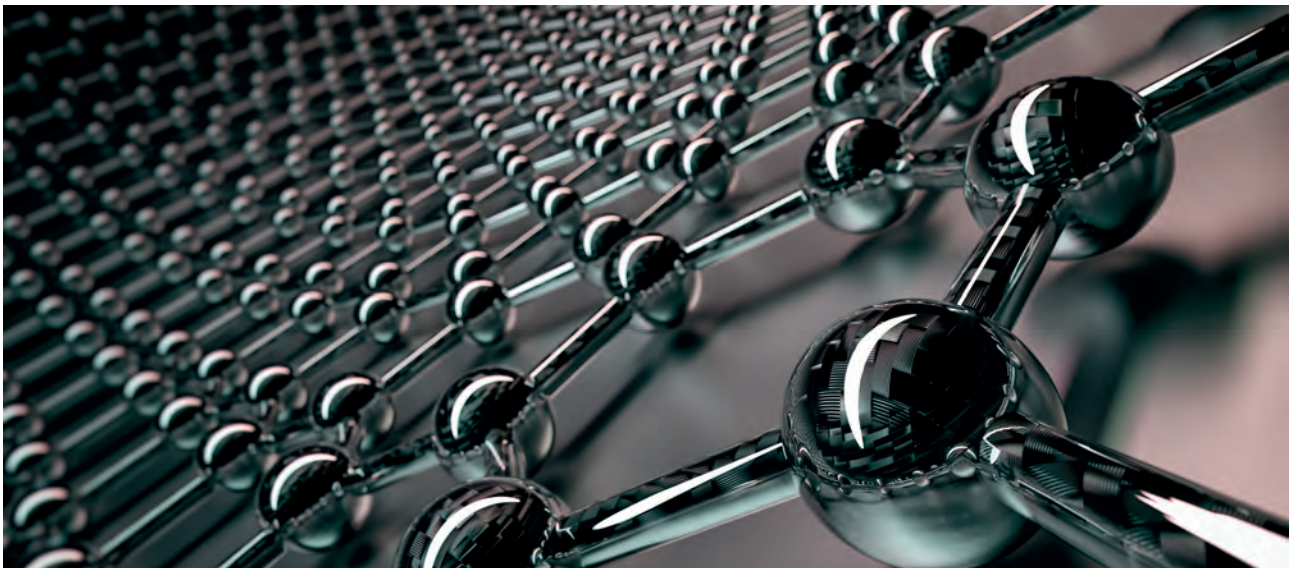
a. en el sector de la electrónica y la energía, pues representa el mejor candidato para sustituir al silicio en la producción de materiales electrónicos de nueva generación; además, puede ser útil para mejorar la seguridad de las baterías de litio aumentando su capacidad de carga, así como su vida útil;

b. gracias a su transparencia, se podría utilizar para fabricar paneles solares innovadores que sustituyan a los cristales de las ventanas;

c. por su ligereza, se podría utilizar para reforzar materiales compuestos sin aumentar su peso, por lo que sería especialmente útil en sectores como el aeronáutico y el aeroespacial;

d. en el sector biomédico, el grafeno se podría utilizar en el diseño de sensores ultrasensibles (narices electrónicas) capaces de detectar cantidades ínfimas de sustancias químicas o biomoléculas, que serían útiles, por ejemplo, para analizar el aliento exhalado por los pacientes y diagnosticar la presencia de una enfermedad respiratoria. Además, este material podría interesar especialmente para el diseño de dispositivos de implante neural para detectar, tratar y monitorizar (mediante estimulación eléctrica) una serie de enfermedades del sistema nervioso central y periférico.

Éstas son sólo algunas de las posibles aplicaciones del grafeno, y la colaboración continua entre el mundo académico y la industria será fundamental para dirigir los esfuerzos de la investigación hacia las aplicaciones más prometedoras.



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