INTERNATIONAL

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Carenze micronutrizionali e allergie
Micronutritional deficiencies and Allergies
Deficiencias de micronutrientes y alergias

Real-World Evidence nell'immunoterapia specifica
Real-World Evidence in Specific Immunotherapy
Real-World Evidence en Inmunoterapia Específica

Nuove modalità di comunicazione nelle malattie allergiche
New modalities of communication in allergic diseases
Nuevos modos de comunicación en relación con las enfermedades alérgicas

SPECIAL EDITION

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2023 • Vol. **41** • **Special Edition**

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La magia degli spettacoli pirotecnici che illuminano il cielo notturno in un tripudio di colori, scie luminose e fontane sfavillanti affascina sempre lasciando tutti con uno sguardo all'insù pieno di stupore e meraviglia. Essi sono generalmente associati al festeggiamento e alla celebrazione rappresentando un simbolo di festa, di gioia, di allegria, ma anche un segno di buon augurio preannunciando l'arrivo di un'occasione speciale da condividere con gli altri.
L'immagine di fuochi di artificio usata per la cover di questo numero del Notiziario Allergologico è stata scelta proprio per preannunciare l'arrivo di un'occasione speciale nella storia di questa rivista rappresentata in questo caso dalla sua internazionalizzazione con la traduzione dei testi in spagnolo e inglese. Tutto ciò nella convinzione che il suo apprezzamento possa estendersi non solo in Italia ma anche in altri Paesi. Finalità della rivista è quella di offrire alla classe medica uno strumento di approfondimento scientifico su tematiche legate al mondo dell'allergologia. Questo grazie alla pubblicazione di articoli di carattere divulgativo da parte di autori di consolidata autorevolezza e competenza che potranno appagare la curiosità, l'interesse, il desiderio di aggiornamento di un numero sempre crescente di medici (questo è il nostro auspicio), non necessariamente specialisti in materia.

The magic of fireworks displays lighting up the night sky in a riot of colours, light trails and sparkling fountains always fascinates, leaving everyone with an upturned gaze full of awe and wonder. They are generally associated with feasting and celebration, representing a symbol of festivity, joy and merriment, but also a sign of good luck heralding the arrival of a special occasion to be shared with others.
The image of fireworks used for the cover of this issue of the Notiziario Allergologico was chosen precisely to highlight a special occasion in the history of this journal, represented in this case by its internationalisation with the translation of the texts into Spanish and English. All this in the belief that its appreciation may extend not only in Italy but also in other countries. The aim of the journal is to offer the medical profession an in depth scientific tool on topics related to the world of allergology.
This is thanks to the publication of popular articles by authors of established authority and competence that will satisfy the curiosity, interest, and desire to keep up to date of a growing number of doctors (this is our hope), not necessarily specialists in the subject.

La magia de los fuegos artificiales que iluminan el cielo nocturno con un derroche de colores, estelas de luz y fuentes centelleantes siempre fascina, dejando a todo el mundo con la mirada al cielo, llena de asombro y maravilla. Generalmente se asocian con la fiesta y la celebración, representando un símbolo de festividad, alegría, jovialidad, pero también un signo de buena suerte que anuncia la llegada de una ocasión especial para compartir con los demás.
Hemos elegido la imagen de los fuegos artificiales utilizada para la portada de este número del Notiziario Allergologico precisamente para destacar una ocasión especial en la historia de esta revista, representada en este caso por su internacionalización con la traducción de los textos al español y al inglés. Lo hicimos porque estamos convencidos de que nuestra publicación puede apreciarse más allá de Italia, también en otros países. El objetivo de la revista es proporcionar a la profesión médica una herramienta de profundización científica sobre temas relacionados con el mundo de la alergología.
Contamos con lograrlo publicando artículos de carácter divulgativo escritos por autores de reconocida autoridad y competencia, que podrán satisfacer la curiosidad, el interés y el deseo de estar al día de un número creciente de médicos (este es nuestro deseo), que no necesariamente deben ser especialistas en la materia.

SUMMARY

Notiziario Allergologico, 2023 Vol. 41, Special Edition

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**

With this issue of Notiziario Allergologico, started some 40 years ago by the late Dr Falagiani, who died prematurely in 2011, a new adventure begins. While the graphic design of the journal has changed several times until arriving at what it is today, on the contrary, the purpose for which the journal was created has never changed, and that is to promote allergological culture as distinct from mere propaganda information, offering readers the possibility of an in-depth analysis and updates on various allergological topics, also aimed at the future, thanks to the competence and authority of the authors of the articles published. The popular nature of the articles has made them accessible to a vast number of specialists, not only allergists but also pulmonologists, paediatricians, dermatologists, etc. working in Italy. Part of the journal also includes the review of a selection of articles or case reports taken from scientific publications, which for their originality are deemed worthy of being brought to the attention of readers.

This issue adds a space (the Lofarma Academy rubric) reserved for future specialists who can use it to talk about their first experiences in the field or to open a dialogue not only with professionals and the industry to improve their knowledge, but also with colleagues from the various Specialisation Schools to exchange information on what they have learnt during their academic career and possibly to propose initiatives to make it as educational as possible.

The most innovative part, however, which represents a sort of challenge for us, is the internalisation of the journal, whereby the texts will also be translated into Spanish and English and the journal will be distributed free of charge not only in Italy but also in other countries, in the hope that it will arouse similar interest and thus become an instrument of allergology popularisation outside Italy, thanks also (so I hope) to the involvement of foreign contributors.

This issue starts with an interesting contribution by Dr Roth-Walter (University of Vienna, Austria) on micronutrients and allergy. 'Let food be your medicine', wrote Hippocrates 2000 years ago. It is well known that food consists of macronutrients (sugar, fat and protein) and micronutrients (vitamins, trace minerals), which are equally essential. It has recently been observed that a prolonged deficiency of certain micronutrients (iron, zinc, vitamin A) could promote a state of chronic inflammation and act on the immune system by making it particularly overactive. All this would favour an exaggerated immune response that would contribute to the development of an atopic state also because, in particular, the absorption of iron by certain immunocompetent cells would be inhibited at the same time. The article goes on to describe an original clinical approach developed by the Austrian group to compensate for iron deficiency and demonstrate how iron replenishment would result in a reduction in symptoms and drug consumption in patients with pollinosis.

ENG

Randomised clinical trials (RCTs) are the gold standard for demonstrating the safety and efficacy of a drug. However, due to the strict inclusion criteria of these trials, the hyper-selected population enrolled may not fully correspond to the real-world population that visits allergy clinics on a daily basis. This is particularly critical in the case of RCTs performed with allergen-specific immunotherapy (AIT), which have treatment times that are different from those applied in real clinical practice. Hence the importance of real-life studies, the subject of Dr Paoletti's (Humanitas IRSSR, Rozzano, Italy) contribution. Such studies are based on data from the 'real world' (Real World Evidence, RWE), collected in real clinical practice on a much larger population. The safety and efficacy results obtained in these studies would therefore be more generalisable, especially if not retrospectively adapted for the purpose.

To underline the growing interest in RWE studies on AIT, the author notes that even the European Medicines Agency (EMA) is reportedly in the process of recognising that data derived from these studies may also represent sources of complementary clinical evidence and could thus be used to support both the pre-authorisation and post-approval phases of AIT products.

We conclude with an article by Prof. Paolini's team (La Sapienza University, Rome, Italy) that describes, in the most informative way possible, the role of particular microstructures released by cells in the extracellular matrix. As knowledge has progressed, it has been shown that these structures (in particular microvesicles and exosomes) represent sophisticated intercellular messengers capable of modulating the behaviour of recipient cells by releasing bioactive molecules of various kinds, thus contributing to the regulation of various biological functions. These microstructures are produced by a wide range of cells and contain a variety of bioactive molecules. The authors focus in particular on those released by mast cells, the main effector cells responsible for the allergic response. Depending on the stimuli provided by the microenvironment, the composition of the vesicles released by mast cells can change and lead to distinct biological responses that may or may not promote the inflammatory process associated with allergic diseases. The authors conclude their article by emphasising that the potential of these microstructures could be exploited both for diagnostic purposes as possible biomarkers of various diseases and for therapeutic purposes by favouring, after appropriate engineering of these microstructures, the delivery of active ingredients only to specific target cells.

As for reviews are concerned, I point out the curious case report on 'meteorites and allergy'.

I wish everyone a good read.

Gianni Mistrello

Micronutritional deficiencies and Allergies

Franziska Roth-Walter

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INTRODUCTION

It is imperative to appreciate the fact that not all people will become allergic. Despite exposure to same food and environmental triggers, only atopic individuals, will respond with their immune system excessively to allergenic molecules. As such, it is pivotal to understand conditions that makes the immune system of atopic individuals "hypersensitive".

As many micronutritional deficiencies are linked with the atopic state, immune priming and inflammation, evidence is given here on the impact of nutritional deficiencies in allergic subjects.

This review will primarily focus on the bioavailability of the micronutrients iron, zinc and vitamin A as key modulators of immune cells, and its prevalence in atopic subjects. The role of allergens will be discussed as nutrient binder, as well as clinical nutritional strategies to prevent micronutritional deficiencies for allergic symptom amelioration.

ABSTRACT

Keywords

• allergy • atopy • micronutritional-deficiencies • iron • zinc • vitamin A • Th2 • IgE • lymph

People suffering from atopic dermatitis, food allergy, rhinitis and asthma are afflicted by micronutritional deficiencies. These deficiencies may contribute to the atopic state as prolonged deficiencies of minerals such as iron, zinc and vitamin A are linked to inflammation, a Th2 signature, the maturation of antigen presenting cells and the generation of IgE antibodies. In contrast, an adequate supply of these micronutrients fosters immune cells with a regulatory and tolerogenic phenotype. The distinct dietary uptake route the blood or lymphatic vessels will be briefly discussed, as well as their bioavailability under healthy and inflamed conditions. In general, iron, zinc and vitamin A uptake occurs via both routes, with inflammation hindering dietary uptake to the blood circulation. The main features of these mineral and vitamin deficiencies will be briefly covered, as well as their known pre-clinical and clinical impact on immune cells. Furthermore, the prevalence of these deficiencies in atopic individuals is summarized. Evidence is presented that the major allergens are indeed able to bind to micronutrients, which may explain the ability of these few allergenic protein families to activate the immune system under nutrient-poor conditions. In contrast, binding to these micronutrients as shown with the lipocalin protein beta-lactoglobulin (holoBLG) – the major whey protein - foster tolerance by providing these precious micronutrients to immune cells.

Finally, we summarize clinical nutritional intervention studies in atopic diseases exploiting the lymphatic route to ameliorate the allergic symptoms in patients with allergic rhinitis against house dust mite, birch- and grass pollen and cats that highlight the lymphatic route represents as a novel and causal dietary approach to combat atopy.

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Figure 1 Macrophages are central for the immune response

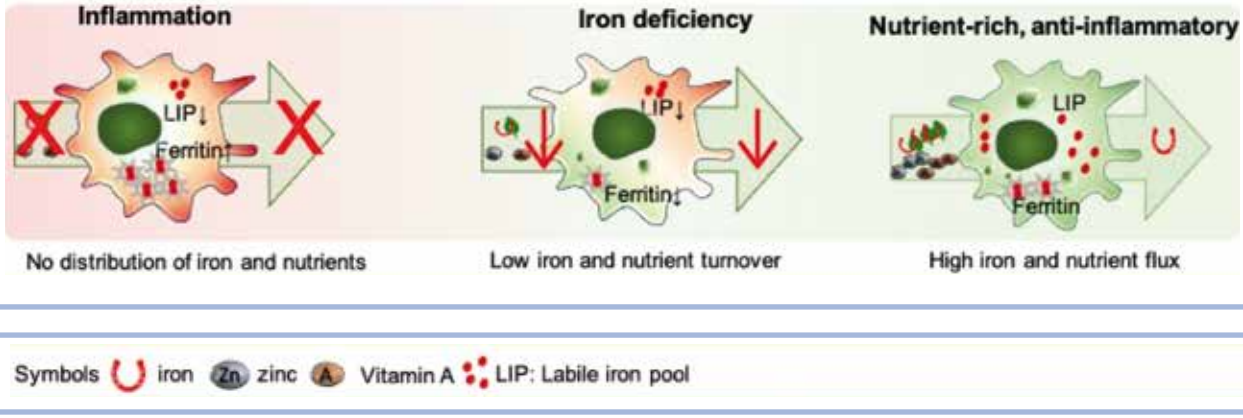

Figure 1. *Macrophages are central for the immune response, but also supply the surrounding tissue with nutrients such as iron. In the case of inflammation, however, the respective uptake and delivery is turned down and also intracellularly the cytosolic iron content, named the labile iron pool (LIP) and representing the metabolic active iron, becomes smaller, due to incorporation into ferritin. Nutritional deficiencies mimic an inflammation as also here the LIP is smaller, which sets macrophages on alarm and more prone to inflammation. In contrast, in nutrient-rich macrophages, macrophages act anti-inflammatory and have a large LIP to supply the surrounding tissues with nutrients*

1. Micronutritional "functional" deficiencies promote inflammation

In general, micronutritional deficiencies are linked to inflammation and disease, which is particularly well seen with iron. The central hub for iron-distribution in our body, are the macrophages. Indeed, only the anti-inflammatory macrophages distribute iron and have a large pool of metabolic active iron. On the contrary, pro-inflammatory macrophages do not partake in iron sequestration or export and decrease intracellularly their metabolic active iron by incorporating into ferritin (1). Intracellularly, the metabolic active iron is represented by the labile iron pool, LIP (Figure 1).

Indeed, nutritional iron deficiency is associated with low-grade inflammation (2). Iron-deficiency is linked to C-reactive protein, CRP, and elevated IgE-levels (3)-irrespective of the cause, which is associated with a more pro-inflammatory signature in the monocytic cell compartment of iron-deficient children (4) and infants (5). Moreover, also skin mast cells are sensitive to iron chelator desferal (desferrioxamine) leading to their degranulation and releases of inflammatory mediators, whereas iron-supply with iron-saturated transferrin, lactoferrin and beta-lactoglobulin (holoBLG) prevents mast cell degranulation (3,6,7).

Also Additionally, zinc deficiency has been linked with loss in oral tolerance and mucosal inflammation (7), with severe zinc deficiency known to result in red and eczematous skin patches (8), while zinc also promotes regulatory T cells and suppresses of pro-inflammatory cytokines (7).

Along these lines, alsoLikewise, Vitamin A deficiency promotes inflammation and is associated with increased

IFNγ and CRP-levels (3,7,9). Severe Vitamin A deficiencies are also associated with a Th2 signature, elevated IgE levels (7) as well as an increased mortality (7). In contrast, oral supplementation with carotenoids inhibits oral sensitization and food allergy in preclinical studies (10,11) with Vitamin A sufficiency promoting T-regulatory cells, immature B-cells, and stabilizing mast cells in vivo (7).

As such, under nutrient-restricted conditions immune cells are primed and rather promote inflammation. In contrast, in nutrient-sated conditions immune tolerance is established with antigen presenting cells such as dendritic cells and B cells kept immature, regulatory T cells being promoted and mast cells being stabilized (Figure 2).

Figure 2 Micronutritional deficiencies prime immune cells

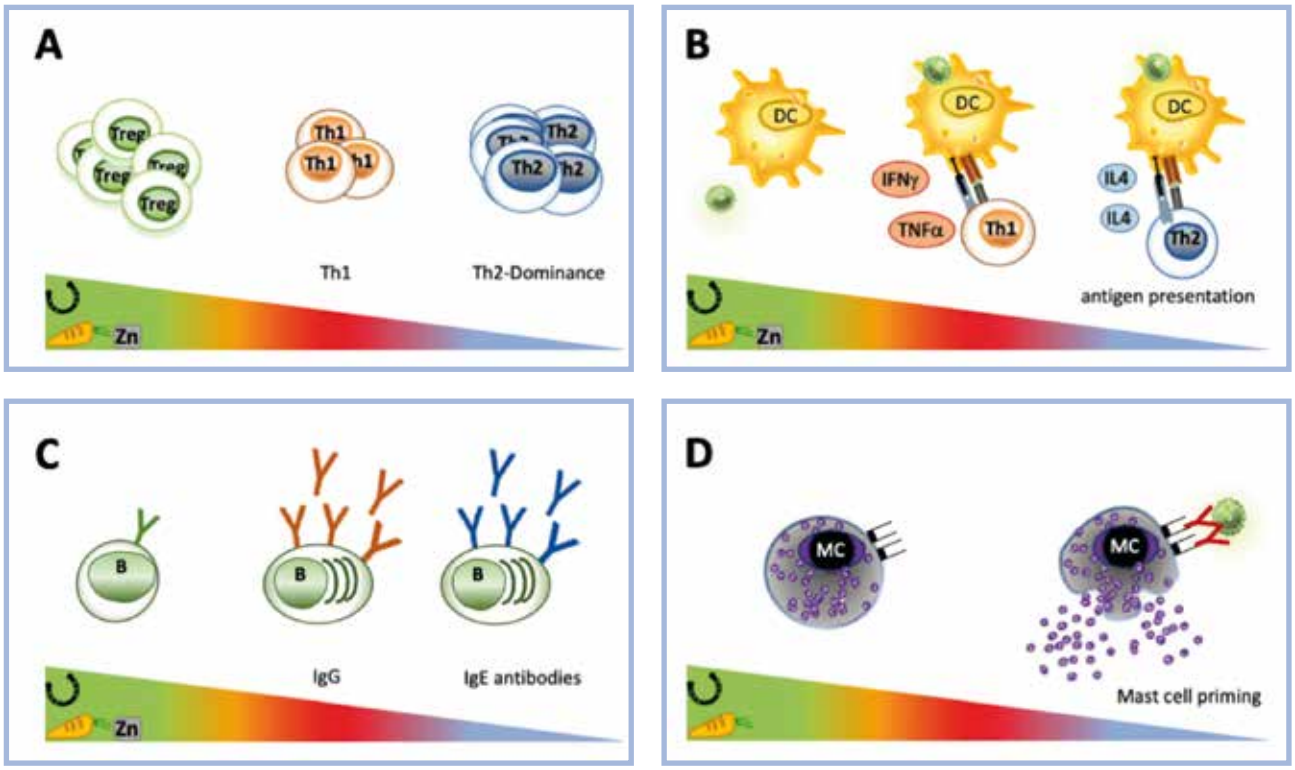

Figure 2. *Micronutritional deficiencies prime immune cells. In a nutrient-rich environment A. regulatory T cells are promoted, while B. Dendritic cells (DCs) and C. B cells don't differentiate and D. mast cells are stabilized and are less prone to degranulate. In contrast, the lack of iron, zinc and vitamin A can lead to immune activation and lead to a* **A.** *Th1 -dominated immune response with* **B.** *effective DC maturation and antigen presentation and* **C.** *the generation of IgG antibodies. Only when these deficiencies persist, a Th2 milieu is generated due to the higher resistance of these cells to survive under nutrient-deprived conditions, with antibody production shifted towards IgE.* **D.** *Locally, iron depletion is sufficient for mast cell priming and evoke degranulation, whereas in nutrient-rich conditions the readiness of mast cells to degranulate is hampered.*

ENG

2. Bioavailability of iron, zinc and Vitamin A in steady-state and inflammation

Dietary uptake of nutrients can usually can follow two main paths: either absorption goes via the enterocytes directly to the blood system and via the portal vein to the liver, or uptake can occur through the lymph system that bypass the liver. Here, the dietary molecules enter the circulation via the subclavian vein.

Most, nutrients are bioavailable through both paths., h However, under inflammatory conditions, the "blood path" is usually blocked for vitamin A, iron and zinc (7). Moreover, many food proteins such as whey proteins usually belonging to the innate defense arm are known to enter the body via the lymph vessels (7).

In general, iron uptake occurs in the duodenum and upper jejunum with Vitamin C, dairy products, but also fat improving iron absorption. In contrast, phytates and tannins bind iron and hinder its uptake (12). Though calcium has been reported to impede iron absorption, this only seemed to be true, when excessively high amount of calcium is consumed (13). Clinical trials have shown that in the presence of low-grade inflammation, the addition of vitamin A improves dietary iron uptake in adolescent girls (14).

Zinc bioavailability improves with proteins and citrates, whereas phytates and dietary calcium inhibit zinc absorption. Zinc can also compete for absorption with other trace minerals, such as iron and copper, for absorption, but only when these minerals are present in large amounts. Under inflammation, zinc levels in the blood (serum) decrease due to increased storage in the liver and reduced dietary uptake (7).

Retinoids and provitamins are absorbed in the intestinal lumen and converted to retinyl esters and packed into chylomicrons for uptake via the lymphatic vessels. The conversion rate of beta-carotene to retinyl-ester is with 12:1 and for other provitamin A carotenoids with 24:1 rather low (7,9). However, the addition of oil can vastly increase their bioavailability by improving their lymphoid uptake. In contrast to retinyl-ester, retinol itself is transported predominantly through the bloodstream to target tissues, such as the retina. Importantly, retinol uptake is reported to be impaired during inflammation.

3. Micronutrient iron

Iron deficiency is the most common nutrient deficiency in the world, affecting an estimated 1.4 billion people worldwide. The prevalence of iron deficiency is highest in children under 5 years, adolescents, and women of childbearing age, with the global pooled prevalence of iron deficiency anemia and iron deficiency being reported for 2022 to be 16% and 18%, respectively (15).

Only in severe cases, iron deficiency leads to anemia, low immune function, cognitive impairment in children, premature birth and low birth weight in babies, and is also associated with an increased mortality (7,9).

It is important to note, that their exist two entities of iron-deficiency: Beside besides the extreme form of iron-deficiency anemia – defined by low hemoglobin-levels-, –, also "functional iron-deficiency" exists. With „ functional iron-deficiency", "metabolic active iron" is reduced and inflammation is always present. Here, body iron stores may be adequate, but iron is not accessible, because iron is stored within ferritin in reticuloendothelial cells – primarily consisting of macrophages and monocytes (3) (Figure 2). Indeed, in subjects with any infectious, inflammatory or malignant diseases, functional iron-deficiency has been reported. Also high performance athletes – due to exercise-induced inflammation – and obese people – because of the presence of low-grade inflammation – are sufferingcan suffer from functional iron-deficiency (3).

For "functional iron deficiency", ferritin-levels are normal or elevated ranging from 30-500 µg/, while transferrin saturation TSAT values are below 20% and dependent on inflammation severity, inflammation markers such as C-reactive protein (CRP) (for low grade inflammation high sensitivity-CRP) or 1-acid glycoprotein (AGP) are elevated (16).

3.1 Iron deficiency and allergic diseases

There is compelling evidence that children and adults suffering from atopic diseases either have " „functional iron-deficiency" ("17) or are anemic (3). Epidemiological studies in the US

(18), Korea (19) and Japan (20) have attested that children with affected by wheeze, allergic rhinitis/conjunctivitis as well as atopic dermatitis, are up to 8 times more likely to be anemic compared to children without allergies. Smaller studies have reported a high prevalence in iron and zinc deficiencies in children with atopic dermatitis, with low serum iron associated with decreased lung function (3,7,9).

The increased risk for allergy is passed from the pregnant women to their children, with a good iron status during pregnancy associated with a lower risk for their children to suffer from atopic dermatitis, or asthma, while a lower iron status during pregnancy being associated with childhood wheeze, decreased lung function and atopic sensitization (3,6,7). In contrast, iron supplementation during pregnancy in combination with folic acid was associated by Fortes C (21) with a fourfold reduced risk for atopic dermatitis, and Shaheen SO (22) calculated in the follow-up of a randomized clinical trial in Finland that iron supplementation during pregnancy significantly reduced the risk of asthma by nearly 70% in the offspring of asthmatic mothers (22). Also, we could show that providing dietary iron for lymphoid uptake for six months in birch and grass pollen allergic women resulted in a 40% improvement of their allergic rhinitis symptoms along with and improved iron status (23).

Not only the prevalence of anemia is greater in allergic individuals, but also the incidence to develop anemia is higher in atopic subjects. Indeed, asthmatics without anemia have a 5 fold greater risk to develop anemia within 5 years (24), and 2 year-old allergic children will nearly double their risk for anemia within a year (20).

To summarize, iron-deficiency is common in atopic individuals, with the risk for anemia being greater in allergic individuals. Importantly an improved iron status has been consistently associated with a decreased in symptoms and allergic diseases.

4. Zinc deficiency

The lack of a reliable and widely accepted marker for zinc status makes it difficult to accurately estimate the number of people who are zinc deficient, with estimated 20% of the world's population may be at risk of zinc deficiency (9). Plasma, serum, hair, and zinc-erythrocyte levels are not sensitive enough to detect mild or moderate zinc deficiency.

Zinc deficiency is closely linked to iron deficiency, as both minerals are found in similar foods (meat, poultry, fish) and are both inhibited by phytates. However, unlike iron, zinc is not affected by blood loss.

4.1 Zinc deficiency and allergic diseases

Studies on zinc deficiency did not associate this mineral with atopy in children (7) nor has maternal zinc intake been shown to reduce the risk of allergic diseases (25), wheezing or eczema in the offspring (26). However, maternal zinc intake during pregnancy is associated with better lung function (7) in the offspring and lower odds of wheezing during childhood, but not with atopic diseases or asthma. Still, low levels of zinc in serum, hair, and red blood cells are consistently reported in people with atopic dermatitis (7). However, zinc levels do not change with the severity of the disease. In people with atopic asthma, low zinc levels are associated with higher levels of total IgE (27). A meta-analysis of studies found that decreased zinc and selenium levels were associated with an increased risk of asthma (28).

As such, although zinc deficiency is not directly linked to the development of allergies, its availability decreases in people with atopic conditions, possibly due to low-grade inflammation.

Zinc supplementations for atopic diseases are still controversial, while some giving evidence for improvement compared to no supplementation in atopic dermatitis, and while others don't. One study showed a beneficial effect on childhood asthma by zinc supplementation for 8 weeks leading to improved clinical symptoms and lung function but not total IgE-levels (29).

Overall, the evidence on the relationship between zinc and atopic diseases is mixed. More research is needed to clarify the role of zinc in the development and progression of these conditions.

5. Micronutrient Vitamin A

The WHO estimates that about 250 million preschool-aged children worldwide have subclinical or clinically relevant low serum vitamin A levels, while Vitamin A supplementation associated with a reduced "all-cause mortality", particularly in children (7,9).
Vitamin A is essential for many bodily functions, including vision, epithelial tissue integrity, and immunity. Severe forms of Vitamin A deficiency can manifest in clinical ocular signs such as night blindness and xerophthalmia. In contrast, subclinical vitamin A deficiency is linked to a worsened outcome and disease course in a variety of conditions and is associated with iron deficiency and inflammation (3,7,9).

5.1 Vitamin A deficiency in allergic diseases

Vitamin A deficiency during infancy and early childhood is associated with the subsequent development of allergies (7). Studies in children suffering from atopic dermatitis reported significantly lower serum retinol levels as well as impaired retinoid-mediated signaling in the skin than healthy controls, while also children with asthma have lower circulating vitamin A levels (7).
Indeed, retinol-deficiency can worsen asthma, allergic rhinitis and atopic dermatitis (7,30). In contrast, retinol supplementation during infancy has not been found to increase the risk of atopy at age 7 (7). Moreover, the intake of the carotenoids beta-cryptoxanthin and alpha-carotene is inversely associated with allergic skin sensitization. However, excess retinol intake (≥2.5 times the recommended intake) during pregnancy was associated with an increased asthma risk in school-age children (7).
For vitamin A, the applied amount and form is decisive for its bioavailability, as too little and too much cause inflammation. Importantly, the bioavailability of carotenes is vastly improved by the addition of oil which promote lymphoid uptake (7,9,31). This is highlighted in the prospective birth cohort study showing that supplementation of children in the first year of life with vitamins A and D in the water-soluble form increased the risk of food allergy and asthma two-fold at the age of four, compared to children receiving the same formulation in oil-suspension (32). Similarly, also in 7-year-old children intake of high dietary preformed vitamin A , but not ß-carotene intake, was associated with higher lung func-

Figure 3 — Dietary lymphoid approach with holoBLG

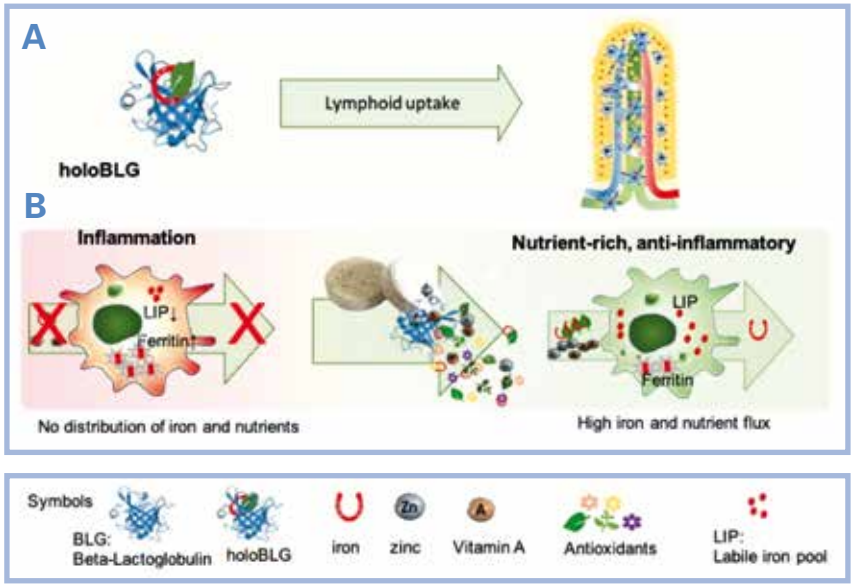

Figure 3. *Dietary lymphoid approach with holoBLG.* **A***: the whey protein beta-lactoglobulin, as a carrier of numerous micronutrients (iron, zinc, vitamins) (holoBLG), with dietary absorption occurring via the lymph* **B***. This allows the supply of micronutrients to immune cells and foster their anti-inflammatory signature.*

tion and lower incident asthma risk (7). Dietary intake of ß-carotene have has been associated with a reduced risk of allergic sensitization and lower IgE levels, in 5- and 8-year-old children and women (33), while others associated ß-carotene intake with an increased risk of hay fever in adults (34).
Hence, while there is evidence that food containing vitamin A may help prevent atopic diseases, the form of vitamin A is essential for its bioavailability and likely explains some of the contradictory findings of different studies.

6. Allergens at the cross-road: nutrient binders as immune primers or tolerogens

Only very few protein families are prone to become allergens. Indeed, allergens are clustered in very few protein families with the major allergens from mammalian systems always belonging to the same superfamilies/protein families. Mammalian allergens usually belong to the "lipocalin" superfamily (3) and major allergens from plant belonging to the pathogenesis-related proteins PR10 family, the prolamin superfamily (seed storage protein families 2S albumins and the non-specific lipid transfer proteins nsLTPs); the Gibberellin-regulated proteins GRPs (35), cupin protein superfamily (legumins-7S and vicilins-11S protein) and Ole e 1 families (36). Something that the majority of major allergens have in common is, that they are able to bind to nutrients such as iron (shown with lipocalins, 7S, 11S and PR10 proteins) (37-45) zinc (46,47), lipids (shown with lipocalins, PR10 and LTPs) (46), Vitamin A and Vitamin D (shown with lipocalins and PR10 molecules) (39-41). A lower immunogenicity upon iron-binding has also been demonstrated with the peanut allergens Arah1 (7S protein), Arah3 (11S protein) (45), egg proteins such as ovotransferrin (48), the lipocalin beta-lactoglobuline(BLG) (42,44) and the birch allergen Bet v 1 (37,43). Moreover, we and others have shown in a series of experiments that PR10 proteins such as Bet v 1 and in particular the whey protein beta-lactoglobulin, representing lipocalins only becomes allergenic in a nutrient-deprived state, whereas under nutrient-sated conditions they were rather tolerogenic and promoted immune resilience in vitro, as well as in preclinical studies in a preventive and therapeutic manner (37-40,42-44).
Moreover, for the vast majority of these protein families, their involvement in the stress response and in nutritional immunity in the respective plant and organism (3,6) is well acknowledged. Recently, we expanded the list of proteins involved in nutritional immunity by adding the major fungal allergen Alt a 1 as a protein capable of binding iron complexes with great affinity (49).
In this regard, our current concept is that due to their function and their ability to bind to nutrients, allergenic proteins can turn into allergens under nutrient-restricted conditions. They can locally deplete their surrounding from nutrients such as iron, lipids or vitamins, thereby triggering a danger signal and evoking an immune response in atopic individuals. In contrast, in nutrient-rich conditions, these proteins will carry micronutrients and are presented with their nutritional binding partner as holo- (loaded) proteins. In these nutrient-rich conditions, they contribute to the nutritional balance of the immune cell by providing these essential nutrients and thereby actively contributing to tolerance.

7. Targeted Dietary intervention using the lymphoid route in clinical studies

The lipocalin and cow milk protein BLG is structurally very similar to human lipocalin 2 LCN2, a protein with decreased concentration in allergics (50), and involved in nutritional immunity in humans. Indeed, LCN2 is able to regulate an immune response depending whether it imports or exports iron from immune cells (3). Indeed, our preclinical studies indeed suggest a similar nutritional and immunomodulatory function as LCN2. BLG harbors some important feature for the dietary route, as is itsit is resistant to digestion, but sensitive to heat (51), and upon ingestion BLG is primarily transported via the lymphatic vessels directly to human immune cells (Figure 3).
Based on our preclinical findings and clinical studies with whey proteins conducted in children with atopic asthma, in which consumption of a whey-based oral supplement for a month reduced IgE antibodies and

improved lung function (52) as well as the RCT from Brazil, in which milk-beverages fortified with micronutrients (Vitamin A, iron, zinc), docosahexaenoic acid, prebiotics (polydextrose, galactooligosaccharides and yeast extract) for 6 months decreased the risk of allergic manifestations by 36% (53), we sought clinical translation and developed a lozenge that combined whey proteins containing predominantly the lipocalin protein beta-lactoglobulin with catechins, iron, zinc, and vitamin A (holoBLG lozenge). The lozenge prototype contained less than 1 mg of iron per lozenge, but contained iron in a form suitable to be carried and transported via the lymph. In a double-blind, placebo-controlled clinical trial with women allergic to birch and/or grass pollen, 6 months of supplementation with the formulated holoBLG lozenge resulted in a 42% improvement in the total nasal symptom score (TNSS) compared to a 13% improvement in the placebo group after nasal provocation. The combined symptom-medication score, which is considered the gold standard of allergen immunotherapy, was also 45% lower in the holoBLG group during the birch pollen peak season and 40% lower during the grass pollen season (23).

In another clinical study with house dust mite allergic patients, 3 months of holoBLG supplementation resulted in a 60% reduction in the TNSS. This reduction was still observed 7-8 months after the participants stopped taking the lozenge (54,55).

Moreover, the findings could also be replicated also in cat-allergic patients, with 3 month supplementation resulting in a reduction of the total symptom score TSS by 50% (56). As such, improving the nutritional immune state by promoting the lymphoid path, where indeed in all conducted clinical studies able to improve allergic symptoms in a completely allergen-unspecific manner.

CONCLUSIONS

Micronutrients are essential, with a lack thereof strongly associated with a hypersensitive immune system. Atopic individuals suffer from micronutritional ("functional") deficiencies particularly of iron and fat-soluble vitamin A, in which dietary uptake of these precious micronutrient seems to be hampered due to low-grade inflammation. To date, specific allergen immunotherapy is considered the only causal treatment option for improving atopic diseases. However, here evidence is given that supplying immune cells with micronutrients shows a strikingly similar efficacy, and this in a completely allergen-independent manner. This underlines that the supply of micronutrients is another causal therapeutic option against allergies that should be included in current practice.

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Real-World Evidence in Specific Immunotherapy

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INTRODUCTION

Specific immunotherapy against inhalant allergens (AIT) is the only treatment available today to modify the course of allergic diseases mediated by type E immunoglobulin (IgE) (1,2).

This class of immunoglobulins is defined by the presence of the epsilon heavy chain and is highly specialised in activating mast cells in epithelial tissues, to which they bind tightly, stimulating powerful inflammatory reactions in the presence of specific antigens such as allergens.

AIT can offer long-term clinical benefits that may persist for years even after treatment is discontinued. As early as 1911, Noon demonstrated the efficacy of subcutaneous injections of a grass pollen extract in patients with allergic rhinitis using an empirical approach. Although the theoretical basis for Noon's intuition that 'vaccination' against 'aerogenic toxins' could induce 'tolerance' was flawed, it served to prove that subcutaneous administration of pollen extracts was effective in reducing the symptoms of allergic rhinitis. This discovery paved the way for the development of modern desensitisation protocols (3).

In 1964, Frankland and his colleagues took an important step in this direction with the first randomised double-blind placebo-controlled trial of subcutaneous immunotherapy (SCIT), and in 1968, Johnstone and Dutton provided evidence that SCIT could alter the natural history of respiratory allergies (4,5).

SUMMARY

Keywords

• **Real-World Evidence** • **specific immunotherapy** • **research methodology**

Specific immunotherapy (AIT) for respiratory allergies can make use of evidence from Real-World Evidence (RWE) studies to evaluate the efficacy and safety of this treatment. AIT is the main approach for modifying the course of E-type immunoglobulin (IgE)-mediated allergies. In the article, the evolution of clinical trials over time from non-randomised to randomised controlled trials (RCTs) is explained, highlighting the challenges and limitations of both approaches.

Diversity in formulations and study methods makes comparison between AIT studies difficult, with the need to standardise clinical endpoints to allow accurate comparison of results. The use of RWE in the context of AIT allows for long-term data on safety and efficacy of treatment. However, it is important to ensure high quality methodology to avoid bias.

A key tool are registries on AIT, a possible source of data for future RWE-based studies, which could ensure approval of AIT treatments for less common allergens by regulatory bodies. In conclusion, RWE-based studies are complementary to traditional RCTs and provide important information on AIT, but methodological challenges need to be addressed to obtain reliable results.

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For over 70 years, SCIT remained the only available form of AIT and was used empirically until the discovery of IgE by Ishizaka in 1965 (6). SCIT may be associated with disadvantages, including the need for repeated injections over time and the increased risk of systemic adverse reactions. Concerns over safety and the need for a simpler administration regimen prompted the search for alternative routes of administration, with the aim of developing effective treatments for allergic rhinitis that could offer better comfort, safety and a reduced chance of human error, compared to conventional SCIT. By the early 1980s, several new routes of administration had been explored, including bronchial inhalation, nasal topical and oral routes; however, these were abandoned due to a lack of efficacy in reducing symptoms or an increased risk of side effects (7).

In the same years, however, there were several landmark studies that demonstrated the safety and efficacy of sublingual immunotherapy (SLIT). In 1986, Scadding conducted the first randomised, double-blind, placebo-controlled trial of a sublingual AIT preparation, demonstrating its efficacy in relieving symptoms in nearly three quarters of patients with perennial allergic rhinitis to house dust mites (8). In 1998, the first mechanistic study of SLIT published in the Lancet (9) revealed that an entirely novel allergoid (monomeric allergoid), compared to traditional allergoids known as polymeric and therefore not administered sublingually (10), was able to induce lower expression of markers of allergic

Table 1 Overview of the most common types of clinical trials with their description

TYPE OF CLINICAL STUDY	DESCRIPTION
OPEN	Clinical study without a control group, as opposed to a controlled clinical study.
CONTROLLED	Clinical study in which there is a treatment group and a control group.
SINGLE BLIND	The patient enrolled in the study, but not the operator, is unaware which of the possible treatments he receives.
DOUBLE BLIND	Both the enrolled patient and the observer ignore the treatment administered.
TRIPLE BLIND	The patient enrolled, the investigator conducting the study and the investigator analysing the data ignore the treatment administered.
CROSSED	Each enrolled patient receives each of the treatments under study consecutively.
ONE-PATIENT	The population is limited to one patient and the order of administration of comparative treatments is determined randomly.
EXPLANATORY	A study in which the objective is basically to gain scientific knowledge and biological explanations of effectiveness.
SINGLE-CENTRIC	Carried out by a single researcher or research team.
MULTI-CENTRIC	Carried out in 2 or more centres with an identical research protocol.
PARALLEL	Each group of patients receives one treatment simultaneously.
SEQUENTIAL	Observations are evaluated step by step when they occur and the total number of participants is not predetermined, but depends on the results obtained.
COMMUNITY	Unpredictably assigned elements are communities or populations, instead of individuals.
PILOT	Initial small-scale study of a study protocol in order to verify whether the project is appropriate and feasible.
OBSERVATIONAL	In this study, the researcher does not determine the allocation of patients to each group, but merely observes what happens.

Table 1 Overview of the most common types of clinical trials with their description

CROSS-SECTIONAL	Each patient represents a moment in time. The purpose of this study is to examine the relationship between different variables in a defined population over a defined period of time.
HORIZONTAL	In this type of study, there is a time lag between the different variables, so that a temporal relationship can be established between them.
ANALYTIC	Designed to examine associations, the aim of which is to identify or measure the effects of risk factors or specific interventions on health.
PROSPECTIVE	Patients are included from the moment you decide to start.
RETROSPECTIVE	The data collected refer to events that have already occurred.
CASES AND CONTROLS	It identifies people with a disease and compares them to a control group without that disease.
COHORT	A study in which there is a group of subjects with a characteristic or set of characteristics in common that are monitored over time
PRAGMATIC	Study designed to find out the effects of a given treatmen on the population in real life.
RANDOMIZED	The allocation of treatment to enrolled subjects must be done by a random method. This increases the likelihood that other variables, not taken into account in the study design, will be evenly distributed in the experimental and control group.
NON-RANDOMISED	In this study, exposure to the agent under investigation is not randomly attributed.

inflammation, associated with a significant reduction in symptoms. In the same year, the World Health Organisation recognised SLIT as a viable alternative to the subcutaneous route for the first time. Subsequently, the European Academy of Allergy and Clinical Immunology (EAACI) guidelines on AIT for the treatment of allergic rhinitis and two World Allergy Organisation (WAO) position papers on SLIT were published (11,12,13).

Both SCIT and SLIT have demonstrated good clinical efficacy for the treatment of allergic rhinitis and allergic asthma, and the availability of both formulations offers physicians and patients a wide choice of treatments. However, due to the wide variety of allergens and extract compositions, it is and will be organisationally and economically difficult to conduct randomised controlled trials (RCTs) for each individual product.

A systematic search on AIT reveals a major problem, namely that many studies are not comparable with each other in many respects. For example, different types of allergen extracts were used in the preparation of AIT, and different dosage schedules were also adopted. In addition, study designs, inclusion criteria and outcome evaluations are also very different. The diversity in the composition of products in use for AIT requires that efficacy must be demonstrated for each individual product, rather than class efficacy, as is the case for other drugs. Furthermore, in the different studies the clinical efficacy of AIT was measured using different tools and scores, and also the primary and secondary endpoints of the studies are very often not comparable with each other. In order to enable comparison of results, greater standardisation of the clinical endpoints to be analysed will also be necessary in the future (14,15).

There is an inherent risk in all this: as the drug registration process is expensive, there may not be sufficient return on investment for pharmaceutical companies in less common allergies in the

population. The increasing need for regulatory authorities to follow and standardise AIT approval studies might push pharmaceutical companies away from investing in research for such products. Ultimately, this situation would lead to the inability to prescribe desensitisation therapy to individuals with uncommon allergies.

Currently, randomised, double-blind, placebo-controlled phase III studies have provided the necessary evidence for the approval of some AIT products as drugs (17,18,19,20,21).

Several meta-analyses (a quantitative clinical-statistical technique that combines data from several studies conducted on the same topic, generating a single conclusive figure to answer a specific clinical question) have been conducted to evaluate the efficacy and safety of SCIT or SLIT for the treatment of allergic asthma and allergic rhinitis in both adult and pediatric populations. The results of the research studies included in the meta-analyses are generally in favour of AIT; but as mentioned earlier, due to differences in the products, doses, protocols and treatment regimens chosen, as well as in the choice of instruments to assess the outcomes, these methodological difficulties may prevent the meta-analyses from reaching solid and definitive conclusions (22,23,24,25,26,27,28).

A product-by-product analysis and evaluation is therefore mandatory when choosing an AIT treatment for clinical use, as clearly stated by the WAO (15) and the EAACI (29,30).

There is a wide range of clinical studies which will be summarised in the most important concepts in table 1.

1. How clinical trials have evolved

Until the 1940s, the development of new drug treatments was based on non-randomised studies. Later, it was increasingly recognised that anecdotal reports based on observations from clinical practice were often misleading. This led to an almost complete replacement of the former non-randomised approach by the use of randomised, controlled clinical trials. The limitations of the anecdotal approach have proven to be real in the history of medicine, which is indeed replete with examples where observational data have been misleading, even with regard to established clinical practices (31). This reinforced the common and widespread idea about non-randomised studies that, irrespective of their magnitude or rigour in analysis, the risk of bias (i.e. confounding factors, such as in the selection of patients or the assessment of causal links) limited the certainty of the data obtained. In contrast, proponents of non-randomised RWE studies believe that randomised RCTs may often not fully represent real-life situations because they employ strict protocol-defined inclusion criteria to identify eligible patients. This could mean that some patients are excluded from such studies based on characteristics such as disease severity, age, comorbidities or concomitant drug use.

2. Real-world evidence and real-world data

The term 'Real World Evidence' (RWE) is often used as a generic definition and has led to misuse of the term. The US Food and Drug Administration (FDA) has provided some guidelines, defining RWE as "clinical evidence regarding the use and potential benefits or risks of a medical product derived from analysis of real-world data (RWD). RWE can be generated from different types of study designs or analyses, including, randomised trials, including large simple studies, pragmatic studies and observational studies (prospective and/or retrospective)'. Similarly, the FDA defines RWD as 'data related to patient health status and/or health care delivery collected routinely from a variety of sources, e.g., electronic health records, electronic billing activities, product and disease registries, patient-generated data, including data collected in home settings, and data collected from other sources that may provide health status information, such as mobile devices'(32).

An important criticism and limitation of this type of study is that RWD are often collected without the initial intention of being used for research into the safety or efficacy of a product, but are instead retrospectively repurposed for this purpose. However, through scientific rigour and a correct prospective view, with the use of tools such as disease registries, real-world data can be obtained to evaluate not only drug treatments not yet approved but also in-

clude drugs already on the market and large and diverse patient populations.

2.1 How do traditional RCT studies differ from non-randomised studies? What are the strengths and limitations of these two types?

Traditional RCTs may answer a specific question more robustly and have a lower risk of bias, but may sometimes be limited in applicability. Furthermore, restrictive enrolment criteria and a concentration of trial sites in certain healthcare systems make it difficult for some patients to participate in RCTs, especially if they have comorbidities or if their mobility or cognitive abilities are impaired.

In contrast, non-randomised studies can evaluate larger and more numerous populations and thus results can be easily generalized, but may be misleading due to a higher risk of bias. Thus, both approaches have their inherent limitations, and a trade-off is clearly necessary in choosing one approach over the other. However, it is a mistake to pit them against each other, instead of considering them as complementary sources of evidence to aid the decision-making process aimed at obtaining reliable clinical indications.

2.2 The degree of evidence of RWE studies in the AIT

The data used obtainable as RWD and reported in a scientific paper may have different weight in the importance of the resulting evidence. In the EAACI position paper published in 2021, we tried to hypothesise and devise a hierarchy of Evidence from real-world studies related to immunotherapy for allergies through the representation of a pyramid where at its tip we placed the most rigorous and impactful tools and at its base the tools that can give evidence of lesser quality. This pyramid was structured on the most recent RWE knowledge and recommendations of authorities and scientific societies (Figure 1) (33).

At the apex of this pyramid we find the 'pragmatic randomised controlled trial'. This type of work is designed in a similar way to classic RCTs, where, however, no inclusion and exclusion criteria are set. In this way, one defines a priori the 'outcomes' to be analysed, how they are to be analysed, but one is able to obtain data on a broader and more varied population. For example, one includes smoking or comorbid patients who are not normally included in traditional RCT studies. Next, another tool that can produce very valuable data is the 'pathology or treatment registry'. This is a system organised to collect uniform data on specific outcomes in a population defined by a particular disease, condition or treatment. Normally, registries allow for what is called 'Big Data' i.e. larger and more complex datasets, so voluminous that normal data processing software cannot handle them. Of course, these huge volumes of data can be used to answer questions that otherwise could not be addressed. Moving one step further down the pyramid are studies derived from 'real-world prospective evidence' data. A type of cohort study, in which participants are enrolled in the study before they develop that specific disease. Other studies with less of an impact on evidence are those derived from multi-centre databases with retrospective data limited by the use of an existing database to answer specific clinical questions, with the inherent risk of bias related to 'fitting' the data in hand. All the way down to the base of the pyramid, where the step related to "Expertise of an expert in the field" was identified.

Against this background, it can be said that RWE studies are an important source of data regarding AIT. Suffice it to say that thanks to these we can predict follow-ups of years after the cessation of medical treatment and understand what happens in our patients who have undergone a given desensitisation therapy.

Although most non-randomised efficacy studies in RWE of AIT are retrospective, and include a limited number of patients, increasingly large databases of prescription and billing data are being used to allow analysis of a larger number of patients than in the past (with the limitations and cautions described above).

Some important results were derived from this approach. For example, work by Wahn retrospectively showed on a prescription database that included patients with allergic rhinitis and/or asthma associated with birch pollen the benefits of AIT up to 6 years after cessation of treatment, with a significant reduction in medication intake for these

Figure 1 RWE's pyramid of evidence in AIT

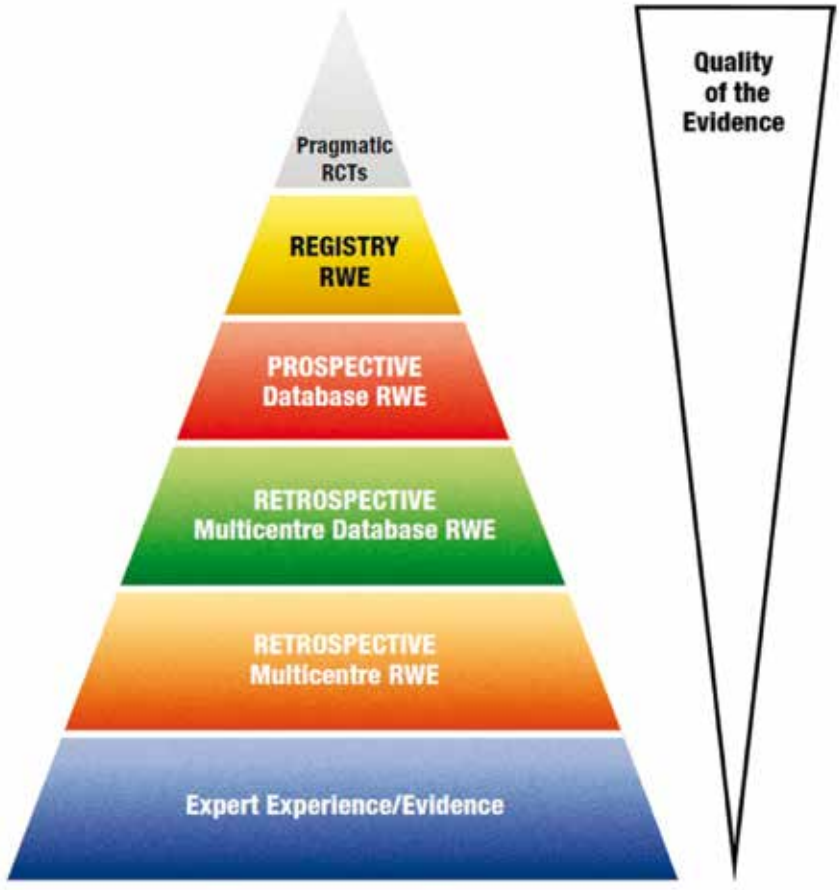

Figure 1. *Proposed hierarchy of real-world evidence on specific immunotherapy towards inhalant allergens from highest to lowest quality - RCT, randomised controlled trial; RWE, real-world evidence. Taken from article (33).*

conditions and a decrease in the risk of using drugs to manage asthma during treatment (34). A similar analysis conducted by Jutel investigated the efficacy in RWE of AIT with allergoids in the treatment of allergic rhinitis and/or dust mite-induced allergic asthma. The work showed a drastic reduction in medication to manage the two diseases in patients undergoing AIT compared to control patients (59.7 % vs. 10.8 %) and also in the treatment group a reduction in the risk of developing asthma with an extended follow-up of up to 6 years (35).

Finally, another example comes from a retrospective study that included more than one hundred adult patients with allergic asthma who had used inhaled corticosteroids (ICS) for more than 1 year in a single hospital in Korea. This compared clinical parameters and outcomes between the AIT and non-IT group and concluded that, regardless of the type of allergen, AIT, in the long run, helps to reduce daily ICS intake and achieve better control in patients with allergic asthma.

2.3 Records on AIT

The RWE approach related to AIT is being increasingly evaluated in the European scientific community, where the first treatment registers on AIT are starting to emerge. At present, the registry promoted by "The British Society for Allergy & Clinical Immunology (BSACI)" called "British Registry for Immunotherapy "(BRIT) and the one promoted by "The Society of Allergology, Asthma and Clinical Immunology (SIAAIC)" called the Italian Registry of Allergen ImmunoTherapy (RIAIT) (36,37) exist.

The BRIT is a registry that collects immunotherapy data from patients served by BSACI consultants practising in the UK, but is rapidly being adopted by all immunotherapy centres in the UK.

This registry focuses on pollen and mite

immunotherapy, the treatment of reactions to wasp and bee venom, and the use of Omalizumab for chronic urticaria.
The youngest Italian RIAIT registry aims to establish a pool of patients treated with specific immunotherapy, with the possibility of having national data, evaluated by centres with specific experience in the field, therefore able to prescribe AIT according to guidelines, and to monitor the evolution of the disease over time in relation to the therapeutic intervention.
From these registers, it is expected that in a few years' time, we will be able to obtain important evidence from Big Data similar to that obtained from severe asthma registers at global, European and national level.

2.4 Quality of RWE studies on AIT

As already addressed in observational research, as well as in RCTs, ensuring high quality methodology is crucial to avoid bias that would compromise the reliability and validity of results.
Although there are various tools to guide the design of observational research in order to ensure systematic and rigorous processes, some of the best known and most widely used, also designed for respiratory diseases and clinical approaches, are the "Risk Of Bias In Non-randomised Studies of Interventions (ROBINS-I)" (38) and more recently the "REal Life EVidence AssessmeNt Tool (RELEVANT)" (39).
The ROBINS-I devised by the Cochrane Collaboration is based on an approach similar to the tools that assess the risk of bias in RCTs.
The ROBINS-I covers seven main domains using questions to help assess the risk of bias within each individual domain. These assessments give an overall score on the risk in all domains for the outcome being examined.
The RELEVANT was created in 2019 by members of the Respiratory Effectiveness Group (REG) and the EAACI and was initially designed to be applied in asthma. The final version of this tool consists of 21 sub-items (11 of which are considered critical and referred to as 'primary sub-items') distributed over seven different domains: Background, Design, Measures, Analysis, Results, Discussion/Interpretation and Conflict of Interest.
Di Bona and colleagues in 2021 applied RELEVANT with the aim of systematically evaluating the quality of published comparative observational studies on AIT. An extensive literature search was conducted on observational studies that analysed and compared AIT with pharmacotherapy for respiratory allergies, assessing symptom reduction and/or reduction in drug use as an 'outcome'. The 14 studies identified supported the real-life benefit of AIT, which persists even after treatment discontinuation. However, none of them fulfilled all 7 main RELEVANT criteria. The main shortcomings were found in the study design (28.6% of the studies), in the measurement methods used and the analysis performed (64.3% of the studies) and finally in the results (78.6% of the studies), due to selection bias and the lack of methods for adjusting controls. Furthermore, another important shortcoming of these studies is that in half of them the conflict of interest of the authors was not reported. Thus, one can see how there is a need for more solid observational research on AIT (40).

CONCLUSIONS

RWE studies on AIT are a useful and necessary tool that should be used together with traditional RCT studies to increase the existing evidence on this therapeutic approach. The European Medicines Agency (EMA) also stated in a recent 2023 report on the use of real-world evidence for regulatory decisions that such data support their use in both pre-authorisation and post-approval assessments of drugs. The document also emphasises that work still needs to be done to anticipate the need for these types of studies and to speed up their initiation, so as to guarantee the various regulatory bodies timely access to real-world evidence (41). This step is essential to allow in particular the less common allergens in the population to be approved as drugs in the future in a sustainable manner but with due scientific rigour.
In conclusion, it can be said that there are currently methodological limitations in much of RWE's work, but these can be a useful starting point for future approaches to more and higher evidence in the conclusions that can be obtained.

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ENG

New modalities of communication in allergic diseases

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INTRODUCTION

Mast cells (MCs) derive from haematopoietic progenitor cells that colonise vascularised tissues where they complete their maturation under the influence of growth factors and local stimuli (1). MCs play a key role in the innate and adaptive immune response and are the main effector cells of the allergic response (2-5).

MCs are, in fact, characterised by the constitutive expression of the high-affinity receptor for IgE immunoglobulins (FcεRI) and the presence of numerous cytoplasmic granules containing a wide range of bioactive molecules. Receptor aggregation, which occurs upon interaction with class E immunoglobulins and multivalent antigen, provides an activating signal that leads to the release of the bioactive molecules contained in the cytoplasmic granules -a process known as mast cell degranulation- and the ex novo synthesis of pro-inflammatory mediators (4).

In addition to releasing mediators in soluble form, MCs secrete extracellular vesicles (EVs) constitutively or in response to different stimuli including IgE and antigen-dependent activation (6,7).

EVs comprise a heterogeneous group of membrane-encircled vesicles that can be divided into three subgroups based on their biogenesis and size: microvesicles, exosomes and apoptotic bodies (8-10). Microvesicles are between 100 and 1,000 nm in size and are formed by budding outwards from the plasma membrane. Exosomes are vesicles of a

SUMMARY

Keywords

• The high-affinity receptor for IgE (FcεRI) • IgE • mast cells • extracellular vesicles • microvesicles • exosomes

Abbreviations

• Dendritic cells (DC) • Multivesicular corpses (CMV) • Bronchoalveolar lavage (BAL) • Inborn lymphocytes type 2 (ILC2) • Thymic stromal lymphopoietin (TSLP) • Mast cells (MC) • Bone marrow-derived mast cells (BMMC) • Extracellular vesicles (EVs)

Mast cells reside in tissues near the putative pathogen entry gates and act by 'patrolling' their environment due to their ability to respond to a plethora of different stimuli. When activated by these stimuli, mast cells orchestrate various immune responses under both physiological and pathological conditions and are the main effector cells responsible for the allergic response.

The best known mechanism of mast cell activation involves the aggregation of the high-affinity receptor for IgE (FcεRI) by allergens and IgE-class antibodies, but certain toxins, lipopolysaccharides and IgG-class antigen-antibody complexes are also very common activating stimuli. Depending on the activation pathway, mast cells respond by releasing a ▶

SUMMARY

wide range of pre-formed and newly synthesised pro-inflammatory mediators.
More recently, mast cells have attracted considerable attention for their ability to release extracellular vesicles (EVs), including exosomes and microvesicles, which represent a new mode of intercellular communication. In fact, these vesicles transport lipids, proteins and nucleic acids derived from donor cells, thus modifying the composition and functional capacity of recipient cells.
Recent evidence shows that the composition of mast cell-derived vesicles changes according to the activating stimuli provided by the microenvironment, making the released vesicles capable of conveying different messages and controlling distinct biological responses.
This article provides an overview of the ability of nano- and micro-vesicles released by mast cells to communicate with other cells located nearby or at distant sites and to modulate the inflammatory process associated with allergic diseases in particular.
Their potential use as therapeutic agents or as biomarkers for the diagnosis and prognosis of allergic diseases and other disorders related to deregulated mast cell activation will also be discussed.

more homogeneous size (30-150 nm) and of endocytic origin: intraluminal vesicles bud within MultiVesicular Bodies (CMVs) and are released into the extracellular space as exosomes following the fusion of CMVs with the plasma membrane. Apoptotic bodies are extremely heterogeneous vesicles (varying on average from 1,000 to 5,000 nm in diameter) that are released from cells undergoing programmed cell death (Figure 1).
Microvesicles and exosomes, once released into the extracellular space, circulate in body fluids (11) and modulate the behaviour of recipient cells, both near and far, through the horizontal transfer of bioactive molecules, including proteins, lipids, DNA, RNA and microRNA (12,13). Thus, they have been implicated in both physiological phenomena and pathological processes including inflammatory processes, metabolic disorders, neurological diseases and cancer (14,15).
EVs released from MCs can convey and transfer biological information, orchestrating both inflammatory processes associated with the allergic response and tumour progression (6,7). In the course of the immune response, MCs themselves are phenotypically and functionally shaped by EVs derived from other cells of the immune system and released at the site of damage (16).
The next sections will briefly illustrate the contribution of microvesicles and exosomes as intercellular messengers and then focus on the role of exosomes released by MCs in modulating the inflammatory response associated with allergic reactions. The potential use of exosomes as therapeutic agents or as biomarkers for the diagnosis and prognosis of allergic diseases and other disorders related to deregulated mast cell activation will also be discussed.

2. Microvesicles and exosomes in cell-cell communication

A new cellular communication mechanism described in the last decade is based on the release of membrane-coated vesicles that carry molecular messengers (8-10). In particular, microvesicles and exosomes have recently attracted much interest. Exosomes are nanovesicles of endocytic origin that are homogeneous in size and morphology, whereas microvesicles are generated by budding of the plasma membrane and vary in size between 100 and 1,000 nm (Figure 1). However, it is important to emphasise that microvesicles and exosomes can also have overlapping dimensions and carry similar identification markers, which often makes both their biogenesis and classification ambiguous. In this regard, according to the new guidelines published by the International Society for Extracellular Vesicles, it is recommended to use the term 'EV' when there is uncertainty about the subcellular origin of vesicles (17).
A growing body of experimental evidence has attributed various physiological as well as pathological roles to these vesicles, and their contribution in regulating neoplastic transformation, neurogenerative diseases and inflammatory diseases is now generally accepted (14,15).

Figure 1 Biogenesis and composition of extracellular vesicles

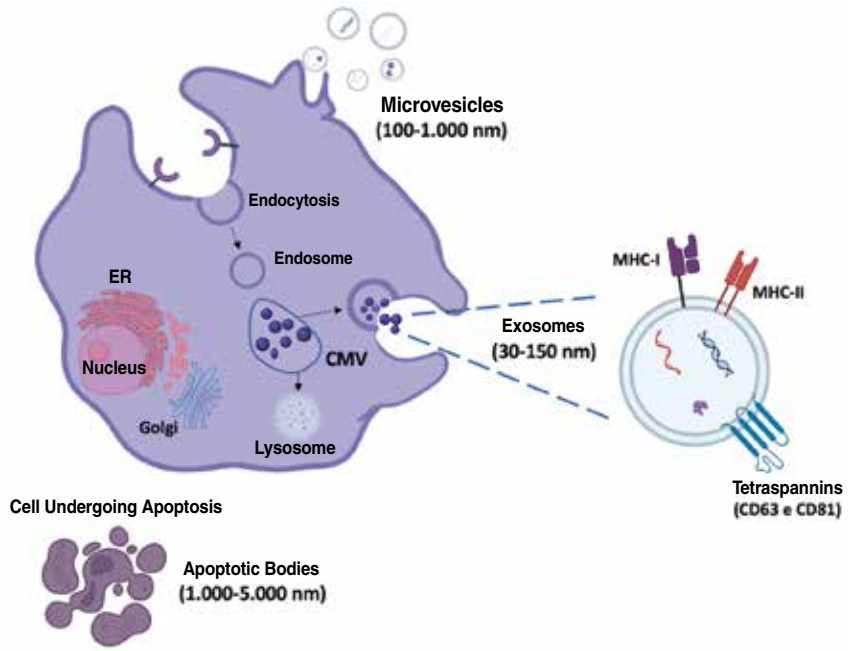

Figure 1. *Extracellular vesicles include microvesicles, exosomes and apoptotic bodies. Microvesicles are released from the plasma membrane through protrusion or budding of the plasma membrane; exosomes are nanovesicles released from specific endosomal compartments, called MultiVesicular Bodies (CMV), following their fusion with the plasma membrane; apoptotic bodies are generated by invaginations of the plasma membrane of cells undergoing apoptosis.*
The enlargement on the right shows the main molecular components of exosomes. Most of them are shared with microvesicles. Modified from Lecce et al. (2020) using Biorender.com.

2.1 Exosomes as vehicles for potential biomarkers and therapeutic agents

Exosomes are secreted by a wide range of cells including both immune system cells and tumour cells, and they are believed to function as intercellular messengers not only locally but also at a distance as they have been found in most biological fluids including blood, urine, saliva and in bronchoalveolar lavage (BAL) (8-11).
Exosomes are made up of a lipid bilayer, contain proteins both exposed on their surface and carried internally along with numerous nucleic acids including DNA, mRNA and microRNA, as illustrated schematically in Figure 1.
Proteins and nucleic acids are protected by a lipid bilayer that gives them a high degree of stability, and the molecular components they carry include numerous micro-RNAs that have proven useful as biomarkers both for diagnostic purposes and for monitoring therapeutic treatments (12-15).
The results obtained from the analysis of the proteomic and transcriptomic profiles of these vesicles, which revealed both the ubiquitous presence of proteins including tetraspannins and of tissue-specific molecules (18), have aroused considerable interest. This feature can be exploited to target engineered exosomes loaded with specific drugs only to specific target cells (Figure 2).
The use of exosomes as a vehicle for drugs ('drug delivery') offers several advantages over other transport systems, including limited immunogenicity and greater stability.

3. Therapeutic approaches based on the use of exosomes in allergic diseases

3.1 Exosomes and allergic diseases

During allergic reactions, exosomes released by different cell types contribute by enhancing or inhibiting different phases of the reaction and could potentially be manipulated in order to devise new therapeutic approaches in the treatment of allergic diseases (19,20).
During the onset of an allergic reaction, the sensitisation phase occurs following

the compromise of the skin or lung barrier at the level of which inflammatory signals from epithelial cells, including those provided by thymic stromal lymphopoietin (TSLP) and the cytokines IL-25 and IL-33, are able to promote the activation of innate type 2 (ILC2) lymphocytes, marking the start of a preferential T helper 2 (Th2) response (21). In this context, it is interesting to note that dendritic cells (DCs) activated by TSLP are able to release exosomes that drive the proliferation of CD4 T lymphocytes+ and the differentiation of the Th2 subpopulation. Moreover, both exosomes released by DCs and B lymphocytes can carry allergens within them and are able to promote the production of Th2-type cytokines (22,23).
When analysing the content of exosomes released in the BAL of asthma patients, the presence of numerous microRNAs promoting the production of Th2-type cytokines was found (24,25).
During the triggering phase, both MCs, as described in detail in the next subsection, and eosinophils are able to release exosomes that positively regulate the allergic reaction.
In particular, eosinophil-derived exosomes act in an autocrine manner by promoting both the migration of eosinophils from the blood to the reaction site tissue and their effector function by inducing production of reactive oxygen species and nitric oxide (26). In asthmatic patients, this contributes to alveolar epithelial cell death, delays the repair process and increases smooth muscle

Figure 2 Exosomes as vehicles for biomolecules and therapeutic agents

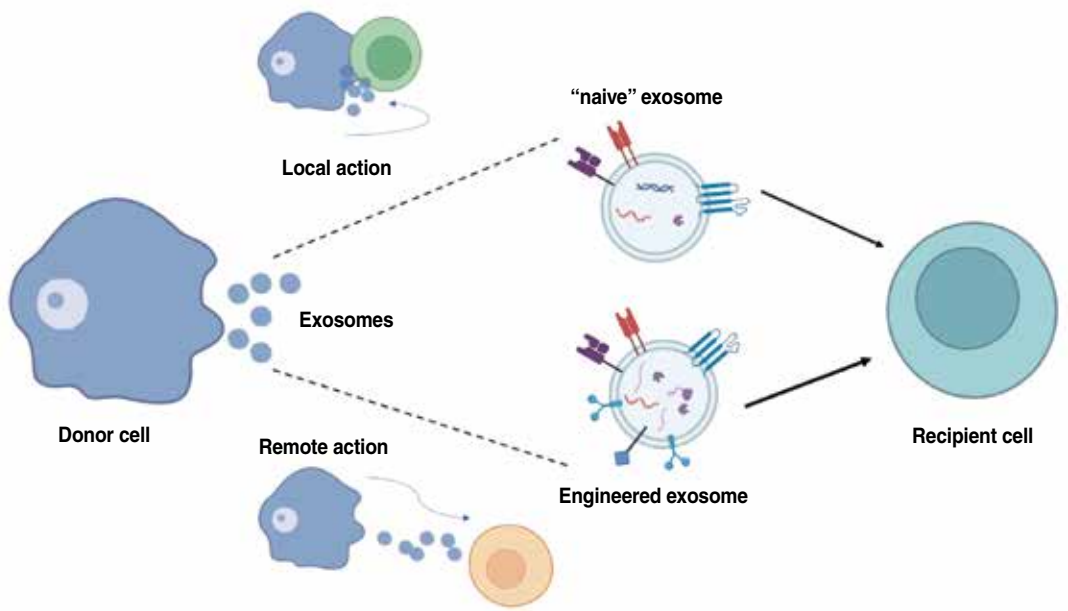

Figure 2. *Exosomes are secreted by a wide range of cells including both immune system cells and tumour cells and function as intercellular messengers not only locally but also at a distance. The 'naive' exosome can be engineered to enhance its ability to carry biomolecules and/or drugs. The engineered exosome can also be picked up by the recipient cell more efficiently than the 'naive' exosome. Created by Biorender.com.*

cell proliferation causing airway obstruction and tissue remodelling (27). Neutrophils can also take part in the late phase of the reaction and act with a mechanism similar to the one just described, which involves the release of exosomes that can act in an autocrine manner, amplifying the inflammatory process and causing further airway remodelling (28).
In this context, the use of drugs to inhibit exosome production could interfere with their deleterious action and alleviate the symptoms associated with the late phase of allergic asthma.

3.2 Role of mast cell-derived exosomes and therapeutic potential

Numerous studies have shown that during an allergic reaction, the dialogue between MCs and other cellular effectors is mediated primarily by EVs produced by the MCs themselves after activation induced by aggregation of the FcεRI receptor (29).
In one of these early studies, Kormelink and co-authors demonstrated that MCs actually release microvesicles and exosomes even in the absence of activation, but that after antigen-dependent stimulation the number of vesicles released into the culture medium increases (30). Furthermore, EVs derived from activated MCs are enriched in the marker CD63, differ in size and lipid composition and contain more biologically active mediators than those derived from resting MCs (30).
By analysing the molecular composition of EVs isolated from primary cultures of MCs generated from bone marrow precursors (BMMCs), the presence of higher amounts of proteases (tryptase and carboxypeptidase A) and IL-4 in EVs released after antigen-dependent stimulation compared to constitutively released vesicles was highlighted (31), suggesting their regulatory function. In relation to the biological activity of EVs released from activated MCs, Valadi and co-authors also reported the presence of miRNAs that can be transferred to recipient cells other than MCs by altering their function (12, 32).
In the case of human MCs activated by the aggregation of the FcεRI receptor, EVs released by MCs are recognised and internalised by ILC2 and are able, by carrying a selective miRNA (miR103a-3p), to increase IL-5 production, thereby enhancing the inflammatory process (33).
By producing exosomes, MCs can indirectly regulate the adaptive response by inducing in vivo DC maturation and antigen presentation to T lymphocytes (34). In addition, exosomes promote in vitro the expansion and differentiation of CD4+ 'naive' T lymphocytes into Th2 lymphocytes via direct binding between the OX40 ligand, exposed on their surface, and the OX40 receptor present on the surface of T lymphocytes (35). EVs derived from MCs of psoriasis patients can also selectively deliver phospholipase A2, contributing to an enhanced specific T response in these patients (36).
Several studies have reported a critical role of MCs, attributable to their production of exosomes, in the regulation of neuroinflammation. In response to infections and toxins, MCs interact with microglia cells that are activated by releasing pro-inflammatory cytokines. It has recently been shown that the interaction between MCs and microglia occurs through the release of EVs by lipopolysaccharide-activated MCs and the selective transfer of miR-409-3p delivered from within the vesicles to the microglia. This causes NF-kB activation as a result of reduced expression of specific nuclear receptors, promoting microglia activation resulting in neuroinflammation (37).
Despite this experimental evidence, the role of MC-derived EVs in the regulation of allergic reactions is still controversial. A recent study has shown that EVs released from murine MCs cultured in vitro expose the FcεRI receptor and that intravenous injection of these vesicles in a mouse model of allergic asthma induced by ovalbumin exposure decreases serum IgE specific for ovalbumin (38). These data suggest that the use of exosomes carrying the free FcεRI receptor (i.e. not bound to IgE) could represent a new therapeutic approach capable of limiting the sensitisation phase of MCs.
More recently, my research group has shown that exosomes isolated from activated murine MCs after antigenic stimulation carry both the FcεRI receptor and IgE/multivalent antigen immunocomplexes on their surface and that these exosomes are able to be picked up by sensitised MCs and promote their degranulation in a dose-dependent

manner and at comparable levels to those induced by multivalent antigen (39). From sera of atopic individuals we have also isolated exosomes that carry IgE in amounts proportional to the antibody levels present in the serum (39). These nanovesicles could therefore act to promote the sensitisation phase.

It is also likely that exosomes released by activated MCs that expose the IgE/antigen complex are also recognised and internalised by monocytes and DCs of atopic subjects expressing the FcεRI receptor (40). Their uptake by DCs could favour the processing and presentation of the antigen to T lymphocytes, further amplifying the allergic response.

As illustrated in Figure 3, it is possible to hypothesise that MCs in the absence of an antigenic stimulus are able to release exosomes that carry the empty FcεRI receptor and are able to bind circulating IgE, negatively regulating the inflammatory response (Figure 3, panel A). Sensitised MCs (i.e. with IgE bound to the FcεRI receptor) activated by recognition of the multivalent antigen, release exosomes that carry the entire FcεRI/IgE/antigen complex and are therefore able to act in concert with the multivalent antigen, amplifying the allergic reaction (Figure 3, panel B).

All in all, the results illustrated and discussed in this section demonstrate that exosomes and microvesicles released by MCs are capable of carrying molecular messengers in recipient cells that can modulate the inflammatory response in a negative or positive direction, and that further studies are needed to clarify their actual role in vivo.

CONCLUSION AND FUTURE PERSPECTIVES

Exosomes, due to their role in both physiological and pathological conditions and recent technological developments allowing their rapid purification and characterisation, have significantly changed many areas of clinical science. The testing phase

Figure 3 Modulation of the IgE-dependent inflammatory response by mast cell-derived exosomes

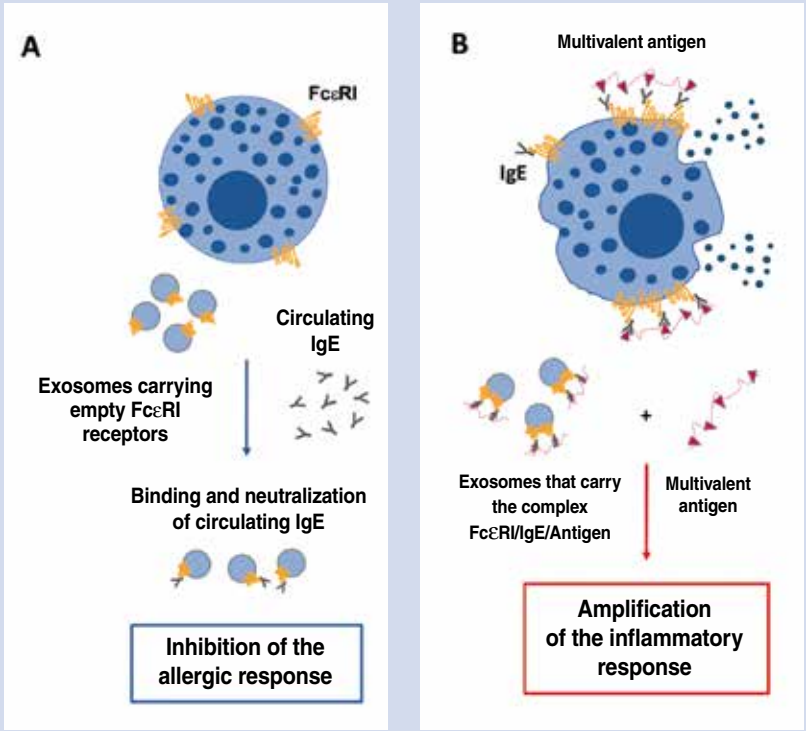

Figure 3. *(A) In the absence of a stimulus, mast cells release exosomes bearing empty FcεRI receptors that are able to bind and neutralise circulating IgE, suppressing the allergic response. (B) After receptor aggregation induced by the binding of a multivalent antigen with FcεRI receptor-bound IgE, mast cells release exosomes that carry the entire FcεRI/IgE/antigen complex on their surface, which acts by cooperating with the soluble multivalent antigen in amplifying the allergic response. Partially created by Biorender.com.*

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is encouraging and exosomes could also represent a valuable therapeutic support in allergic diseases, which are becoming a major health threat worldwide and also represent a significant economic burden.

Allergic reactions can be seen as an overreaction of the immune system characterised by a massive and deregulated production of IgE antibodies in response to a foreign but harmless antigen. The use of exosomes engineered to carry the empty FcεRI receptor capable of binding free IgE present in serum could have beneficial immunomodulatory effects, suggesting their potential promising use for therapeutic approaches aimed at inhibiting the IgE-mediated inflammatory response in atopic subjects. Furthermore, the development of vesicles modified to have a greater propensity to fuse with the membranes of specific recipient cells could enhance therapeutic capabilities.

From another point of view, allergies can be seen as a response characterised by an underproduction of high-affinity antigen-specific IgG antibodies capable of protecting the body from IgE-mediated effector functions. In this case, exosomes engineered to deliver molecules that can correct the response by favouring the differentiation of Th1 lymphocytes at the expense of Th2 could be administered to allergic individuals as an alternative to current hyposensitising therapy.

Finally, another approach under development as a possible therapeutic strategy in particular for asthmatic individuals involves the use of inhibitors of EVs production to prevent the vesicles from amplifying the inflammatory response.

The full implementation of what has been described is not easy and immediate and further studies are needed, which, based on their results, could ensure future therapeutic innovations.

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Vegan diets from an allergy point of view

Vegan diets from an allergy point of view
Position paper of the DGAKI working group on food allergy

Reese I. et al. Allergologie select vol. 7, 57–83, 2023

The vegan diet is a diet that completely excludes the consumption of animal products and seems to be increasingly popular. For example, it is estimated that the number of vegans in Germany has risen from less than 80,000 in 2008 to almost 1.6 million in 2022, and that the production of meat substitute products has increased by 37% in just one year between 2019 and 2020. According to a 2016 market survey, the choice to follow a vegan diet is mainly linked to animal welfare (in 60% of cases) and only to a lesser extent (8%) to health reasons, but other factors can also be decisive, such as religious reasons or the desire to adopt a more sustainable lifestyle. Plant-based diets are generally considered to be healthier, especially in relation to cardiovascular diseases, type 2 diabetes and cancer, but often risk being deficient in certain macro- and micronutrients. Furthermore, the use of vegan substitutes, which are often ultra-processed, may also carry allergy risks, as accurate information on the ingredients present is not always available.

These issues are discussed in depth in a position paper of the German Society for Allergology and Clinical Immunology (*Deutsche Gesellschaft für Allergologie und Klinische Immunologie*, DGAKI). In this paper, the authors present benefits, limitations and risks of vegan diets, especially in relation to allergies. With regard to nutritional aspects, they emphasise that in order to ensure the recommended daily intake of various nutrients it is essential to consume legumes, especially soy, nuts, seeds, potatoes and other vegetables, and whole grains on a daily basis, and present an example of a balanced vegan diet plan (for adults; Table 1 of the original paper). The vegan diet is usually low in calcium, iron, vitamin B12, zinc, iodine, selenium, and long-chain omega-3 fatty acids. In addition to supplementing these important elements, it is essential to ensure an adequate protein intake, especially in terms of quality. This is related to the proportion of essential amino acids in the protein source, and is generally significantly higher in animal sources than in plant sources. Useful strategies for meeting protein requirements include consuming larger amounts of the protein source, combining different plant protein sources, using amino acid supplements or protein isolates and concentrates in the production of vegan products.

The risk of not meeting nutritional needs by using substitute products is well exemplified by cow's milk substitutes. In fact, there is a wide variety of alternative drinks to milk available on the market, obtained from different plant sources (e.g. soy 'milk', rice, almond, cashew, oats, barley...), but the nutritional profile of these products is quite different from that of milk, especially in terms of protein, calcium and vitamins. The authors discuss these aspects and, in Table 4 of the original paper, present a comparison of the nutritional values (energy value, protein, carbohydrates, fat and calcium) of cow's milk and yoghurt with those of some alternative plant-based products. The recommendation for those who decide to switch to a vegan diet is to replace milk with calcium-enriched soy drinks. In addition to nutritional aspects, the allergenic potential of plant-based drinks should also be considered. This is not only related to the main ingredient, but also to the presence of other components, such as plant protein isolates (used to increase the protein content of drinks) that may not be indicated on the label.

Reese and colleagues also discuss other plant substitute products from the perspective of allergies. One of the risks is that patients allergic to animal proteins choose vegan products considering them safe. However, the label 'vegan' only refers to the absence of

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animal components among the ingredients, but does not exclude possible production-related contamination, as shown by a study of 30 vegan foods, which detected the adventitious presence of milk in some confectionery products. Furthermore, major plant protein sources, such as legumes and nuts, are among the most important causes of anaphylactic reactions. The authors assess in detail the various groups, such as legumes (peanuts, soy, lupine, peas), nuts and seeds (cashews, hazelnuts, sesame, hemp seeds) and wheat, providing information on their use, prevalence, allergens, possible cross-reactivity, and labelling.

Another interesting aspect that the authors point out is that many ready-to-eat vegan products are highly processed and processed (ultra-processed foods) in order to make them more palatable and more similar to animal products in taste, appearance, and texture. This leads to foods that are too high in salt, sugar and starch, but low in protein, fibre and micronutrients, thus compromising nutritional quality and reducing or negating the benefits associated with a vegan diet. Furthermore, due to intensive processing, the allergenic potential of these products cannot be fully assessed and further studies are needed. It should also be considered that the reduced dietary fibre content in ultra-processed foods could alter the gut microbiome and have negative effects on immune responses. The authors conclude by emphasising that people who choose to opt for a vegan diet should be aware of the limitations and health implications, and recommend that they seek expert nutritional advice.

An extraordinary case of nickel contact dermatitis

Contact allergy to a meteorite: An interesting consequence of nickel allergy

Malinauskiene L. Contact Dermatitis, vol. 79, issue 1, 36-37 Jul 2018. doi: 10.1111/cod.12974.

This rather curious case report appeared in the journal *Contact Dermatitis* in 2018. A young man (28 years old) had been suffering for six months from dermatitis on his right hand, specifically on the inner side of his little finger, with erythematous and itchy lesions in the area in contact with his wedding ring. The man also had dermatitis on his abdomen, at the belt buckle. What made what may have seemed a rather common case of contact dermatitis particularly interesting was the particularity of the ring. The jewel, made of white gold, was in fact decorated with inserts from a meteorite.* The ring is shown in Figure 1 of the original work, and the article only states that it had been purchased in a specialist boutique.

Figure 1. Image of ferrous meteorite Widmanstatten

The patient was referred to the Centre for Pneumology and Allergology at Vilnius University (Lithuania), where he underwent patch tests with European Standard Series and Metal Series (Chemotchenique Diagnostic). Readings on day 2 (D2) showed a very strong reaction to nickel (++; nickel sulphate 5% pet.) and clearly positive reactions to cobalt and palladium (++; cobalt chloride 1% pet.; palladium chloride 2% pet.).

The ring was thoroughly examined at the Department of

Geology and Mineralogy of Vilnius University, using a binocular microscope and energy dispersive spectrometer coupled with a scanning electron microscope. The meteoritic material was identified as being of the ferrous type (class IVA), consisting of 90% iron and 7% nickel, and containing phosphorus (0.3%) and cobalt (0.7%). According to the work, these were fragments of the meteorites Gibeon (discovered in Namibia in 1836) and Muonionalusta (discovered in northern Scandinavia in 1906).

To assess the release of nickel and cobalt from the ring, commercial tests were used, respectively, the Chemo Nickel Test™ (dimethylglyoxime test) and the Chemo cobalt test™ (containing nitrous salt), both produced by Chemotchenique Diagnostic. The test for cobalt was negative, while the nickel test was positive but only in the part with the meteorite fragments, confirming that nickel was the cause of the contact dermatitis in the hand and, possibly, also the reaction in the abdomen. The patient then stopped wearing the ring and the dermatitis resolved.

This is actually the second case reported in the literature of contact allergy due to a piece of jewellery made from meteorite parts. The first was published in 2014 by a group of Swiss dermatologists and reported a strikingly similar case of a 31-year-old man who for eight weeks had dermatitis on his ring finger where he wore his wedding ring, and on his belly near his belt buckle. Patch tests showed positive for nickel sulphate (+++) and the dimethylglyoxime test on the ring detected the presence of nickel. Again, the ring, purchased in Paris, had been made from a meteoritic material (Gibeon meteorite).

Both works represent 'extraordinary' cases of contact dermatitis, but linked to sensitisation to a rather 'ordinary' allergen, so to speak, such as nickel.

**In recent years, a very fruitful market has developed and thousands of meteorite fragments are being offered for sale online. In addition to specialised sites, bids end up on various sales channels and even Christie's recently held an auction with several lots of meteorites that soon sold out.*

An unusual case of occupational respiratory allergy

Unusual allergen in a butcher with respiratory symptoms

Sander I. et al. Allergologie select vol. 4, 105-109, 2 Dec. 2020

This paper describes an unusual case of occupational allergy. It concerns a 37-year-old man, a smoker for 20 years and with a history of pollen allergy and bronchial asthma, who for eight years also suffered from respiratory problems, not seasonal, related to his work in a large meat processing company in Frankfurt (Germany). The patient presented with attacks of dry coughing and sneezing during the preparation of sausages and the use of spice mixtures. In the afternoons, when he switched to the production of chicken nuggets (Figure 1), his symptoms worsened to the extent that he had to double the dosage of the asthma spray (beclomethasone dipropionate and formeterol fumarate 200 µg/6 µg per delivery).

Possible triggering factors included certain spices (e.g. paprika and pepper) and certain powdered additives containing transglutaminase of bacterial origin. Transglutaminase (or 'meat glue') is an enzyme capable of inducing cross-linking of proteins and is increasingly used in the production of meat, fish and dairy products as it improves the texture and appearance of processed products. In addition to transglutaminase, additives may also contain other components that can facilitate stabilisation, such as wheat or milk proteins.

The patient underwent instrumental and laboratory examinations. The pulmonary function test (body plethysmography) showed mild obstruction and bronchial hyperreactivity. Using electrophoretic techniques, the proteins of two samples of the enzyme mixture used in the plant were analysed. Since the composition of the mixture was unknown and, therefore, the presence of other components capable of inducing sensitisation could not be excluded, a commercial preparation specifically containing the transglutaminase of the bacterium Streptomyces mobaraensis (Activa) was also included in the analysis. In the preparations used in production, the presence of several bands

Figure 1. Chicken croquettes

at 67, 40, 33, 16, 15, 13, and 12 kDa was evident, whereas in the Activa sample, only three bands at 67, 40, and 12 kDa were visible. The immunoblotting results showed that only the 40 kDa band was recognised by the patient's serum, both in the mixture used at work and in the Activa sample. Serum inhibition experiments with transglutaminase at 0.7% or 2.8% (w/v) showed inhibition of binding already at the lowest concentration. Serological analysis confirmed the positivity towards both transglutaminase preparations. (7.12 and 7.48 kU/l). The same analysis performed on samples of various species (coriander, mace, nutmeg, cardamom and paprika) showed a positivity, albeit weak, only towards coriander 0.74 kU/l.

Prick tests with the transglutaminase sample used in Production were positive, as were nasal and bronchial provocation tests with paprika. For safety reasons, provocation tests with transglutaminase were not conducted. Symptoms improved after transfer to a company where no enzymes were used.

Cases of occupational allergy to meat proteins, spices or enzymes (papain) in workers exposed to these substances have been reported in the literature, but this is the first case of a meat processor developing allergic sensitisation to transglutaminase.

Skin prick tests or molecular tests in respiratory allergy screening?

Allergy screening with extract based skin prick tests demonstrates higher sensitivity over in vitro molecular allergy testing

Gureczny T. et al. Clinical and translational allergy vol. 13,2 (2023): e12220

The skin *prick test* (SPT) is a first-line method for allergological diagnosis and a significant percentage of European allergists consider it to be the most effective diagnostic tool in the case of respiratory allergies (1). The SPT has the advantage of providing an immediate result that can be discussed immediately with the patient and requires specialist expertise for correct interpretation. in some cases, reading the results can be made difficult by certain factors, such as sensitivity to non-clinically relevant panallergens or conditions of excessive skin reactivity, such as dermographism, while the use of certain drugs can give false negatives (e.g. antihistamines).

In vitro tests for allergen-specific IgE (sIgE) in patient serum are also widely used for diagnostic purposes. However, the test may give false negatives if the patient is sensitised to minor allergens and the analysis only includes the major allergenic molecular components. Usually, both SPT with the extract and the sIgE assay for the major molecular allergens are routinely performed. However, should only one of the two methods be usable, how many patients would be falsely identified as non-allergic? This is the question behind the study by Gureczny and colleagues.

These authors conducted a retrospective analysis of a large cohort of patients who presented in 2018 at the Floridsdorfer Allergie Zentrum, a large allergy centre in Vienna. Only patients with a clear history of respiratory allergy and with complete SPT test pairs with extract and sIgE to the major allergens birch (Bet v 1), cat (Fel d 1), grasses (Phl p 1; Phl p 5) and HDM (Der p 1; Der p 2; Der p 23), for a total of 2646 patients (mean age 32.7 years; 53.7% female), including individuals sensitised to multiple allergens (1281 birch allergies, 1362 to HDM, 1577 to grasses and

709 to cat). The main symptoms were allergic rhinoconjunctivitis (35%), bronchial asthma/allergic cough (11%) and atopic dermatitis (7%). In order to compare the sensitivity of the two tests, only cases with a positive result in at least one of the two tests (Ø wheal ≥ 3 mm and/or sIgE ≥ 0.35kU/L) were considered. The analysis showed that for all respiratory allergen sources, the skin test alone had a higher sensitivity (from 15.2% for birch to 27.8% for cat) than the *in vitro* test alone (from 2.5% for HDM to 8.1% for grasses) (Figure 1). For all four allergens, a positive correlation between wheal size and sIgE levels was observed. The authors also showed that including the sIgE assay for Der p 23 for the diagnosis of HDM allergy did not significantly increase the sensitivity of the tests (sIgE alone or in combination with SPT).
In the discussion, the authors emphasise the advantages and disadvantages of using natural extracts in diagnostic tests, critically compare their results with those of other studies, and discuss some limitations of their study (e.g. the lack of provocation tests due to the high number of patients involved).
In conclusion, the study confirms the diagnostic importance of SPT in clinical allergology, emphasising that it cannot be replaced by in vitro sIgE analysis due to the lower sensitivity of the latter, the use of which is nevertheless recommended to supplement skin test results and ensure optimal sensitivity.

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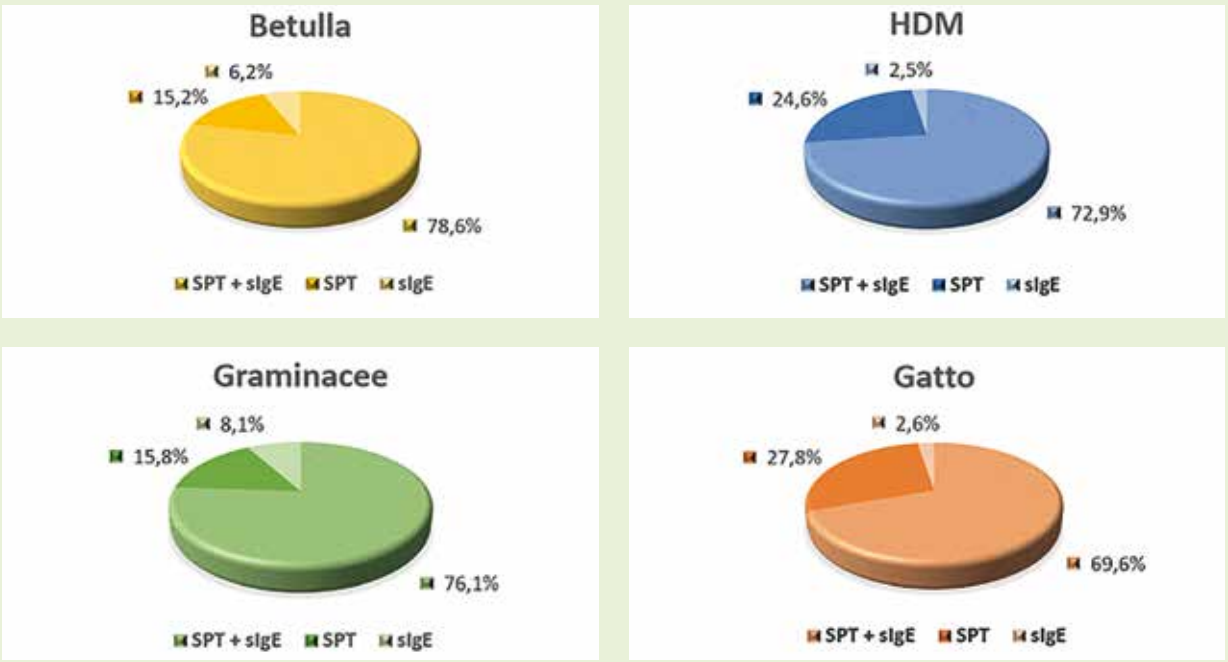
Figure 1 Results of comparisons between SPT, sIgE and SPT+sIgE



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Provide information, create a profession

Edited by **Franco Frati**

Specialist in Paediatrics, Allergology and Clinical Immunology Director Lofarma Academy

***Lofarma Academy* is a scientific information project for Allergology and Clinical Immunology specialists. Notiziario Allergologico dedicates this Column to *Lofarma Academy*, offering young allergologists a cultural space to compare and share their experiences.**

In this issue:

Innovative approach to cultural outreach on AIT for young specialists in Allergology and Clinical Immunology in Italy (EAACI 2023)

F. Frati, N. De Beni, C. Ferrero, L. Ladina, L. Gentile, E. Compalati

Lofarma S.p.A, Milan

During university courses, allergen-specific immunotherapy (AIT) is sometimes approached in a theoretical manner, far removed from clinical practice. In the absence of a didactic pathway specifically dedicated to AIT for specialists in Allergology and Clinical Immunology, there is a need to implement alternative educational pathways that allow theoretical knowledge to be integrated with clinical practice skills. The survey presented at EAACI explored the impact of a training project aimed at future allergists, focusing on AIT-related topics, from the diagnostic pathway to the treatment of the allergic patient, as well as the management of possible side effects.

Specifically, the survey concerned Lofarma Academy, a training course for future allergists, created in 2021 and consisting of remote webinars and in-person meetings held by experts in the field, on clinical topics and real-life cases of clinical practice in the context of allergies and immunotherapy, free from commercial influence. This pilot project involved 18 of the 21 Italian Specialisation Schools of Allergology and Clinical Immunology, with a total of 191 specialists enrolled. At the conclusion of the training, participants were invited to answer a short web questionnaire in order to collect their feedback on their learning experience.

The majority of respondents expressed great satisfaction with the training experience at Lofarma Academy, considering it an excellent educational tool to support the traditional academic course in order to acquire more in-depth and practical knowledge of AIT. Among the proposed activities, clinical case discussion and nasal cytology were the most appreciated. All participants highly valued the opportunity to exchange views, both with specialists from other schools in Italy and with the expert speakers, and would recommend this educational course to their colleagues.

These initial results highlight the importance of an innovative teaching approach for future allergists, useful for increasing the competence of professionals in the field of AIT, in synergy with what is learned in the traditional academic curriculum, with the aim of optimising the management of the allergic patient in clinical practice.

Sharing the know-how of pharmaceutical companies in the field of dissemination in partnership with institutions such as scientific societies and universities may represent a future perspective to optimise existing training paths relating to AIT, taking into account the needs of specialists.

Specific immunotherapy: certainties and expectations for young allergists

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In recent years, there has been a significant increase in the number of patients who consult an allergy specialist for symptoms of an allergic nature, with oculorhinitis, allergic asthma, food allergy and hymenoptera venom allergy being among the most frequently reported clinical symptoms.

Allergic diseases are nowadays a major public health problem, not only because of the recorded incidence but especially because of the impairment of the affected person's ability to work. It is therefore essential to intervene as early as possible to limit sequelae and complications.

Specific immunotherapy (SIT) appears to be the only therapy available today, a valuable ally in the daily work of an allergist. The ultimate aim of using SIT is not only to reduce the patient's specific symptoms, but above all to modulate the clinical history of the disease (disease modifying effect).

Real-life use has demonstrated the overall effectiveness of the therapy. SIT remains a powerful weapon at our disposal, especially in a healthcare universe that aims for precision medicine 'customized' to the needs and requirements of the individual.

Our efforts in the future should aim at further therapeutic specificity, in order to achieve a reduction in the pool of patients who only partially respond to therapy.

Attention should also be focused on polysensitive allergic patients, whose number is increasing.

It may be helpful, in order to settle any doubts about the desensitising approach, to formulate appropriate treatment algorithms in order to optimise therapy.

It could be possible, in the future, create a territorial network for the management of allergic patients who would benefit clinically from SIT but are prevented from doing so for socio-economic reasons.

Finally, achieving standardisation of refundability across the Italian territory could widen the catchment area, giving everyone the opportunity to access the therapy.

It is essential to raise public awareness of allergic issues in order to optimise the resources available in the territory and reduce healthcare expenditure related to allergic disease complications in the long run.

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