EMJ EUROPEAN MEDICAL JOURNAL

ALLERGY & IMMUNOLOGY

ISSN 2398-9130 Vol 3.1 • July 2018 • europeanmedical-journal.com

INSIDE

Review of

EAACI 2018

Munich, Germany

Contents

"...I take great pleasure in welcoming you to this edition of EMJ Allergy & Immunology, a publication, like the annual EAACI Congress, dedicated to the development of this ever-growing field."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

Editorial Expertise

EMJ is supported by various levels of expertise:

- Guidance from an Editorial Board consisting of leading authorities from a wide variety of disciplines.
- Invited contributors are recognised authorities from their respective fields.
- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
- An experienced team of editors and technical editors.

Peer Review

On submission, all articles are assessed by the editorial team to determine their suitability for the journal and appropriateness for peer review.

Editorial staff, following consultation with either a member of the Editorial Board or the author(s) if necessary, identify three appropriate reviewers, who are selected based on their specialist knowledge in the relevant area.

All peer review is double blind.

Following review, papers are either accepted without modification, returned to the author(s) to incorporate required changes, or rejected.

Editorial staff have final discretion over any proposed amendments.

Submissions

We welcome contributions from professionals, consultants, academics, and industry leaders on relevant and topical subjects.

We seek papers with the most current, interesting, and relevant information in each therapeutic area and accept original research, review articles, case reports, and features.

We are always keen to hear from healthcare professionals wishing to discuss potential submissions, please email: editorial.assistant@emjreviews.com

To submit a paper, use our online submission site: www.editorialmanager.com/e-m-j

Submission details can be found through our website: www.europeanmedical-journal.com/contributors/authors

Reprints

All articles included in EMJ are available as reprints (minimum order 1,000). Please contact hello@europeanmedical-journal.com if you would like to order reprints.

Distribution and Readership

EMJ is distributed through controlled circulation to healthcare professionals in the relevant fields across Europe.

Indexing and Availability

EMJ is indexed on DOAJ, the Royal Society of Medicine, and Google Scholar®; selected articles are indexed in PubMed Central®.

EMJ is available through the websites of our leading partners and collaborating societies.

EMJ journals are all available via our website: www.europeanmedical-journal.com

Open Access

Congress Notice

Staff members attend medical congresses as reporters when required.

This Publication

European Medical Journal Allergy & Immunology is published once a year. For subscription details please visit: www.europeanmedical-journal.com

Front cover and contents photograph: Munich, Germany, home of the EAACI 2018 Congress.

EMJ Allergy Immunol.

Welcome

Munich, Germany, this year played host to the fantastic international celebration of every allergy and immunology advancement made over the last 12 months. With this in mind, I take great pleasure in welcoming you to this edition of *EMJ Allergy & Immunology*, a publication, like the annual European Academy of Allergy and Clinical Immunology (EAACI) Congress, dedicated to the development of this ever-growing field. This is achieved through an enthralling Congress Review, fascinating peer-reviewed articles, and captivating Abstract Reviews.

Held in the Bavarian capital, this year's immensely successful EAACI Congress was bigger and better than ever before, and the *EMJ Allergy & Immunology* team and I were there to see it all. Within the comprehensive Congress Review, we have highlighted the key events from the 5-day spectacular, including a detailed report on an innovative new pollen indicator set to change the lives of people with pollen allergies across Germany. Discover the latest results from ongoing and recently completed research projects in abstract summaries authored by the researchers themselves within our dedicated Abstract Review section. *EMJ Allergy & Immunology* welcomed four new Editorial Board members this year, interviews with three of whom, Dr Philippe Bégin, Dr James Woijoo Kim, and Dr Michael Rudenko, can be found inside, and a video interview with our fourth newest member, Dr Stefan Wöhrl, will soon be available on our YouTube channel. These field-leading experts give their opinions on the latest developments, discuss their career highlights, and outline their hopes for the future. This excellent opportunity to learn directly from the experts should not be missed.

As always, the eJournal contains high-quality, peer-reviewed articles, providing you with a wide breadth of updates and developments from an extensive range of allergy and immunology topics, from insect stings to food allergies, and anaphylaxis to autoinflammatory diseases. In this edition's Editor's Pick, Foong et al. review the established link between food allergies and asthma, highlighting the need for early diagnosis and presenting a number of clinical testing and treatment strategies; this is a fascinating read for any food allergy or asthma specialist. A second offering is the paper penned by Wilson and Platts-Mills, which focusses on the role of galactose-α-1,3-galactose in α-Gal syndrome and anaphylaxis, and the authors assess the very latest research and provide us with a cutting-edge review.

I would like to conclude by thanking everyone involved in the production of this edition: the authors of the high-quality abstract reviews and articles, our esteemed Editorial Board members, and the entire EMJ team. I sincerely hope that you enjoy this edition as much as we did creating and collating it.

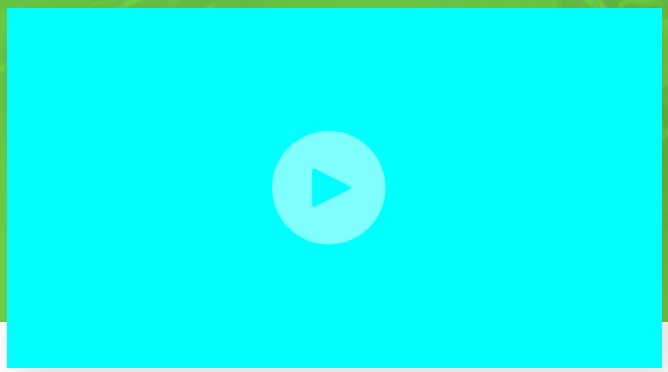
Spencer Gore

Chief Executive Officer, European Medical Group

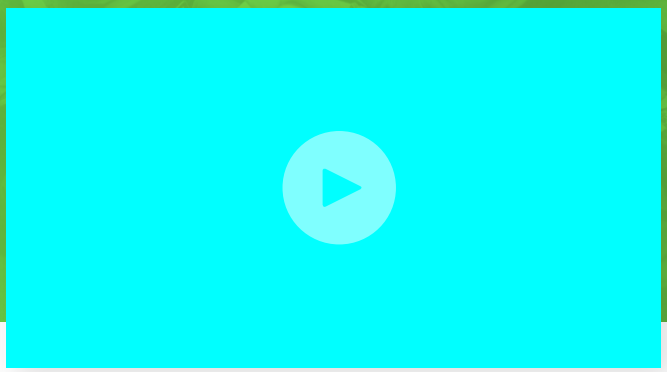
Our latest videos:

XXX

XXX



XXX



XXX

Subscribe free to our YouTube channel for the latest videos in the field of allergy & immunology, and many more from our archive.

EUROPEANMEDICAL-JOURNAL.COM /VIDEOS

Foreword

Dear friends, colleagues, and readers alike,

I am very proud to introduce to you this year's edition of *EMJ Allergy & Immunology*. As my third year as Editor-in-Chief for this fascinating journal, I have seen many changes both across the allergology and immunology medical fields, and within the European Medical Journal, and I am excited for what lies ahead.

This year brings us changes for the better, with many advances in our knowledge of various allergies. Specific allergen immunotherapy is a particularly lively topic this year, with allergy treatments moving towards personalised methods that favour the patients' needs more thoroughly.

The latest European Academy of Allergy and Clinical Immunology (EAACI) Congress highlighted these changes, with some of the hottest topics being covered for healthcare professionals to debate to their heart's content. Some of these topics can be relived in the Abstract Review section of the journal, brought to you straight from the pens of the EAACI presenters.

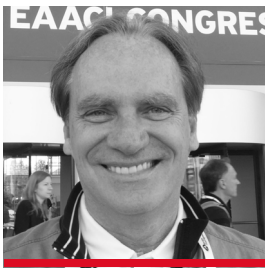
Many of the *EMJ Allergy & Immunology* Editorial Board members, including myself, came together for an informal meeting at this year's EAACI Congress, a networking opportunity that is not always manageable with such a vast array of experts located all around the world. Taking advantage of moments like these is something that I can highly recommend, as they provide a platform to embark on a journey of discovery and innovation, which may not otherwise be possible.

Within this edition of *EMJ Allergy & Immunology*, we have chosen a selection of exciting articles that I am certain will pique your interest. My Editor's Pick for this edition is that by Foong et al., which discusses the role of food allergy and eczema in the development of asthma. Identification of these allergic situations in early childhood, and introduction of either some form of early intervention or treatment, may play an important role in preventing asthma. This is a particularly important area for allergologists and immunologists alike, because the recognition of this state could modulate our treatment plans. Also, the new emerging role of immunomodulators for children with both food allergy and asthma would probably be helpful, but further research is still needed to evaluate these effects over a period of time.

I would like to thank all those who have contributed to the creation of this journal, and I hope that the information inside will inspire you to move forward, network, learn, and discover.

With my warmest wishes, I bid you good reading.

Kindest regards,



Prof Dr Jacques Bouchard
Laval's University, Québec, Canada

Congress Review

Review of the European Academy of Allergy and Clinical Immunology (EAACI) Congress 2018

Location: Munich, Germany – Internationales Congress Center München (ICM)

Date: 26.05.18–30.05.18

Citation: EMJ Allergy Immunol. 2018;3[1]:10-21. Congress Review.

Buzzing and bustling from day one, this year's European Academy of Allergy and Clinical Immunology (EAACI) Congress, held from the 26th–30th May, was one that will be talked about for months to come. The sun beamed down over this year's host city, Munich, Germany, as attendees flocked from around the world, creating the perfect atmosphere for all to enjoy. The Bavarian capital was a fitting choice for the event, being one of the most research-intensive cities in Germany.

As the EAACI doors opened, members made their way to the Opening Ceremony to delight in the announcements to come. Special attention was paid to the host city, with related highlights from the field of allergy and immunology. Networking was encouraged throughout the event, and the ceremony attendees were invited to introduce themselves to a person they had not met before and get to know them. A distinctive video was created specifically for the ceremony, featuring facts and insights galore, adding a unique twist to this fantastic event.

Munich is home to the largest public park in the world, the Englischer Garten, and the high pollen levels in the city were highlighted in the video. A brand-new pollen indicator was present at the congress, which reads and relays the amount and type of pollen present in the air. This was just one of the pollen indicators that will form a network across Germany, providing those with pollen allergies the information that they need to medically prepare for the day to come. It was also highlighted that, alongside its 3.7 km^2 of land, the Englischer Garten features an artificial stream, where individuals, including EAACI Vice-President Prof Carsten Schmidt-Weber, gather with their surfboards to catch the waves.

Attention then moved to Munich's agricultural community, with a vast number of farms being commended for their part in combatting grass allergies. Other allergens were discussed, including air pollution particles, which were reported to alter immune

responses. With global events such as the Olympics drawing worldwide attention to air pollution, and the increased use of vehicles across the world, air pollution was a primary focus across the entire EAACI Congress.

Following the video, Prof Schmidt-Weber took to the stage, EAACI surfboard in hand, and the lively scene was set for the event to come.

Prof Ioana Agache, President of EAACI, then made her way to the stage to bring joy to the crowd and announce the achievements made over the previous year, including the publication of the EAACI White Paper on Research, Innovation, and Quality Care. She described the EAACI Congress as the "crown jewel" of the EAACI Congress, expressing how proud her and the organisers of the event were that the EAACI Congress was "the largest and most influential allergy, asthma, and clinical immunology meeting in the world."

Munich is home to the largest public park in the world, the Englischer Garten, and the high pollen levels in the city were highlighted in the video. A brand-new pollen indicator was present at the congress, which reads and relays the amount and type of pollen present in the air in that area.

The focus then shifted to the awards, given to those who have contributed significantly to the development of therapeutic strategies and innovative diagnostic techniques for the control and prevention of allergic diseases. The Daniel Bovet Award for Treatment and Prevention was presented to Prof Gideon Lack (UK), whose research focusses on peanut allergies and prevention of food allergies through the use of oral intolerance induction. Next, the Paul Ehrlich Award for Experimental Research was awarded to Prof Sebastian Johnston (UK). Prof Johnston, who has revealed many insights into the viral aetiology of asthma exacerbations, contributed to the discovery of novel mechanisms in viral infection susceptibility in asthma, and played a lead role in the first ever mouse model of rhinovirus infections and rhinovirus-induced exacerbation of allergic airway inflammation. The Charles Blackley Award for the Promotion of the Allergy Specialty was given to Prof Pascal Demoly (France), who has published >700 articles, research papers, and abstracts that focus on allergen immunotherapy, respiratory diseases, and drug allergy. Prof Demoly has been the driving force behind the implementation of an allergology and clinical immunology medical speciality in his home country of France. Finally, the Clemens von Pirquet Award for Clinical Research was granted to Prof Joaquín Sastre (Spain), for his contributions to the understanding of airway allergies. He has been involved with 400 abstracts at various scientific conferences and published 285 PubMed-indexed articles.

Other announcements made at the Opening Ceremony included the declaration that the EAACI organisation had completed their mission to 'Go green' for the 2018 congress, with the programme only available this year via the EAACI app, removing the old paper version, and with all posters being presented digitally. EAACI TV was released for the first time, providing watchers with a free-to-access method of keeping up-to-date with all the goings-on at the event. As an additional achievement, abstract submissions hit a record high this year, with a total of 1,918 abstracts submitted.

The ceremony concluded with Prof Schmidt-Weber announcing the arrival of the EAACI beer, brewed at his own institute. Attendees were invited to try the flavoursome beverage, alongside different food dishes from across Germany, including a mango curry from Berlin, which highlighted the multicultural hub the central European nation has become.

The night concluded on a networking high, leaving attendees enthusiastic for the days ahead. Mixing tradition and innovation, the event had something for everyone to enjoy: the Clinical Village was alive with attendees testing out clinical techniques, junior members were able to present their research in a variety of formats, plenary sessions provided discussion on the congress' hot topics, and the final day provided an update of all the latest advances in allergology and immunology medicine.

The following Congress Review section will allow you to re-live the most remarkable moments captured at the event, including a selection of abstract reviews written by the respective presenters, straight from the event. Next year's EAACI Congress will be held in Lisbon, Portugal, and we look forward to experiencing the fantastic event once again. We hope to see you there.

‘Diesel Gate’: Should More be Done to Combat Air Pollution?

ATTENDEES from across the globe were invited to take part in lively debates throughout the EAACI Congress in Munich, Germany that addressed the most pressing concerns for healthcare professionals and patients today. Topics discussed included allergic disease and asthma, with the aim of drawing together the expertise and experiences of healthcare professionals from across the board: allergists, pulmonologists, dermatologists, internists, paediatricians, gastroenterologists, clinical immunologists, primary care physicians, and more.

“...it was already clear 25 years ago that diesel particles are a really strong driver of allergy...”

One such debate, ‘The Diesel Gate Affair’, focussed on the important topic of air pollution in cities. The Diesel Gate debate began several years ago following the revelation that Volkswagen had cheated in an emissions test. The scandal culminated, most recently, in the banning of heavily polluting cars from inner-city areas of Germany to decrease the levels of harmful pollutants in the air, such as nitric oxide, a move celebrated by environmentalists and medical professionals alike. The rise in allergic disease and asthma in recent years has been linked to the increase in pollutants through work with both animal models and human immune cells; this increase in allergic and asthmatic disease is a burden on healthcare systems around the world. For example, allergic diseases cost an estimated €55–150 billion every year in Europe through employees taking leave due to allergy-related illnesses. The question posed to attendees, as reported in an EAACI press release dated 28th May 2018, was: should more be done to address these issues, or will these steps help to significantly ease the burden of allergic diseases in cities?

Speaking about the session, *EMJ Allergy & Immunology* Editorial Board member Dr Stefan Wöhrl, Floridsdorf Allergy Center (FAZ), Vienna, Austria, commented on the importance of continuing to focus on this topic, since “it was already clear 25 years ago that diesel particles are a really strong driver of allergy, so, although it’s not really new, [this session] was my personal highlight. I really appreciated this popular topic being brought to an allergy congress.”

Innovative Pollen Detector Keeps Bavarians One Step Ahead of their Allergies

THOUSANDS of specialists visited Munich, Germany in May 2018 to attend the EAACI Congress, but what many did not realise was that innovation was waiting for them before they even entered the congress centre. A strange device, looking somewhat akin to a spaceship's fuselage, towered >15 feet above the entrance to the congress, with numbers flashing from a digital display at the top. This intriguing structure was revealed during the Opening Ceremony to be a revolutionary form of pollen indicator, displaying online how much and what type of pollen is in the air in real-time.

This device is the first of a network being set up throughout Bavaria, known as the electronic Pollen Information Network (ePIN), which aims to keep those with allergies well informed. The network measures the quantity and intensity of pollen in the air and reports it online, giving allergic individuals the latest key data relating to their condition. "If you are allergic, you want to know how much pollen is there for your medication and in case you need to react. Normally you would never know [how much pollen is in the air]: this [ePIN] makes it visible," explained Prof Jeroen Buters, Zentrum Allergie und Umwelt (ZAUM), Helmholtz Zentrum München and the Technische Universität, München, Germany.

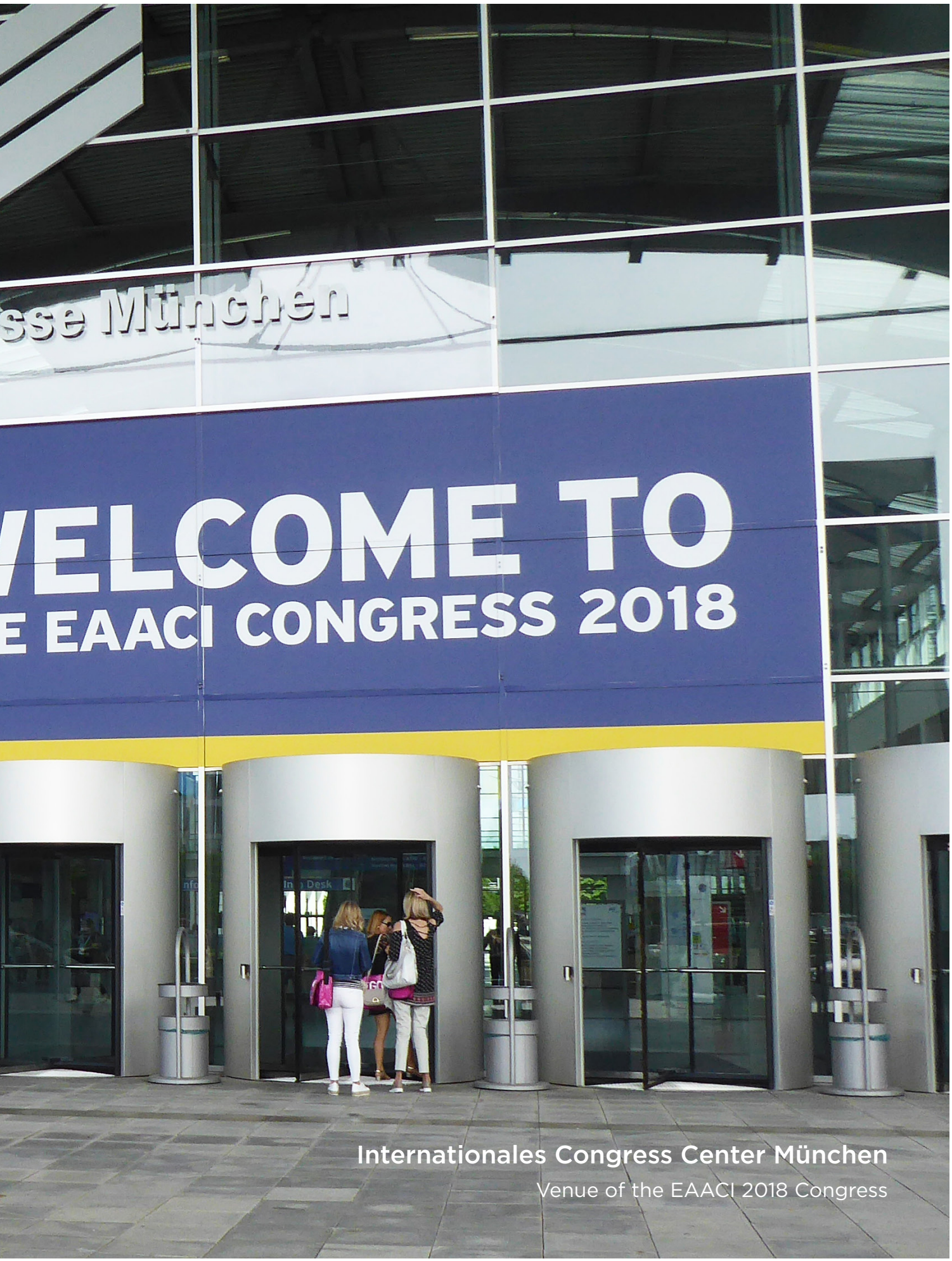
With 1 in 4 Bavarians allergic to pollen, ePIN represents a useful tool for thousands of people during the height of the pollen season. With foreknowledge of the pollen in a certain area, allergic individuals can better manage their condition, avoiding potential hospitalisations because of severe allergic reactions.

The next step is to use this pollen indicating network in conjunction with weather measurements to better predict the levels of pollen on subsequent days, giving a more accurate pollen forecast. Researchers are hopeful that the data gathered could also prove useful for exploring the effects of climate change on pollen levels.

In a light-hearted twist, the large device also doubled as a handy seat for congress delegates. Upon hearing this at the EAACI Opening Ceremony, Prof Carsten Schmidt-Weber, EAACI Vice-President, joked: "I didn't know that people used this €100,000 thing as a seat, but I hope they enjoy sitting on it!"

"If you are allergic, you want to know how much pollen is there for your medication and in case you need to react."


EAACI
2018
Pollen Indicator



Internationales Congress Center München

Venue of the EAACI 2018 Congress

The EAACI Clinical Village

REFERRED to as one of the largest and most influential allergy meetings in the world, the EAACI Congress is known to be unique and thought-provoking. One aspect that makes the EAACI Congress so special is the Clinical Village, which made its way to EAACI 2018 to spread intrigue once again.

The Clinical Village really was a fantastic opportunity to learn and develop, and we very much hope that it will make a reappearance at next year's EAACI Congress.

The Clinical Village provided EAACI attendees with the opportunity to combine theory with practice, taking part in various clinical allergy diagnosis techniques and practising with equipment for themselves. Though a particularly good opportunity for trainee doctors and clinicians, even the experts were raving about this unique stand, as it provided them with the opportunity to update their own skills and knowledge of the latest techniques in the field.

There was a total of 17 stands included in this year's Clinical Village, each one managed by an expert in a particular technique. These included pollen counts; *in vitro* diagnosis using the basophil activation test; a mobile health app; ocular allergy; severe drug reactions; patient organisations; modern drug testing; lung function and sports medicine; anaphylaxis-emergency-medicine; skin function testing, including transdermal water loss and corneometry; nasal and food challenges; exhaled nitric oxide management; standardised patch testing; a paediatric allergy school; and photo allergy.

The interactive postgraduate courses at the event were merged with the Clinical Village this year, allowing attendees to gather what they had learned and put the techniques to the test. The Clinical Village really was a fantastic opportunity to learn and develop, and we very much hope that it will make a reappearance at next year's EAACI Congress.

Congress Technology at its Finest

TECHNOLOGICAL solutions were all around at this year's EAACI Congress, providing unmissable scientific updates direct to visitors in a simple, smart, and environmentally friendly way. From e-posters, a Virtual Congress Hub, and the EAACI App, the EAACI experience was fully enriched by technology, as well as greatly enhanced by the novel introduction of EAACI TV. In a field as fast-paced as allergy and immunology, prompt dissemination of the most recent advances is highly important to enhance clinical care and research.

EAACI TV was used to provide near-instantaneous coverage of the top-quality science delivered at the event. As a moderated, continuous, free-to-access broadcast, the introduction of this novel technology brought all participants closer to the congress activities, including key sessions, interviews, and presentations. With such a large and broad programme, it is impossible to attend all sessions, and therefore EAACI TV provided a vital source of coverage to ensure no key sessions or study results were missed. Live-streaming, morning planning, evening recaps, and behind the scenes footage were some of the features of this new technology that benefitted many.

Redefining the EAACI experience, the official EAACI App was also available for all attendees, bringing the congress programme direct to the delegates' fingertips. By allowing attendees to browse the programme, access congress abstracts, and participate in voting polls, the EAACI App was an important resource to complement the event and enhance learning and development. In addition, all 2018 posters and abstracts were shown via digital e-poster stands in a new, environmentally-sustainable format.

At this year's meeting, three EAACI Virtual Congress Hubs were available where delegates could make the most of these digital resources on offer, including access to EAACI TV, the EAACI newsletter, webcasts of selected sessions, and e-posters and abstracts. The combined effect of these technological solutions on delivery of key information was vast and, as well as supporting EAACI's sustainable approach to the planet, enriched the learning experience for many visitors.

From e-posters, a Virtual Congress Hub, and the EAACI App, the EAACI experience was fully enriched by technology...

Congress Feature

Prof Jacques Bouchard

La Malbaie Hospital, Canada

The EAACI 2018 Congress may have closed, but we remember it as if it were only yesterday. We asked our *EMJ Allergy & Immunology* Editor in Chief, Prof Jacques Bouchard, what his five take-home messages were from this year's fantastic event.

1. Basic Science

It is important, now more than ever, to look at the importance of the fundamentals, genetics, epigenetics, phenotypes, biomarkers, cytokines, and so on, to gain a better understanding of the disease in every aspect and allow physicians to target a more appropriate investigation and treatment course. There are some new concepts emerging and it will be interesting to follow the evolution of these new sciences in the near future.

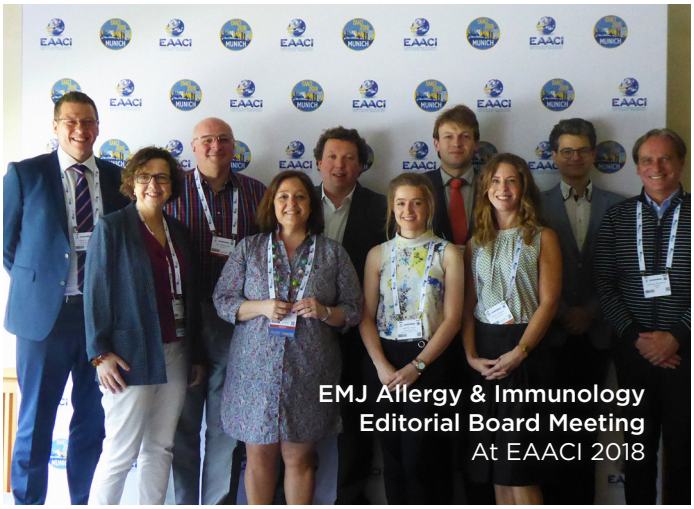
Our Editor-in-Chief
At EAACI 2018

2. Diagnosis

Making a good allergy diagnosis is now more challenging than ever. Skin tests, blood tests for specific antibodies, molecular allergology, cellular testing, oral and provocative challenge test; from old techniques to the new ones, every test plays a role. However, it should always be in relation to the clinical evaluation, including the patient's history. Some presentations at this year's EAACI Congress showed the positive and negative aspects of these tests.

3. Prevention

The EAACI Congress demonstrated the importance of early allergic prevention in patients with some high-risk factors, especially in children. This prevention could also include early exposure to foods such as cow's milk or peanuts. The preventative methods could minimise the risk of developing allergies to these food types. Allergen avoidance remains the gold standard in terms of preventing allergic reactions. Environmental care is also essential and continues to play an important role in controlling most respiratory allergic diseases.



EMJ Allergy & Immunology
Editorial Board Meeting
At EAACI 2018

4. Treatment

Some new treatments, or new methods of treating patients, either for allergy or other related diseases, are available or will become available soon. The use of biologics is growing. Some concepts of allergen immunotherapy and new treatments for severe asthma or hereditary angioedema were widely exposed and discussed. Better understanding of the physiopathology of disease gives clinicians some tools that aid in the adjustment of multiple therapies, focussing on improving the patient's quality of life.

5. Education

Education is well known as playing an important role in every chronic disease. In the allergic and immunologic pathologies, allied healthcare providers are essential to support every patient and their family, in terms of helping the patient to understand their disease and being compliant with their treatment plan, and with regard to what should be done in cases of exacerbation. There are also still some challenges in implementing the guidelines that come from our scientific societies.

Education is well known as playing an important role in every chronic disease. In the allergic and immunologic pathologies, allied healthcare providers are essential to support every patient and their family...

VIEW MORE CONTENT ONLINE ←

Interviews

Take the opportunity to learn more about the opinions and thoughts of some of our esteemed Editorial Board members

Featuring: Dr Michael Rudenko, Dr James Woijoo Kim, Dr Philippe Bégin, and Prof Nikos Papadopoulos

Dr Michael Rudenko @Allergy_London

UKallergy, UK

Firstly, who or what had the greatest influence on your career choice as a medical professional?

My parents, who are both surgeons, influenced my choice of career in the first place, but later on the achievements of my teachers and colleagues directed me to my goal.

You are a prolific researcher, acting as the principal investigator for many clinical trials. What do you enjoy most about overseeing work such as this?

I enjoy placing my small input into the global progress of the discovery of new things; the process is captivating, and the realisation that our day-to-day research routine serves the higher goal keeps us running. It would not be possible to achieve so much without my team and research partners, to whom I am very thankful.

The incidence of asthma is increasing in many countries around the world. To what extent do you associate this with the 'hygiene hypothesis'?

This is an interesting question that has been looked at many times by many researchers. My personal view is that this observation is only superficial, and the true roots of the problem lie deeper within genetics, interactions between various immune mechanisms that are combatting viruses, bacteria, and fungi, as well as the presence of allergic inflammation.

Are there any areas of asthma research currently underway that you believe will drastically change the way in which this condition is treated in the next 5 years?

Current research is mostly focussing on biologics; new therapeutic agents that target the mechanisms used by cells to 'talk' to each other. There are already many drugs on the

market and many more in the pipeline. I think that we will be able to change the way we treat inflammation and use individualised drugs with better safety and efficacy profiles, with a focus on the prevention of remodelling.

In 2011, you founded the award-winning London Allergy and Immunology Centre (LAIC). Could you tell us more about this institute and what inspired you to create it?

We built the LAIC's infrastructure using examples of leading centres in Germany and Switzerland, with the goal of using the best scientifically proven methods of allergy testing and treatment that our patients can benefit from.

The staff members of the LAIC currently include specialists in allergy, immunology, dermatology, and ear, nose, and throat disorders, alongside paediatricians. How important is a multidisciplinary approach when dealing with allergic and immunological conditions?

We try our best to obtain the full picture in regard to what is making our patients unwell and attempt to generate answers beyond just the disease in question. It is necessary to go beyond the scope of practice of an individual specialist, and a multidisciplinary approach works nicely to achieve this.

The LAIC was the first centre in the UK to receive accreditation by the Urticaria Centers of Reference and Excellence (UCARE) network. What makes conditions like urticaria and angioedema difficult to treat effectively, and how do you believe therapy can be improved?

There are many factors that play a role in helping these patients, one of which being the robust diagnostic processes that help to exclude severe conditions like hereditary angioedema and diagnose autoimmune problems. We have a full range of diagnostic tools and the best experts in the field who follow the Global Guidance on Urticaria.

"We try our best to obtain the full picture in regard to what is making our patients unwell and attempt to generate answers beyond just the disease in question."

You have worked closely with a wide variety of societies during your career, including the American Academy of Allergy, Asthma and Immunology (AAAAI) and the European Academy of Allergy and Clinical Immunology (EAACI). What do you consider to be the greatest strengths of large medical societies such as these, and how could their influence on the field of allergy and immunology be enhanced?

Yes indeed, big societies can have a significant influence on governments and regulatory bodies that make health-related decisions. As an example, I want to put forward campaigns to both the AAAAI and EAACI to increase awareness of the only treatment that can change the cause of respiratory allergy and allergic asthma: desensitisation or specific immunotherapy. They also provide a platform for explaining the burden of allergic disease and how allergy testing and treatment can improve quality of life for these patients.

As part of the EAACI Taskforce, you provided questions for the latest EAACI–Union of European Medical Specialists (UEMS) Knowledge Examination in Allergology and Clinical Immunology. How did you become involved with the EAACI Taskforce and how do you go about creating questions for this important exam?

The EAACI European examination is an important task that I have been supporting for many years. The speciality changes with new research achievements in science and constant updates are required to ensure the exam remains a robust tool.

Finally, what advice do you have for current medical students looking to specialise in allergy and immunology?

Our speciality requires a lot of thinking and modulating, and can easily be likened to detective work. The field is rapidly growing and there is a lot of interest in addressing the needs of allergy and immunology patients from pharmaceutical companies who are developing new drugs and diagnostic methods.

"...big societies can have a significant influence on governments and regulatory bodies that make health-related decisions. They also provide a platform for explaining the burden of allergic disease..."

Dr James Woijoo Kim @LinkedIn

Family Physician Airway Group of Canada, Canada

What inspired you to pursue a career in medicine, and what continues to motivate you to this day?

I grew up in a very humble background in South Korea, which, at a very young age, made me realise that money can dictate one's health and health management. My family then moved to South Africa, where I saw a huge inequality and gap between the public and the private healthcare systems. I had difficulty accepting the fact that such massive gaps between healthcare systems, differentiated by socioeconomic status, should exist. There was also a notion that a doctor working in a public health sector was not good enough to work in the private sectors, and therefore the poorer patients were treated suboptimally. To change this, I decided to pursue a career in medicine and become the best doctor that I could be, working with patients with a low socioeconomic status and providing the best evidence-based medicine for them. To this day, this is still my main motivating factor.

"Being on the advisory boards has been an interesting experience; the best part is networking and meeting with the other top players in the country."

You have developed an extremely successful career, with your most recent achievement being your new position as an Assistant Professor at the Faculty of Medicine, University of Calgary, Calgary, Canada. What does your new role entail, and how will it enhance your career?

Oh, thank you for such kind words. Assistant professorship is an entry level professorship at the University of Calgary, so I still have a long way to go. My role mainly involves teaching family medicine residents. In the near future, I will most likely facilitate the group discussion sessions, work as an examiner for the students and residents, and take part in some of the lectures. Interacting with younger minds is refreshing, and they keep me on my toes! I hope to slowly climb the ladder within the faculty of medicine.

You are a member of many advisory boards for different pharmaceutical companies. What is it that interests you most about these positions, and how did you first get involved with this side of your work? How does your role impact the pharmaceutical industry?

So far, I have only been on advisory boards for diabetes, as that has been my main speciality.

"I believe these organisations have a very important role in improving the standard of care for respiratory conditions through identifying the knowledge gaps..."

Being on the advisory boards has been an interesting experience; the best part is networking and meeting with the other top players in the country. This may sound nerdy, but sometimes I feel like I am at the Oscar Awards night for medicine, sitting and sharing ideas with some of the country's medical celebrities!

Being on advisory boards also forces me to be up-to-date with the latest key findings and advances in diabetes. This helps me to stay on top of my game and, sometimes, it also allows me to get a first look at medications that are in the pipeline. For example, about 3–4 years ago I was part of the market research for various medications, and it was exciting to see them being released within the following 2 years; it has also been enjoyable using them in the clinic.

I believe I was identified as one of the key players by the numerous pharmaceutical companies within the world of primary care mainly for the work I have been doing as a physician with a special interest in diabetes. I believe that is how it all started. To be honest, I am not entirely sure how much impact I have within the pharmaceutical industry because my advisory role is to provide my opinions on various topics of medicine. My role may help the pharmaceutical companies' marketing strategies; for example, through me giving them practical tips on how the medications should be utilised.

You are also a board member and a regional representative for the Family Physician Airway Group of Canada and the Respiratory Medicine Program committee within the College of Family Physician of Canada. Can you describe these organisations and explain how important you think organisations such as these are for the development of modern medicine?

As I write these answers I am sitting on the aeroplane after finishing a whole day's meeting with the Respiratory Medicine Program committee! We are a group of family physicians who have a special interest in respiratory conditions. Although the Family Physician Airway Group of Canada and the Respiratory Medicine Program committee function slightly differently, the main purpose of both groups is to provide education for family physicians on respiratory conditions, focussing more on common conditions, such as asthma and chronic obstructive pulmonary disease.

I believe these organisations have a very important role in improving the standard of care for respiratory conditions through identifying the knowledge gaps and practical barriers and creating strategies to close these gaps and overcome the barriers, if possible. These are achieved mainly through various educational sessions and being involved with the national guideline committees. For example, we have identified the underutilisation of spirometry as an issue, and we have been promoting the use of spirometry, especially a simple but effective in-office spirometry that can, and should, be utilised more to correctly diagnose, manage, and monitor numerous respiratory conditions. It is believed that chronic obstructive pulmonary disease is the most overdiagnosed and underdiagnosed condition in Canada; we have developed an educational programme and workshops for spirometry for this reason. Recently, we carried out a nationwide survey on asthma management and found that a huge number of asthmatic patients were treated without clear evidence to support their management plans. We will be addressing these issues and findings through various conferences and presentations.

You have given various presentations at numerous locations throughout your career. Has there been one particularly memorable presentation you could tell us about?

It has to be one of my first presentations in Canada, which was a South-East Asian-focussed diabetes conference. There were numerous very well-known endocrinologists who happened to be articulate speakers, and I had to do my

presentation after theirs. I did not sleep much the night before and was obviously nervous before my presentation started. Anyhow, I delivered the presentation and received much positive feedback on my ability to pitch to the high-level primary care physicians and that they managed to get a lot out of my presentation. That is when I realised that since I am also a primary care physician, I have insight as to what is happening at the primary care level, what the physicians' needs are, and what they need to hear, and I have been using this to my advantage ever since. It was definitely a confidence booster.

During your spare time away from these esteemed positions, you can often be found working in emergency departments. What has been the most interesting case you have seen while working with patient emergencies?

There are many interesting cases that I get to see in the emergency room (ER) frequently, but there was a particularly interesting shift a few years ago. I was working in a small town with a population of roughly 6,000, and I was the only physician available for 72 hours. I was doing a round for the inpatients when I was called to the ER immediately. I saw two ambulances pulling a patient each into our two trauma/cardiac bays. One of them had stopped breathing completely and was being bag-mask ventilated by the paramedics, and the other was having a massive heart attack. We collected most of the healthcare professionals in the hospital and divided the work so that paramedics and nurses were supporting the airway and breathing of the apneic patient, while one nurse and I attended the patient with the heart attack. I gave a thrombolytic agent to the heart attack patient and, while the nurse was monitoring the post-thrombolytic patient, I jumped to the other trauma bay and intubated the apneic patient right away. Once the patients were stabilised, we called the tertiary centres for the transfer of care, and, in the next hour, both an ambulance and a helicopter arrived to take the patients to two different tertiary hospitals. Although severe ER cases are common, it was definitely an adrenaline-pumping hour!

Do you have any research projects that you are keen to get involved with in the future? If so, what do they involve?

I will be starting quality improvement work for diabetes in next few months. I have also been asked to join the research group related to lipid management, and I may start this line of work once diabetes quality improvement takes off.

What are your aims for your career progression and where do you see yourself in 10 years' time? How do you propose to get there?

My main aim is to become one of the key players in the management of chronic diseases, especially diabetes, at a national level. In 10 years' time, I hope to be involved with diabetes guideline committees.

What advice would you give to a trainee medical student hoping to develop a career in allergy and immunology?

This is more general advice that I give to medical students and residents across all disciplines.

We, as doctors, are in a privileged position to listen to other's stories in the clinic or at the hospital, which often they would otherwise not have told us and, sometimes, you may be the first person to hear about their stories. We get to see people when they are in a vulnerable state. With this in mind, please treat every patient with respect and gratefulness. Please do not see them as a disease entity or just a number (e.g., when in hospital, refrain from referring to a patient as 'bed number three', which I have experienced before). This will help you earn their respect, and once mutual respect is formed, you will become a far better physician.

"...treat every patient with respect and gratefulness. Please do not see them as a disease entity or just a number [...]. This will help you earn their respect, and once mutual respect is formed, you will become a far better physician."

Dr Philippe Bégin

Université de Montréal, Canada

What first spurred your interest in allergic diseases and, more specifically, what led you to focus on oral immunotherapy (OIT) research?

I think that, like many other physicians, passionate and dedicated mentors had a huge impact on my early career orientation. This said, during training, I would say patients quickly became the main source of motivation to pursue work in the field of allergy. In food allergy especially, there was a blatant need for better management options, and it was obvious that our recommendations for strict avoidance and epinephrine training did little to alleviate the distress experienced by most families. Seeking extra training in OIT was an obvious choice for me at the time, and looking back I am very glad I made it. To echo many of my colleagues, my work satisfaction has increased tremendously since I have started researching and practicing OIT, because the patients let me know every day just how much it changes their lives.

In 2013, you were awarded a $250,000 Emerging Clinician-Scientist Research Fellowship Award from AllerGen NCE Inc. to fund your research on OIT for patients with food allergies. What impact did this achievement have on your research?

The fellowship award offered an incredible opportunity for me to complete my research training with Dr Kari Nadeau at Stanford University, Stanford, California, USA, and supported my transition as a junior clinician scientist back in Canada afterwards. It allowed me to not only acquire invaluable research experience at a world-leading centre but also the experience and expertise gained gave me the credibility to competitively position myself for subsequent funding opportunities in my institution, as well as in provincial and national competitions. Allergy and immunology is a small speciality, and it can be hard to compete with larger specialities that are backed by huge national foundations. Funding opportunities that are restricted to junior investigators and/or to allergic disease help level the field when later competing against other specialities and can have a huge impact when starting a research career.

As a therapeutic technique, what does OIT involve? How does it work for individual food allergies?

The simple answer is that OIT consists of slowly reintroducing the food a patient is allergic to and building up their tolerance to it. OIT is obviously more complex than that, as no two patients are alike. First, the patient's personal objectives need to be assessed: are we looking for a partial desensitisation to an amount that will protect against traces, a complete desensitisation that will allow full reintroduction of the food in the diet, or are we hoping for clinical remission with sustained protection after stopping treatment? There are multiple protocols to choose from, including the 'standard' slow schedule, medication-enabled accelerated protocols, or 'simplified' protocols for patients with high baseline reaction thresholds. Then, there is the decision as to what food to include in the treatment mix, especially if there are too many allergies to treat them all at once; how does the patient dose at home, e.g., powders or suspensions; and when and how to switch to actual food equivalents. The dosing schedule, target maintenance dose, challenges of cross-reactive food, and sustained remission testing all need to be tailored to the patient's specific allergies and treatment response but also to their personal objectives and comfort level. If anyone was looking for a good example of personalised medicine, OIT is it.

"Direct health costs and risk of reaction are already relatively low in food allergic patients; however, the risk is real, and it is always there"

How do you think OIT will affect the lives of patients with food allergies?

OIT can be life-altering for patients and their families. When we look at the overall goal of OIT, it cannot be to save costs or to reduce the amount of allergic reactions in a given number of years. Direct health costs and risk of reaction are already relatively low in food allergic patients; however, the risk is real, and it is always there. It is living with the constant risk and the associated social limitations that is the true burden of food allergies, and this is what OIT addresses. While the patient may actually react more often than before during food dosing at home, it happens in a safe environment with the patient and family ready to recognise symptoms and intervene, which is completely different from an unexpected reaction in a social event where panic takes over. The true benefit is the other 22 hours of the day when the patient is not taking the dose when they are completely protected against accidental contacts. If you eat eight peanuts a day, the likelihood that you would react to a peanut inadvertently added to your meal is quasi-inexistent. All in all, it is a question of control over your life. With OIT, the patient controls their allergies and not the other way around. The more a patient feels impacted by their allergy, the more there is to gain from treatment. Conversely, it would make little sense to offer it to a patient that is not concerned by their allergies.

You are currently working on transferring OIT from the research to clinical setting at the Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada. Can you describe the process of this transfer and explain when patients will be able to take advantage of this novel technique?

I would tend to say it is a continuous process and that it is definitely not straightforward. One major challenge is resources. It is important for administrators to understand that our practice is completely changed by this and that the patient that you would typically see for one appointment every other year, and do nothing for, will now require multiple visits requiring clinical observation, a system to supervise home dosing, and a technician to prepare the food dose following the food industry standard, among other requirements. And all for what? For quality of life, which can be quite subjective. The first barrier to address is the education of food allergy and OIT. We need to educate patients, the medical community, institutions, the public, and government on why this is justified. This is something we have been doing over the last 3 years, working with parent groups in Québec, Canada, through an awareness and fundraising campaign that led the health minister to directly sponsor our pilot public OIT clinic, which received the mandate of initiating a public clinical offer outside of research focussing on the most severe cases. The clinic opened officially in September 2017 and has a projected objective of treating 275 severe or multi-food allergic children per year for 3 years. The clinic will serve to provide performance indicators on clinical, administrative, and quality of life outcomes to direct future public investments and establish a global strategy for an eventual implementation throughout the province. Transferring clinical expertise to colleagues and staff takes time, but well-designed clinical tools (dosing diaries, OIT action plans, consent forms, standard visit forms, and food dose prescription forms), standard operating procedures (for dosing schedules, food preparation, or patient training), a consensus-based referral teamed with a prioritisation system, a hospital hotline, and clearly defined roles for allergy nurses, food technicians, nutritionists, and paediatricians can go a long way in enabling clinicians and improving patient safety.

The clinical application of this technique at the Centre Hospitalier Universitaire Sainte-Justine will focus on the treatment of children. Will OIT eventually be used for adult patients too? How does the therapeutic technique differ between adult and child patients?

Indeed, there is an important demand from adults with food allergies. We have focussed on children for the pilot because the demand was greater and because more research has been conducted in this age group. We do perform OIT in adults but mostly in the context of research for the moment. The general principles are the same in adults as in children, but there are some

differences. The first difference is that there are no parents involved, and as a result we do see more issues with compliance and risk-taking behaviours, although this is highly variable between patients. Another difference is that the type of allergy differs. Adults are more likely to have long-lasting persistent allergies that may be less likely to attain sustained remission compared to newly diagnosed toddlers. They also have more pollen-food syndromes for which the utility of OIT is less obvious. Adults do offer an advantage for research because they can consent themselves and are generally willing to provide more clinical samples for translational research. This is not trivial considering that the development of biomarkers to better inform and personalise treatment is an area that needs active research.

"...talking with patients is the single most important thing you do as an allergist [...] it is through your discussions that you keep the patient safe and prevent one of the three, four, five, or sometimes even 20 allergic atomic bombs in them from going off."

Will you attend any congresses this year? How important do you feel congresses are for the future of medical research and clinical practice?

I usually attend one international meeting every year. For me, these are essential to meet and catch up with colleagues. During congress, I enjoy poster sessions the most. I also always attend our provincial and national meetings in Québec and Canada, respectively. A key to any successful project is to engage colleagues and make sure the planned strategy addresses their needs. It means you also need to reciprocate when colleagues need you to participate in their projects. As doctors, our first loyalty is to our patients, and, ultimately, concrete consensual initiatives from regional or national societies or a local change in policy will have greater impact on them than the latest high impact publication.

How do you think allergic medicine will change over the next 5 years? What areas do you think the medical community should be focussing on?

I am obviously biased, but I do think new recommendations for early prevention and immunotherapy for food allergy are going to completely transform the way we practice. Although I am very excited by many other cutting-edge avenues, I think one priority is to develop a strategy to engage stakeholders, lobby governments, and develop the health economics argument, so we have the capacity to offer these options to all patients, which is currently not the case. Comparative studies assessing clinical standards and strategies used to provide these services in different countries with public healthcare would be incredibly useful.

What advice would you give to a trainee medical student hoping to pursue a career in allergology?

From a strategic point of view, as for any speciality, I would recommend they do at least one optional rotation in allergy to see what the clinic is like and, if possible, get involved in a research project over the summer. Even if they change their mind, any experience in research at that level is beneficial. I would also suggest that they enrol in trainee programmes at national or international conferences. From a more personal point of view, I think the thing they should really be focussing on is learning to talk and connect with patients. If a resident knows nothing of immunology when entering our programme, we will teach them. However, talking with patients is the single most important thing you do as an allergist; it is how you obtain a diagnosis, how you sort through and debunk all the misconceptions, how you find the hidden culprit no one else thought of, and, most importantly, it is through your discussions that you keep the patient safe and prevent one of the three, four, five, or sometimes even 20 allergic atomic bombs in them from going off.

Prof Nikos Papadopoulos

University of Manchester, UK

Your main research focusses on the role of infections in respiratory conditions, as well as food allergy. What infection is of most concern to an individual suffering a respiratory condition?

Well, surprisingly, among the many infections that can be quite serious, the one that affects more people with asthma, and possibly also with food allergy, is the common cold, which is the rhinovirus infection. For several reasons, people who have this allergic tendency seem to overreact; the system is primed to react too much to this common cold virus. They start from very mild symptoms and can progress all the way up to very severe exacerbations. We also have the suspicion that some food allergy reactions are associated with such a viral infection, possibly as a cofactor, an additional factor that can help the development of the food allergy reaction.

Can you briefly describe how these infections work as a cofactor for food allergies?

We have this hypothesis, in the case of respiratory allergy but also in food allergy, that you must have more than one factor in order to have a clinical reaction. In the case of food allergy, we know that if you eat enough of the allergen, then you have a reaction; however, we also know that the level of reactivity, what we call a 'threshold', can change. So, it is possible, and we see it in clinical practice, but it has not been proven in research and a mechanism has not been shown for the lowering of the threshold when you have an infection. So, something happens when you react to the viruses that make you more susceptible, so you can have the reaction but with much less food.

"We have this hypothesis... that you must have more than one factor in order to have a clinical reaction."

"For several reasons, people who have this allergic tendency seem to overreact; the system is primed to react too much to this common cold virus."

What does your research currently involve that will help combat these kinds of infections?

We are looking into the ecology of the respiratory tract. So, not only specific infections, but also all the microorganisms that live within our respiratory tract. This is the microbiome, which has become famous, but we are focussing more on the viral aspect of it: the virome. This means that there are perhaps possibilities to re-establish a balance in a system that has been unbalanced because of the immunity, but also because of the wrong micro-organisms being there. There is a possibility, and this is what we are proposing in a recent project we are running, a European project called CURE (www.cureasthma.eu), that we might use some viruses in order to combat bacteria and re-establish the balance. So, we might even use an infection to combat an infection!

How will your research encourage further study in this area?

The programme that I have just described, about the possibility of manipulating different viral populations, is extremely novel. There are lots of possibilities, and now there are some other groups looking into these balances as well. There are different possibilities in terms of conditions and diseases; we are focussing mostly on asthma, but by the same reasoning you can apply this strategy to food allergy, rhinitis, conjunctivitis, and many other diseases. You can also have different types of approaches, for example, prognostic, preventive, and therapeutic approaches, depending on which type of patient might benefit more. So, there are several possibilities, and I think

we are opening a field for these possibilities to be explored.

EAACI have announced the installation of pollen indicators around Germany, which record the levels of pollen in the area for patients to access and adjust their medication and day accordingly. How much of an impact do you think this will have on the day-to-day lives of those people suffering from pollen allergies?

Information about pollen can be very important for those patients who know that they are allergic to that pollen, because they can plan their day. But it is not only about planning, it is about the information per se, and knowing what your problem is and knowing that you have an increased risk of having this problem, makes you be more careful and ensures you take your medication. I think this is very important information to have. It is commendable that both the local science team and EAACI has helped to establish this information. This is something that should happen and should be available everywhere.

"You can also have different types of approaches, for example, prognostic, preventive, and therapeutic approaches, depending on which type of patient might benefit more. So, there are several possibilities, and I think we are opening a field for these possibilities to be explored."

WATCH IN FULL ONLINE ←

This is an abridged version of our interview with Prof Nikos Papadopoulos, click here to watch it in full.

VIEW MORE INTERVIEWS ONLINE ←

Available now.

Inside this journal with a difference, you will find exclusive content straight from EuroPCR, as well as material written by EMJ's very own reporting team.

Read *EMJ Interventional Cardiology* for free today.

EUROPEANMEDICAL-JOURNAL.COM /SUBSCRIBE

Take a Breath of Real-World Evidence

This company-sponsored symposium took place on 29th May 2018, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress 2018 in Munich, Germany

Chair People: Stefan Zielen[1] and Pascal Demoly[2]

Speakers: Adam Fox,[3] Stefan Zielen,[1] Pascal Demoly,[2] Ulrich Wahn[4]

1. Division of Allergology, Pulmonology and Cystic Fibrosis, Department for Children and Adolescents, Goethe University Hospital, Frankfurt, Germany
2. Department of Pulmonology and Addictology, Arnaud de Villeneuve Hospital, Montpellier University, Montpellier, and Sorbonne Universités, France
3. Guy's and St Thomas' NHS Foundation Trust, London, UK
4. Department of Paediatric Pneumology and Immunology, Charité Medical University, Berlin, Germany

Disclosure: Prof Zielen has received lecture fees from Stallergenes Greer, Allergopharma GmbH, Allergy Therapeutics, Boehringer Ingelheim, GlaxoSmithKline GmbH, Novartis, AstraZeneca, Lofarma GmbH, and IMS HEALTH GmbH & Co. OHG; and research grants from Biotest GmbH, Vifor Pharma Deutschland GmbH, ALK Arzneimittel, and Bene-Arzneimittel GmbH. Prof Demoly has been a speaker for Stallergenes Greer, Thermo Fisher Scientific, ALK, AstraZeneca, and Mylan; has consulted for Stallergenes Greer, IQVIA, ALK, Chiesi, and Sanofi; has been on a panel of experts for Stallergenes Greer, Chiesi, Thermo Fisher Scientific, Ménarini, Bausch&Lomb, and Allergopharma; and is the President of the French Federation of Allergology. Dr Fox has received lecture fees from Stallergenes Greer, ALK-Abello, and Allergy Therapeutics; and has received research grants from ALK Abello. Prof Wahn has been a speaker for Stallergenes Greer, Allergopharma, ALK-Abello, Novartis, Nestlé, MEDA, and Pfizer; has consulted for Stallergenes Greer, Allergopharma, ALK-Abello, Novartis, Nutricia, Biomay, and Berlin Chemic; and has been on a panel of experts for Stallergenes Greer, Allergopharma, ALK-Abello, Danone, Sanofi Aventis, and IQVIA (Former IMS, Quintiles IMS).

Acknowledgements: Writing assistance was provided by Janet Fricker.

Support: The symposium and publication of this article were funded by Stallergenes Greer.

Citation: EMJ Allergy Immunol. 2018;3[1]:34-39.

Meeting Summary

Allergen immunotherapy (AIT) in the form of subcutaneous or sublingual immunotherapy (SCIT/SLIT) is the only treatment for allergic rhinitis (AR) and/or allergic asthma with long-term efficacy.

Dr Fox considered the benefits for using real-world (RW) evidence in AIT. RW evidence provides the opportunity to explore a wide range of patients, estimate evolving risk benefits, and obtain data on clinical and economic value, as well as allowing comparisons of multiple alternative interventions. In clinical settings, such information allows doctors to provide allergy patients with the best advice, because most patients do not fit the narrow inclusion/exclusion criteria of clinical trials.

The benefits of RW research can be illustrated by two studies that are part of the Bringing Real-World Evidence to Allergy Treatment for Health (BREATH) programme, which was launched by Stallergenes Greer.

Prof Zielen provided an overview of the design of the German Birch AIT and French Grass SLIT Tablets RW studies. The studies are retrospective cohort studies based on IQVIA™ longitudinal prescription databases allowing patient follow-up. Follow-up was up to 9 years in Germany. Both studies share three objectives: looking at progression of AR after treatment cessation, initiation of new asthma medication in patients with AR (not asthma) at baseline, and progression of asthma medication use in patients with asthma (with or without AR at baseline).

Exploring the studies in greater detail, Prof Demoly presented the French Grass SLIT Tablets RW study, which compared 1,099 patients treated with SLIT with 24,475 controls not treated with SLIT. The results for the SLIT cohort versus the control cohort demonstrated long-term benefits for AIT (up to 2 years after treatment cessation), significantly reduced AR medication intake ($p<0.001$), significantly reduced asthma medication intake ($p=0.003$), and significantly decreased initiation of asthma medication ($p=0.0013$).

Prof Wahn presented the German Birch AIT RW study, which compared 9,001 AIT patients with 45,005 control patients not taking AIT. The results showed that AIT patients were significantly more likely to be AR medication-free ($p<0.001$), had reduced risk for initiation of asthma medication during the study ($p=0.001$), and were more likely to be asthma medication-free during 6 years of follow-up ($p<0.001$). Notably, when different types of AIT were compared to control, SLIT was not found to be any less effective than SCIT, opening the way for wider use of sublingual treatments.

Introduction

David Tomlinson

The symposium addressed the question of how to optimise the quantity and quality of RW data for the benefit of patients. For example, the BREATH programme gathered information from >150,000 patients, tracked for 8 years. The audience discussed how RW data like this will change practice.

Time to Think Bigger? Is Real-World Evidence a Game-Changer?

Doctor Adam Fox

While randomised controlled trials (RCT) are considered the gold standard for assessing safety and efficacy, their lengthy inclusion and exclusion criteria have created concerns that it may be difficult to generalise results to wider populations.

Recently, it has become possible to use RW evidence derived from sources outside typical clinical research, with examples including electronic hospital records, billing data, disease registries, and prescription databases. Such sources complement RCT by reflecting use in clinical practice. The approach offers cost-effective possibilities to look at interventions over extended periods of time, creating new data gathering opportunities and changing the way clinicians think about the treatments they prescribe routinely. While RW evidence is increasingly recognised as an important source of information by organisations, such as the National Institute for Health and Care Excellence (NICE) and the U.S. Food and Drug Administration (FDA), in allergy the approach is still in its infancy.

RCT play a critical role in achieving product licences, with RW studies exploring what happens beyond product registration. Real-life populations may vary according to sex, age, ethnicity, comorbidities, disease severity, concomitant medications, and compliance.[1] How such factors affect outcome needs to be explored; this will result in the possibility of using this information to design the next round of RCT.

The benefits of exploring RW data in the context of AIT include:

- A wide range of patients, with possibilities to investigate diverse populations reflecting the range and distribution of patients observed in clinical practice; e.g., polysensitised patients.
- The ability to estimate evolving risk–benefit profiles of AIT, including long-term clinical benefits and risks, such as whether hyposensitising children for AR influences later asthma outcomes.
- Provide evidence related to the clinical and economical value of AIT in addition to safety and proper use. With RW data, it is possible to explore not just whether interventions are effective but where they are most and least effective.
- Possibility for assessment of multiple alternative interventions to inform identification of optimal treatments.

In clinical settings, such information allows doctors to provide patients with the best advice, since most patients do not fit the narrow inclusion/exclusion criteria of clinical trials.

Studies have highlighted the challenges physicians face when treating AIT patients in real practice, which include the problem of selecting the right patient,[2] generalising results from studies to primary care,[3] and the possibility that efficacy may only be achieved for patients with severe symptoms.[4] Even if the right patients are selected, questions remain about whether they will take treatments. It is widely acknowledged that patients in study settings are well motivated and good at taking medications, with a meta-analysis involving 81 SLIT studies and 9,998 patients showing excellent adherence, with only 14% dropping out.[5] Such data are in sharp contrast to a Dutch pharmacy study, which showed only 7% of 3,690 SLIT patients completed their 3-year course.[6]

Study nurses can have a beneficial impact on adherence, with Italian research showing the combination of education, contact, and follow-up reduced drop-out to 5% at 4 months and 12% at 1 year.[7] Such data provide a plausible explanation for differences observed between clinical trials and RW situations.

Despite such challenges, the benefit of AIT treatment in clinical practice was shown recently in a large-scale retrospective RW prescription database analysis using the German longitudinal prescription database, the IQVIA HealthLRx database. The study, which assessed the effectiveness of two grass pollen SLIT tablets, provides a good example of the use of big data. The BREATH large-scale retrospective analysis, which analysed data from 2008–2016, identified 2,851 SLIT patients. They were compared to 71,275 control patients who had seasonal AR; they had been prescribed nasal steroids during the grass pollen season but had not received AIT treatment. The study showed RW treatment of AR patients with grass pollen SLIT tablets versus control was associated with an additional 19% improvement in progression in the use of AR medication, a 30–40% risk reduction of initiating asthma medication, and an additional 17% reduction in asthma medication. These data show grass pollen tablet SLIT prolongs the time to getting asthma and reduces the need for asthma medication.[8]

In summary, RCT remain the gold standard and RW evidence provides data complementing their findings. RW evidence shows how RCT findings can be generalised to broader populations and reflect actual use in practice. However, while RCT evidence supports SLIT efficacy, poor patient selection or poor adherence may impact on effects in clinical practice. BREATH represents the first initiative to develop a substantial RW evidence base around AIT and demonstrates insights into its effects.

What is the Impact of Allergen Immunotherapy on the Disease Evolution of Respiratory Allergy Patients?

Professor Stefan Zielen

Prospective study designs generally require primary data collection, providing a high degree of control over data collected. Disadvantages include studies taking longer and costing more than retrospective designs. Retrospective database studies, looking back in time using secondary data, have the potential to generate

large RW sample sizes quickly and efficiently. Limitations include the fact that the data already exist, allowing for no control over the information collected.

Both the German Birch AIT and the French SLIT Grass Tablets RW data studies are secondary data retrospective studies based on IQVIA longitudinal prescription databases. Patients have a unique ID across all their physicians and the database. Diagnoses are not recorded but are instead inferred from prescriptions. The German study (which retrospectively analysed data from 2008 onwards) involved data from >60% of German pharmacies, while the French study (which retrospectively analysed data from 2012 onwards) involved data from around 35% of French pharmacies.

The main difference was that the German study used birch AIT (in the form of drops, natural SCIT, or chemically modified allergoids) and the French study used grass tablet AIT. Individuals receiving these prescriptions were compared with control patients receiving only symptomatic drugs. For both studies, the three objectives were:

- Progression of symptomatic AR medication after treatment cessation.
- Initiation of new asthma medication in patients with AR (not asthma) at baseline during and after treatment cessation.
- Progression of asthma medication use in patients with asthma (with or without AR at baseline).

For the AIT group, inclusion criteria were ≥5 years of age, ≥2 seasons of treatment with AIT, AR with or without asthma (grass tablets), AR and/or asthma (birch AIT), and ≥1 (grass) or 2 (birch) years follow-up after AIT cessation. The exclusion criteria for the AIT group were perennial and/or severe asthma, and to have received any other AIT in the past. For the control group, inclusion criteria were ≥5 years of age; AR with or without asthma (grass tablets); AR and/or asthma (birch AIT), defined as ≥3 prescriptions of AR; and/or asthma medication for 3 successive grass/birch pollen seasons. The exclusion criteria for the control group were a previous history of AIT and perennial and/or severe asthma.

The German study involved 9,001 AIT patients and 45,005 control patients, and the French study involved 1,099 AIT patients and 27,475 control patients. The key study periods were pre-index (1 year before AIT started representing baseline), index date (date of first AIT delivery), treatment period, and follow-up period (from date of expiry of the last AIT until end of study).

The strengths of the studies are that they reflect clinical practice and the use of AIT, they are nationwide studies representing large cohorts, they allow comparisons of AIT versus standard of care, and different formulations can be tested with the same methodology. Additionally, longitudinal data collection allows patient follow-up over time and the data covers a 9-year period, allowing assessment of long-term effectiveness. Weaknesses include that they are retrospective analyses, the clinical information was obtained via proxies (use of asthma and AR prescription data), and the ability to only detect reimbursed drugs.

New Results from a French Study with Allergen Immunotherapy Tablets for Grass Pollen Allergies

Professor Pascal Demoly

The French study with SLIT tablets for grass pollen allergies was based on a prescription database involving data from one-third of French pharmacies.

Overall, 1,099 AIT patients who received grass pollen tablet SLIT for AR (62% with AR and 38% with AR and asthma) were compared to 27,475 controls who did not receive grass pollen tablet SLIT but had access to symptomatic AR (and asthma) medication (61% with AR and 39% with AR and asthma). For AIT patients, 27.7% were followed for three seasons and 72.3% for two seasons, and controls were followed for a minimum duration of 1 year and a maximum duration of 2 years. The shorter follow-up compared to the German study can be explained by the French prescription database being younger.

Regarding age, for SLIT patients, 43% were aged 5–17 years, 47% 18–45 years, and 10%

>45 years; for the controls, 6% were aged 5–17 years, 24% 18–45 years, and 70% >45 years. The data demonstrate that, overall, AIT patients were younger than controls. However, a post hoc analysis found that even when subjects were paired according to age, the results remained strong.

Regarding the first objective (AR medication progression), the results showed a 50% reduction in SLIT group for AR medication prescriptions after treatment cessation. This was compared to a 30% increase for AR medication use in the control group ($p<0.001$). Additionally, it was found that 37.4% of AIT patients did not use AR symptomatic drug prescriptions during follow-up, compared to 4.5% of controls. This led to the conclusion that SLIT tablets for grass pollen AR lowered the number of patients using AR symptomatic medication by the end of the study.

Regarding the second objective (initiation of asthma medication), the results showed an additional 36.6% reduction in initiation of asthma medication for the AIT group versus the control group ($p=0.003$) in the treatment period. In the follow-up period, there was an additional 62.5% reduction in initiation of asthma medication for the AIT group versus control group ($p=0.0025$). Furthermore, a Cox regression analysis found a significant difference in the length of time AR patients without asthma at baseline did not initiate asthma medication for AIT patients versus the control group (hazard ratio: 0.36; $p=0.0013$). The findings led to the conclusion that SLIT tablets for grass pollen AR significantly reduce the relative risk of starting asthma medication in real life.

Regarding the third objective (progression of asthma medication), results showed that, during the treatment period, 16% of SLIT patients with asthma at baseline did not use treatments, in comparison to 7.1% of controls. In the follow-up period, 43.1% of the SLIT group with asthma did not use asthma symptomatic medication compared to 10.8% of controls. Overall, there was a 40% reduction in asthma medication in the AIT group after treatment cessation, compared to a 20% increase in the control group ($p<0.0001$).

In conclusion, the French investigators confirmed the previous German results[8] in a study looking at long-term benefits of grass pollen SLIT tablets with up to 2-years follow-up. The French study showed AR medication, asthma medication, and initiation of asthma medication were all significantly reduced.

New Results from a German Study with Allergen Immunotherapy for Birch Pollen Allergies

Professor Ulrich Wahn

Allergy research is now leaving the ivory tower of academic studies and entering the real world for use in real patients. In the German study on birch pollen allergic patients with AR and/or asthma, investigators compared the six birch-family pollen AIT products available in Germany (one natural SLIT, one natural SCIT, and four allergoid SCIT preparations) with symptomatic drugs. The study set out to understand whether AIT can help patients with AR get better, reduce the 'allergic march of asthma', and influence seasonal asthma; see earlier for the three study objectives.

In the German study, 9,001 AIT patients were matched to 45,005 control patients. The age distribution for both AIT patients and controls was 5–17 years (19.9%), 18–35 years (21.6%), 35–50 years (34.2%), and >50 years (24.3%). The number of seasonal cycles in the treatment period were two (45.1%), three (40.2%), four (13.3%), and five (1.5%). The follow-up duration of the study was an average of 4.4 years, with a minimum of 2 years and a maximum of 6.6 years.

Results for the first objective (AR medication progression) showed significantly more AIT patients (65.4%) than non-AIT patients (47.4%) were AR medication-free (overall response [OR]: 0.51; $p<0.001$). Furthermore, the proportion of AIT patients not using any AR medication was significantly higher than the control patients for all six different interventional groups.

Additionally, the proportion of patients not using AR symptomatic medication during follow-up was 65.4% for all AIT patients versus 47.4% for controls (OR: 0.51; $p<0.001$), and the significance was maintained in all AIT treatment groups. After covariate adjustment, the additional reduction in AR medication prescription during

follow-up was -28.6% greater for AIT patients than non-AIT controls (p<0.001).

Taking the second objective (initiation of asthma medication), results showed that during treatment AIT users had a significantly reduced risk of initiation of asthma medication than non-AIT users (OR: 0.83; 95% confidence interval: 0.740–0.930; p=0.001). When different AIT intervention groups were analysed, the effect versus control was stronger for some of the AIT therapies, notably allergoid SCIT-1 (p=0.016) and natural SLIT (p=0.013).

Up to 6 years after stopping treatment, none of the products prevented the occurrence of new-onset asthma medication intake in non-asthmatic patients (OR: 1.02; 95% confidence interval: 0.884–1.182; p=0.765). Over the combined treatment and follow-up period, only SLIT showed a significantly reduced risk of initiating asthma medication use versus non-AIT patients.

Taking the third objective (progression of asthma medication in patients with asthma with or without AR at baseline), at up to 6 years of follow-up, 49.1% of patients in the AIT group using asthma therapy at baseline were asthma medication-free, in comparison to 35.1% of non-AIT patients (OR: 0.60; p<0.001). The difference was statistically significant for all AIT groups. Such data demonstrate it is possible to reduce asthma medication among patients with allergic asthma.

In conclusion, both the German and French studies show that AIT changes the natural history of the patients in the real world, with robust and consistent evidence for reducing both AR and asthma medication intakes and reducing the risk of new asthma medication initiation in those who did not previously have it. AIT is a treatment that now needs to be discussed with patients.

Take-Home Messages

Finally, each of the speakers provided take home messages from the seminar:

- Dr Fox said that RW AIT studies change the way clinicians use the data they produce to inform practice and represent the birth of genuine personalised medicine in allergy.
- Prof Demoly stressed the importance of studies including asthma patients.
- Prof Zielen highlighted the finding that SLIT and SCIT are equally effective.
- Prof Wahn said RW studies show AIT modifies disease and interferes with the ‘allergic march’, providing long-term benefits.

References

1. Cziraky M, Pollock M; Applied Clinical Trials. Real-World Evidence Studies. 2015. Available at: www.appliedclinicaltrialsonline.com/real-world-evidence-studies. Last accessed: 21 June 2018.
2. Murphy K et al. A Phase 3 trial assessing the efficacy and safety of grass allergy immunotherapy tablet in subjects with grass pollen-induced allergic rhinitis with or without conjunctivitis, with or without asthma. J Negat Results Biomed. 2013;12:10.
3. Röder E et al. Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care. J Allergy Clin Immunol. 2007;119(4):892-8.
4. Bufe A et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: A double-blind placebo controlled study. Allergy. 2004;59(5):498-504.
5. Makatsori M et al. Dropouts in sublingual allergen immunotherapy trials - A systematic review. Allergy. 2014;69(5):571-80.
6. Kiel MA et al. Real-life compliance and persistence among users of subcutaneous and sublingual allergen immunotherapy. J Allergy Clin Immunol. 2013;132(2):353-60.e2.
7. Savi E et al. Causes of SLIT discontinuation and strategies to improve the adherence: A pragmatic approach. Allergy. 2013;68(9):1193-5.
8. Zielen S et al. Sublingual immunotherapy provides a long-term relief in allergic rhinitis and reduces the risk of asthma: A retrospective, real-world database analysis. Allergy. 2018;73(1):165-77.

Allergoids: Ready for the Future

This symposium took place on 28th May 2018, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Munich, Germany

Chair People: Carsten Schmidt-Weber,[1] Petra Zieglmayer[2,3,4]

Speakers: Jörg Kleine-Tebbe,[5] Marek Jutel,[6] Matthias Kopp[7]

1. Center of Allergy and Environment (ZAUM), Technical University of Munich; Helmholtz Center Munich, Munich, Germany
2. Vienna Challenge Chamber, Vienna, Austria
3. Allergy Center Vienna West, Vienna, Austria
4. Thermo Fisher Diagnostics Austria, Vienna, Austria
5. Allergy & Asthma Center Westend, Outpatient Clinic & Clinical Research Center, Berlin, Germany
6. "ALL-MED" Medical Research Institute, Wroclaw, Poland; Department of Clinical Immunology, Silesian Piasts University of Medicine, Wroclaw, Poland
7. Department of Paediatric Allergy and Pulmonology, University of Lübeck, Airway Research-Center North (ARCN), Lübeck, Germany

Disclosure: Prof Kleine-Tebbe has acted as a consultant for AllergenOnline, Allergy Therapeutics, ALK-Abelló, Circassia, Leti, Merck, CLSI, and Novartis; as a lecturer for Allergopharma, ALK-Abelló, AstraZeneca, Bencard, HAL Allergy, Leti, Novartis, Roxall, Stallergenes Greer, clinical laboratories, and Phadia Thermo Fisher; and has received industry-sponsored grants from Allergopharma, ALK-Abelló, HAL Allergy, Leti, Stallergenes Greer, Phadia Thermo Fisher, and Dr Fooke Laboratories. Prof Jutel has received research grants and other research support from Anergis SA; and has acted as a consultant/advisory board member and/or received speaker fees from Anergis SA, Allergopharma, Stallergenes ALK, GSK, and Novartis. Prof Kopp has received research grants from Allergopharma and Vertex, and has acted as a consultant/advisory board member for ALK-Abelló, Allergopharma, Boehringer-Ingelheim, Chiesi, GSK, Infectopharm, Meda, Novartis, and Vertex. Prof Schmidt-Weber has received grants from DFG, BMBF, Bavarian Research foundation, Allergopharma, LETI Pharma, Novartis, Pfizer, Zeller AG, and PLS Design; has been a consultant for Regeneron, Allergopharma, Leo pharma, and PLS Design; and is a shareholder of PLS Design. Dr Zieglmayer is medical director of Thermo Fisher Scientific, scientific director of Vienna Challenge Chamber, chief scientific officer of Allergy center Vienna West, and lecturer at the MCCA of the Medical University Vienna; has received lecture fees from Alk Abello, Allergopharma, Allergy Therapeutics, Novartis, Stallergenes, and Thermo Fisher Scientific; has received scientific and educational grants from Allergopharma, Allergy Therapeutics, Biomay, Calistoga, GSK, HAL, MSD, Ono, Oxagen, RespiVert, Stallergenes, and VentirX; and is an advisory board member for Bencard, MEDA, Merck, and Stallergenes.

Acknowledgements: Writing assistance was provided by Terri Penfold, Ascend, Manchester, UK.

Support: The symposium and the publication of this article was funded by Allergopharma GmbH & Co. KG, Reinbek, Germany. The views and opinions expressed are those of the authors and not necessarily those of Allergopharma.

Citation: EMJ Allergy Immunol. 2018;3[1]:40-49.

Meeting Summary

Improving therapy for people with allergies is a continuously evolving area and, due to the increasing prevalence of allergic diseases, options such as pharmacotherapy and allergen avoidance are inadequate alone to control these diseases. Worldwide, approximately 400 million people are affected by allergic rhinitis (AR) and 300 million people by asthma.[1] Unlike anti-allergy medications, a unique feature of allergen immunotherapy (AIT) is that it modifies the underlying cause of disease,[2] suggesting that it may be an optimal treatment approach. Guidelines, such as those from the European Medicines Agency (EMA) in 2009,[3] provide the basis for optimising trial design for the development of new AIT preparations. A wide range of treatment modalities, including recombinant allergens, have been developed, and results from several studies, some only published in trial registries, provide clarity and insights into optimising clinical trial design even further.[4-13] Lessons learned from these studies, which are scientifically informative for the community, were explored in this session.

In addition, the latest results were discussed from a dose-finding trial and a Phase III trial of a new allergoid treatment in development for patients with house dust mite (HDM)-induced asthma with or without AR or allergic rhinoconjunctivitis (ARC).[14,15] Since AIT is recommended to be administered for 3 years, successful AIT requires adequate patient adherence over the long-term. The last section of this review focusses on strategies to optimise existing AIT and patient care, with a particular emphasis on reducing the number of injections during dose escalation when performing subcutaneous immunotherapy (SCIT) using pollen allergoids.

Development of Recombinant Allergen Immunotherapy: Lessons Learned

Professor Jörg Kleine-Tebbe

AIT, a well-known treatment option for immunoglobulin (Ig)E-mediated AR, ARC, and allergic asthma, is impeded by a lack of standardised extracts for many allergens and batch-to-batch variation in allergen concentration within available extracts because of the use of natural sources.[16] Therapies that use proteins synthesised with recombinant DNA technology provide the opportunity to administer fully characterised molecules with reliable pharmaceutical quality in consistently identical vaccines.[16-18] This unique approach has sparked much interest because it offers the potential to tailor vaccines to the specific major allergens of each patient, facilitates more precise administration of optimal allergen doses, and it allows the structure of IgE-binding allergen epitopes to be modified.[16]

All AIT products must undergo rigorous assessment before clinical use. The 2009 EMA guidelines for the development of AIT products to treat allergic diseases require early-phase studies to evaluate safety and tolerability, studies designed to establish a dose–response relationship for clinical efficacy, and at least one confirmatory trial using a randomised, double-blind, placebo-controlled design.[3]

In the early 2000s, a clinical trial programme of fully characterised, standardised, high-quality recombinant products for SCIT was initiated. The development programme for a recombinant grass allergen mix (rPhleum, Allergopharma GmbH & Co. KG, Reinbek, Germany) of equimolar concentrations of five different major allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, and Phl p 6) from *Phleum pratense* (Timothy grass) consisted of five studies performed between 2003 and 2009 (Figure 1).[4-9] Four studies performed between 2003 and 2010 (Figure 1) made up the development programme for the hypoallergenic recombinant folding variant (FV) of the major allergen Bet v1 of *Betula verrucosa* (rBet v1-FV).[10-13] The results of these studies, some of which are so far only reported in clinical trial registers, can aid in understanding the nature of recombinant AIT and improving clinical trial design, enabling optimal selection of patients and endpoints. Most of the studies had a baseline period (1 year of observation to confirm a sufficient level of symptoms and evaluate medication use before randomisation) and a 2-year treatment period. This allows calculation of the change in efficacy between baseline and after 1 or 2 years of AIT, as well as the placebo effect. This contrasts with most AIT trials, which do not have a baseline period.

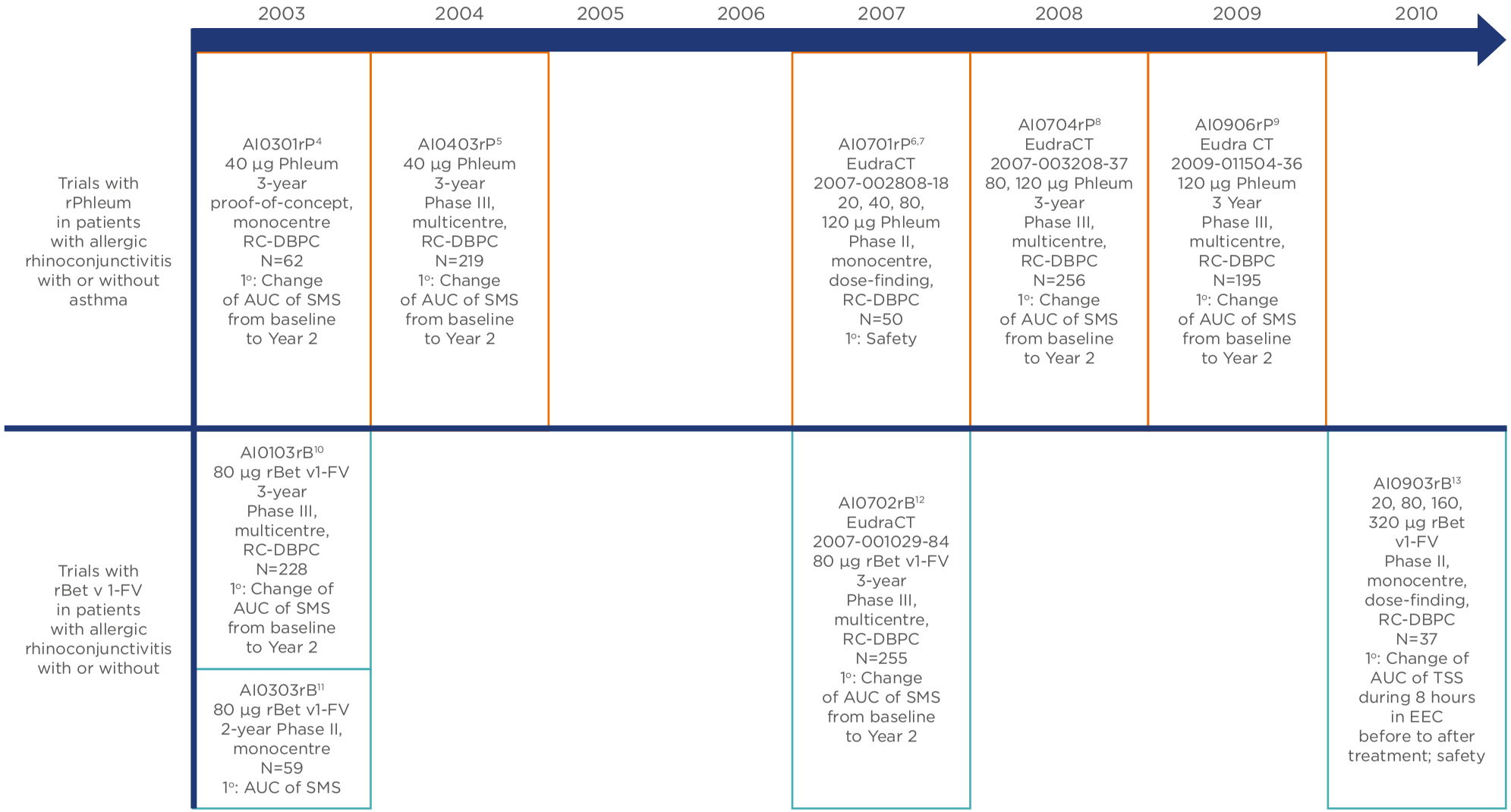


Figure 1: The clinical development programmes, including study design, patient numbers, doses, and primary endpoint, of the recombinant *Phleum pratense* (Timothy grass) pollen allergen mix (rPhleum) and the recombinant folding-variant of the birch (*Betula verrucosa*) pollen major allergen Bet v 1 (rBet v 1-FV) preparations.

1°: primary endpoint; AUC: area under the curve; DBPC: double-blind placebo-controlled; EEC: environmental exposure chamber; RC: randomised controlled; SMS: combined symptom medication score; TSS: total symptom score.

SCIT with rPhleum

The first proof-of-concept study (AI0301rP) for this preparation evaluated a 40 µg dose of rPhleum in 62 patients with grass pollen allergy.[4] Symptom medication score (SMS) was 4.6 for active treatment versus 7.5 for placebo after 2 years (38.5% difference; p=0.051 [full analysis set]). Active treatment led to increases in IgG1 (60-fold) and IgG4 (400-fold) *P. pratense*-specific antibody concentrations, whereas specific IgE concentrations were significantly lower than with placebo. Overall, rPhleum demonstrated clinical efficacy in the per protocol set (p=0.044), was well tolerated, and induced strong allergen-specific IgG responses.

The Phase III AI0403rP trial,[5] using the same dose (40 µg) of rPhleum, found improvement in SMS with both active treatment and placebo after 2 years, but the difference between the groups was not significant (data on file). The safety of increased doses was therefore investigated in the dose-finding study AI0701rP,[6] which reported no systemic reactions with placebo, two with the 20 µg allergen mix, and three each for the 40, 80, and 120 µg allergen mix, indicating the safety of rPhleum even at high doses.[7] The subsequent Phase III randomised trials AI0704rP,[8] investigating 80 and 120 µg rPhleum, and AI0906rP,[9] using 120 µg rPhleum, also found no significant difference between active treatment and placebo in change in SMS after 2 years (p=0.4153 and p=0.1124, respectively). In summary, rPhleum lacked clinically convincing data despite promising results early in the development programme. Being a fixed cocktail, it may not have been as effective in all patients due to individual sensitisation profile heterogeneity.

SCIT with rBet v1-FV

The preliminary AI0103rB trial investigating 80 µg of rBet v1-FV found a significant difference in change of SMS between active treatment and placebo after 2 years (p=0.014).[10] The second trial (AI0303rB)[11] compared the efficacy of 80 µg rBet v1-FV with a registered native birch pollen preparation. After 1 year of SCIT, SMS was lower for rBet v1-FV; however, the difference was not maintained at 2 years, with both treatments achieving reduced SMS scores. Therefore, rBet v1-FV may act more rapidly than SCIT with a native preparation, but ultimately the efficacy is equal. In 2007, the Phase III AI0702rB trial[12] found no significant difference between 80 µg rBet v1-FV and placebo in change of SMS (p=0.1094). Lastly, a Phase II dose-finding study (AI0903rB)[13] investigating doses of up to 320 µg rBet v1-FV found that, in an exposure chamber, total changes in symptom score from before to after SCIT for 10 weeks were significantly decreased and the level of IgG1 significantly increased in all active groups versus placebo. However, a clear dose–response relationship was lacking.[13] The programme concluded that rBet v1-FV was more effective than placebo but not necessarily more effective than approved SCIT products derived from native birch pollen extracts.

Lessons Learned

Overall, recombinant preparations were neither more effective nor safer than already available preparations. However, negative results are not necessarily failures, and the data gathered is scientifically informative for the community, providing the opportunity to test new alternatives and improve trial design.

Firstly, these trials showed that allergic individuals display wide heterogeneity, which can obscure conclusions; raw data from these types of trials are useful to gauge the heterogeneity of the cohorts involved and should be made publicly available. Secondly, an inherent challenge of Phase III trials is to demonstrate specific effects that exceed nonspecific placebo effects. This can be hindered by regression to the mean; extreme observations tend to move towards the mean because subjective expectations influence results, and the natural course of the disease may result in changes in symptom burden during therapy and the Hawthorn effect (under observation, subjects behave differently). Thirdly, current endpoints required by EMA guidelines appear suboptimal; for example, SMS is limited by its subjective nature. To improve accuracy, SMS can be combined with other validated immunological parameters. Lastly, it is difficult to standardise exposure to therapies, the most accurate method for diagnosis remains uncertain, and there is a paucity in knowledge of what happens to recombinant peptides following administration.

These general problems have also occurred with other recombinant candidates, resulting in a halt in development. These include recombinant Bet v 1 sublingual immunotherapy (SLIT),[19] a recombinant Amb a 1-CpG construct (ragweed pollen antigen),[20] and two fairly long Fel d 1 peptides (which caused late-phase side effects).[21] The recombinant grass pollen construct BM32, first tested in an exposure chamber, was well tolerated and achieved significant reductions in total nasal symptom score at 20 µg (p=0.03) and 40 µg (p=0.003).[22] However, a recent Phase II field study found no significant difference between BM32 and placebo.[23] Results from the Phase III trial for this product are anticipated for confirmation of efficacy.

With these new learnings in place, research is now focussing on AIT products that more accurately resemble natural allergens, which are available on the market and have confirmed efficacy and safety. Knowledge gained from this research may enable successful development of recombinant therapy principles in the future.

House Dust Mite SCIT: Focus on Patients with Asthma

Professor Marek Jutel

HDM sensitisation is important in AR, in which 49% of patients are HDM-sensitised, and in allergic asthma, with 50–85% of patients being HDM-sensitised.[24-27]

Current Guidelines for Allergen Immunotherapy in Allergic Asthma

Thus far, there is little guidance available on AIT in allergic asthma. However, an expert working group of the European Academy of Allergy and Clinical Immunology (EAACI) are currently preparing more complete recommendations using the AGREE II international tool.[28] The group recommend SCIT or SLIT for adequately controlled mild-to-moderate disease and AIT should be implemented to reduce symptoms, improve quality of life, and minimise future risk.[28] SCIT has been found to significantly reduce symptoms and medication use but for HDM SLIT there is only weak evidence available for achieving asthma control and moderate evidence for decreasing exacerbations.[28]

Unmet Needs in House Dust Mite Allergen Immunotherapy

Unmet needs in HDM AIT include a lack of adequately powered, randomised, controlled studies and well-characterised allergen preparations. Management of exposure monitoring is a major limitation of HDM studies due to uncertainty around patients collecting samples correctly and how to account for differences between households. Allergen exposure chambers may be more accurate but require validation in Phase III studies. Managing exposure is easier for pollen studies; pollen chambers, which can be very useful in paediatric studies, should be used to gain an understanding of immunotherapy efficacy and the identification of biomarkers that need to be validated. Another large unmet need is well-defined outcome measures in HDM-induced asthma; a potential outcome measure is the reduction of inhaled corticosteroid (ICS) use while retaining asthma control. In a total of 65 HDM-allergic children and adolescents (aged 6–17 years) with controlled bronchial asthma (mild-to-moderate, as classified by the Global Initiative for Asthma [GINA])[29] requiring ICS, after 1 year of HDM SCIT, mean fluticasone propionate dose decreased to 190 µg/day, below the level thought to result in growth suppression (200 µg/day). At baseline, all patients required ICS but after the first, second, and third years, 30.3%, 54.5%, and 60.6% of patients, respectively, no longer required ICS.[30]

Dermatophagoides pteronyssinus Allergoid

For SCIT, an aluminium hydroxide-adsorbed depot allergoid preparation of standardised, high concentration *Dermatophagoides pteronyssinus* allergens modified with formaldehyde and glutaraldehyde has been developed.[31] AL1009ac, a dose-finding study of this HDM allergoid, included adult patients (aged 18–40 years) with HDM-induced asthma with or without AR/ARC and requiring ICS (fluticasone equivalent; maximum daily dose of ≤500 µg).[14] The primary endpoint was late-phase response 6 hours after intracutaneous testing (IC) with *D. pteronyssinus* extract. Secondary endpoints

included early-phase response 20 minutes after intracutaneous testing, minimal asthma control dose of ICS, and safety. Overall, 146 patients were randomised to placebo or to 600, 1,800, 3,000, or 5,400 protein nitrogen units (PNU)/mL of HDM allergoid.

A significantly reduced late-phase IC response was observed with all doses compared with placebo ($p<0.001$). In patients with mild-to-severe asthma, as classified by GINA,[29] a significant reduction in swelling area was reported in all dose groups versus placebo.[14] Compared with placebo, statistical significance in the reduction of early-phase IC response was only achieved with 3,000 PNU ($p<0.01$) and a significant difference in the number of patients who did not need ICS after treatment was only found with 5,400 PNU (6 versus 11 patients; $p<0.05$; unpublished data). From baseline to post treatment in the 5,400 PNU group, no patients increased their ICS dose and 69% of patients had a dose reduction by two steps (unpublished data). The proportions of patients with at least one adverse event (AE) related to study medication were 6.3%, 16.7%, 19.4%, 14.3%, and 35.5% in the placebo and 600, 1,800, 3,000, and 5,400 PNU groups, respectively.[14]

Overall, the most effective doses were 3,000 and 5,400 PNU, with 5,400 PNU being more effective in the reduction of ICS needed to retain asthma control. All tested doses were well tolerated, although more AE were observed in the 5,400 PNU group, but with no greater severity.[14] Therefore, 5,400 PNU was chosen as the dose with the most favourable benefit–risk ratio for further investigation.

AL1402ac[15] is an ongoing Phase III, multicentre, randomised, double-blind, placebo-controlled clinical trial evaluating the efficacy and safety of SCIT with this investigational allergoid in patients with allergic bronchial asthma with or without AR or ARC (Figure 2). Currently, 1,038 patients have been screened, 666 have entered details into their eDiary at baseline, and 426 have been randomised. The primary endpoint is the change between baseline and completion of AIT in dose steps of the minimum daily ICS (budesonide) dose required to ensure asthma control according to an asthma control questionnaire (ACQ6; score ≤1.0). Secondary endpoints include quality of life, combined rhinitis SMS, and the first timepoint at which moderate or severe asthma exacerbation is noted.

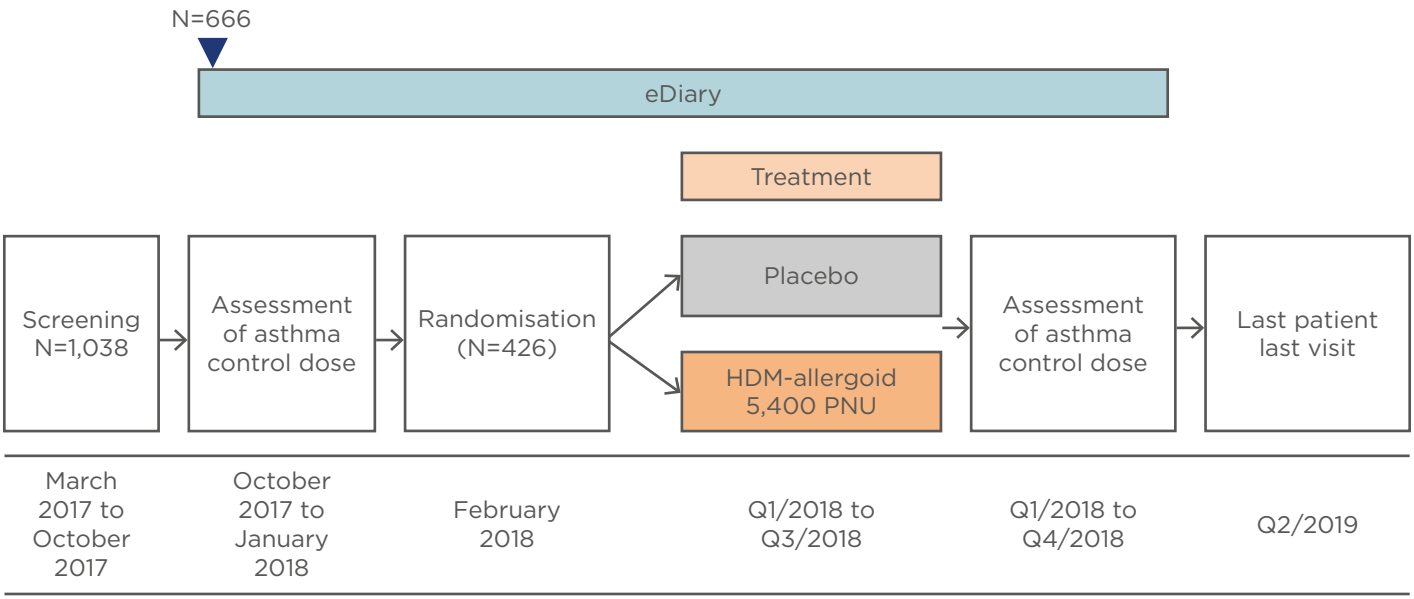


Figure 2: Study design of the AL1402ac Phase III clinical trial evaluating safety and efficacy of subcutaneous immunotherapy with the house dust mite allergoid in patients with house dust mite-allergic asthma and allergic rhinitis or rhinoconjunctivitis.[15]

HDM: house dust mite; PNU: protein nitrogen units/mL; Q: quarter.

Overall, investigational HDM allergoid SCIT is effective and well tolerated, and the dose with the most favourable benefit–risk ratio has been identified. Outcome measures of asthma AIT trials should be harmonised, with ICS reduction or asthma exacerbations being preferable parameters.

Shortcuts in Pollen SCIT: A Step Towards Better Patient Care

Professor Matthias Kopp

When considering the future of AIT and improving patient care, there are numerous potential strategies, including development of new SLIT products, application route optimisation, use of purified allergens, and development for other indications (e.g., food allergy). Two particularly promising avenues are initiation of pollen AIT during the respective pollen season and accelerated dose escalation.

Successful AIT is impeded by a lack of patient adherence.[1,32] AIT is highly effective in AR and allergic asthma and is known to modify the underlying cause of the disease.[2,32] AIT has been shown to have a long-lasting benefit, can prevent the onset of new sensitisations, and can prevent asthma onset in children with AR/ARC.[32] Patient adherence can be improved by good communication with the patient, educational programmes for patients and healthcare providers, and improving AIT convenience.[1,32]

Accelerated dose escalation may reduce the requirement for doctor visits, thus increasing patient adherence.[32] Moreover, accelerated dose escalation is possible without increased number and/or severity of AE, and AIT can be initiated throughout the year, even during the pollen season. Additional data have recently become available for SCIT with birch and grass pollen allergoids.

Birch Pollen Allergoid

The Phase II, open-label ASTOR trial[33] compared the safety and tolerability of accelerated (four injections, n=63) and standard (seven injections, n=67) dose escalation schedules of birch pollen allergoid in adult patients (18–65 years) with seasonal AR with or without controlled asthma. The primary outcome was incidence of systemic AE related to SCIT, graded according to the World Allergy Organization (WAO) system.[34] Overall, 57.1% of accelerated and 58.2% of standard scheme patients experienced at least one AE, with local AE being experienced by 54.0% and 56.7%, respectively. At least one Grade I–II systemic AE was reported in 6.3% of accelerated and 3.0% of standard scheme patients. No Grade III–IV systemic or serious AE were observed. Overall, 85.5% of accelerated and 95.4% of standard group patients rated the tolerability of their treatment as 'good to very good'.[35]

Grass Pollen Allergoid

The Phase III randomised, open-label SuBITo trial[36] compared the safety and tolerability of intraseasonal (n=158) and standard preseasonal (n=73) dose escalation schedules of the six-grasses allergoid. At least one AE was experienced by 68.4% of intraseasonal and 56.1% of preseasonal patients. Incidence of local AE was not statistically different between intraseasonal (64.6%) and preseasonal (54.8%) patients (p=0.1907), with most events being mild (intraseasonal: 55.9% versus preseasonal: 60.0%) or moderate (intraseasonal: 36.3% versus preseasonal: 30.0%). At least one systemic Grade I–II AE was observed in 3.2% of intraseasonal and preseasonal patients. Tolerability was rated as 'good to very good' in 85.0% of intraseasonal and 88.6% of preseasonal patients.[37] These results indicate that starting SCIT during the season is appropriate, with timing being less important than whether or not SCIT is started, since clinical benefit does not occur until the year after initiation and delays to initiation may result in the patient electing not to begin AIT at all.

A previous Phase II trial[38] assessed accelerated dose escalation of a six-grasses pollen allergoid preparation consisting of four weekly injections (200, 600, 2,000, and 6,000 TU/mL). No difference between accelerated and standard escalation regarding the intensity and number of local and systemic AE was found. This raises the question of whether further increases in the rate of escalation would provide additional benefits. It is hypothesised that further escalation from the four weekly injections to three injections (1,000, 3,000, and 6,000 TU/mL)

using only the 10,000 TU/mL vial will allow patients to reach the cumulative dose earlier and minimise risk of confusion between different strength vials.

This is being investigated in the ongoing Phase II ONSeT trial,[39] underway at 13 European sites. The study aims to investigate the safety and tolerability of standard (seven injections) and 'high-speed' (three injections) escalation schedules of the six-grasses allergoid in adult patients (aged 18–65 years) with moderate-to-severe seasonal AR or ARC with or without asthma (Figure 3). Preliminary results were presented during the EAACI 2018 Congress.[40]

Findings and Future Investigation

Accelerated dose escalation of grass and birch pollen allergoids demonstrated comparable safety and tolerability in adult patients with AR with or without asthma. However, patient numbers in individual trials were low. When combining the ASTOR,[33,35] Chaker et al.,[38] and ONSeT[39,40] preliminary data (N=338), there was no significant difference in local AE after accelerated, 'high-speed', or standard dose escalation, but a higher number of Grade I–II systemic AE in the accelerated and 'high-speed' schemes was found.[33,35,38-40] No Grade III–IV systemic AE were observed with any of the escalation schedules.

Conclusion

These clinical development programmes emphasise that a significant challenge of Phase III trials is demonstrating specific effects beyond the universal placebo effect. Also, dose-finding Phase II trials are challenging since clear dose responses to all investigated endpoints are often not demonstrated. However, even negative results (i.e., no statistically significant superiority for active treatment versus placebo) are scientifically informative, allowing improvements in future trials. Investigational HDM allergoid SCIT has shown efficacy and tolerability in adult patients with HDM-induced asthma with or without AR/ARC in a dose-finding trial, and the 5,400 PNU dose has been selected for further investigation.

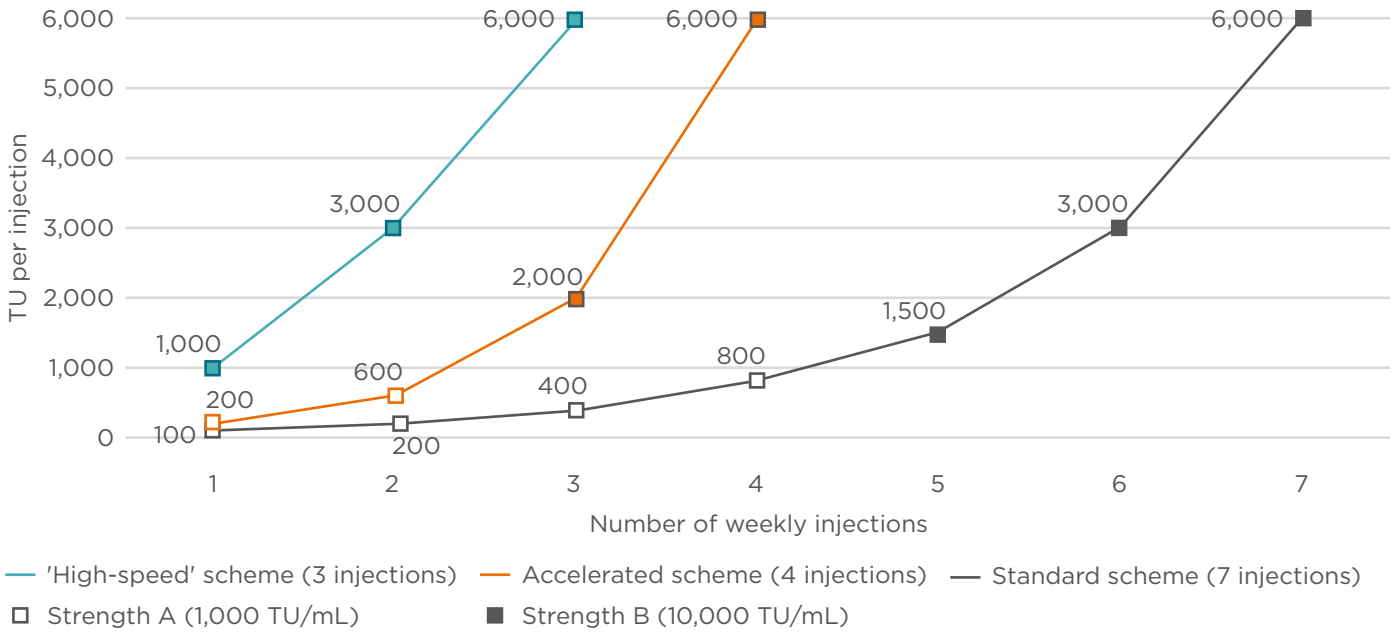


Figure 3: Different dose escalation schemes tested in clinical trials using pollen allergoids with the standard scheme (grey), consisting of seven injections of strength A (1,000 TU/mL) plus B (10,000 TU/mL);[33,35,38-40] the accelerated scheme (orange), consisting of four injections of strength A plus B;[33,35,38] or 'high-speed' scheme (blue), consisting of three injections of strength B.[39,40]

TU: therapeutic units.

Large studies, including the ongoing Phase III AL1402ac trial,[15] should greatly improve our understanding of AIT in asthma. Accelerated dose escalation with grass and birch pollen allergoids has displayed comparable safety and tolerability to standard escalation schedules in adult patients with AR with or without asthma, resulting in reductions from 6 to 2 weeks of escalation. Initiation of SCIT with a standard regimen of the grass pollen allergoid is well tolerated in adults during the grass pollen season. Accelerated dose escalation is expected to attract more patients to SCIT and to increase adherence.

References

1. Pawankar et al.; World Allergy Organization. The WAO White Book on Allergy. Update 2013. 2013. Available at: http://www.worldallergy.org/UserFiles/file/WhiteBook2-2013-v8.pdf. Last accessed: 26 June 2018.
2. Larché M et al. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol. 2006;6(10):761-71.
3. European Medicines Agency. Clinical development of products for specific immunotherapy for the treatment of allergic diseases. 2009. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001212.jsp&mid=. Last accessed: 26 June 2018.
4. Jutel M et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol. 2005;116(3):608-13.
5. Allergopharma GmbH & Co. KG. Safety and efficacy of recombinant grass pollen allergen cocktail in the treatment of allergic rhinoconjunctivitis. NCT00309036. https://clinicaltrials.gov/ct2/show/NCT00309036.
6. Allergopharma GmbH & Co. KG. Dose-response-study with a recombinant cocktail of Phleum (rPhleum) allergens. NCT00666341. https://clinicaltrials.gov/ct2/show/NCT00666341.
7. Klimek L et al. Specific subcutaneous immunotherapy with recombinant grass pollen allergens: First randomized dose-ranging safety study. Clin Exp Allergy. 2012;42(6):936-45.
8. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. Clinical Study Report: A multicentre, multinational randomised placebo-controlled double-blind pivotal clinical trial for evaluation of safety and efficacy of specific immunotherapy with a cocktail of recombinant major allergens of Timothy grass pollen (Phleum pratense) adsorbed onto aluminium-hydroxide in patients with IgE-mediated allergic rhinoconjunctivitis with/without controlled asthma (AMETHYST). 2013. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-003208-37/results. Last accessed: 26 June 2018.
9. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. Randomised double blind placebo controlled pivotal study to evaluate efficacy and safety of rPhleum in adult and adolescent patients suffering from rhinoconjunctivitis +/- controlled asthma (EudraCT 2009-011504-36). 2017. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2009-011504-36/results. Last accessed: 26 June 2018.
10. Jost K et al. Specific immunotherapy with a hypoallergenic recombinant birch pollen allergen rBet v 1-FV is clinically efficacious: Results of a Phase III study. Allergo J. 2007;16(Suppl 1):S90.
11. Klimek L et al. Allergy immunotherapy with a hypoallergenic recombinant birch pollen allergen rBet v 1-FV in a randomized controlled trial. Clin Transl Allergy. 2015;5:28.
12. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. Clinical study report: A multicentre randomised placebo-controlled double-blind pivotal clinical trial for the evaluation of safety and efficacy of specific immunotherapy with an aluminium hydroxide-adsorbed recombinant hypoallergenic derivative of the major birch pollen allergen, rBet v1-FV. 2013. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-001029-84/results. Last accessed: 26 June 2018.
13. Meyer W et al. Double-blind, placebo-controlled, dose-ranging study of a new recombinant hypoallergenic Bet v 1 in an environmental exposure chamber. Allergy. 2013;68(6):724-31.
14. Rudert M et al. A multicentre randomised placebo-controlled double-blind clinical trial for evaluation of the dose-dependent effect of a hypoallergenic house dust mite preparation (Dermatophagoides pteronyssinus) for subcutaneous immunotherapy (SCIT). Abstract 1017. EAACI Congress, 11-15 June, 2016.
15. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. A multicenter randomized double-blind placebo-controlled clinical trial for evaluation of efficacy and safety of specific immunotherapy with an aluminium hydroxide-adsorbed allergoid preparation of house dust mite (Dermatophagoides pteronyssinus) in patients with allergic bronchial asthma and with allergic rhinitis or rhinoconjunctivitis (EudraCT 2015-000188-15). 2016. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-000188-15/LT. Last accessed: 26 June 2018.
16. Mohapatra SS et al. Immunotherapy for allergies and asthma: Present and future. Curr Opin Pharmacol. 2010;10(3):276-88.
17. Pauli G, Malling HJ. Allergen-specific immunotherapy with recombinant allergens. Curr Top Microbiol Immunol. 2011;352:43-54.
18. Ebner C et al. Immunological changes during specific immunotherapy of grass pollen allergy: Reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. Clin Exp Allergy. 1997;27(9):1007-15.
19. Nony E et al. Development and evaluation of a sublingual tablet based on recombinant Bet v 1 in birch pollen-allergic patients. Allergy. 2015;70(7):795-804.
20. Creticos PS et al.; Immune Tolerance Network Group. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med. 2006;355(14):1445-55.
21. Norman PS et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med. 1996;154(6 Pt 1):1623-8.
22. Zieglmayer P et al. Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy. EBioMedicine. 2016;11:43-57.
23. Niederberger V et al. Safety and efficacy of immunotherapy with the recombinant B-cell epitope-based grass pollen vaccine BM32. J Allergy Clin Immunol. 2018. [Epub ahead of print].

24. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J. 2004;24(5):758-64.

25. Nelson RP et al. Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in nonasthmatic control subjects. J Allergy Clin Immunol. 1996;98(2): 258-63.

26. Platts-Mills TAE et al. Dust mite allergens and asthma - A worldwide problem. J Allergy Clin Immunol. 1989;83(2 Pt 1):416-27.

27. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. Trends Immunol. 2011;32(9):402-11.

28. European Academy of Allergy and Clinical Immunology (EAACI). EAACI Guideline on allergen immunotherapy – allergic asthma (version 4). 2017. Available at: http://www.eaaci.org/documents/AIT/Allergic_asthma_AIT_03_06_17.pdf. Last accessed: 26 June 2018.

29. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2006. Available at: http://www.who.int/respiratory/asthma/GINA_WR_2006_copyright[1].pdf. Last accessed: 26 June 2018.

30. Rudert M et al. Effects of the third year of specific immunotherapy with a high-dose hypoallergenic house dust mite preparation in children with allergic asthma. Abstract 1400. EAACI Congress, 16-20 June, 2012.

31. Zielen S et al. Steroid-sparing effects with allergen-specific immunotherapy in children with asthma: A randomized controlled trial. J Allergy Clin Immunol. 2010;126(5):942-9.

32. Roberts G et al. EAACI guidelines on allergen immunotherapy: Allergic rhinoconjunctivitis. Allergy. 2018;73(4):765-98.

33. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. Open label Phase II multicenter clinical trial to evaluate safety during accelerated dose escalation of an allergoid birch pollen preparation in patients with IgE mediated allergic rhinitis or rhinoconjunctivitis with or without controlled bronchial asthma (EudraCT 2015-005400-28). 2016. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-005400-28/DE. Last accessed: 26 June 2018.

34. Cox L et al. Speaking the same language: The World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System. J Allergy Clin Immunol. 2010;125(3):569-74.

35. Zielen S et al. Compatibility of an accelerated dose escalation with a high-dose, hypoallergenic birch pollen allergoid. Abstract 896. EAACI Congress, 26-30 May, 2018.

36. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. A multicenter, randomized, open-label clinical trial to evaluate safety and tolerability of a year-round initiation of specific immunotherapy with an aluminum hydroxide adsorbed allergoid preparation of 6-grasses in patients with moderate to severe seasonal rhinitis or rhinoconjunctivitis with or without controlled asthma. (EudraCT 2015-002409-13). 2015. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-002409-13/DE. Last accessed: 26 June 2018.

37. Mahler V et al. Safety and tolerability of a year-round initiation of specific immunotherapy with a high-dose hypoallergenic grass pollen allergoid. Abstract 611. EAACI Congress, 26-30 May, 2018.

38. Chaker AM et al. An accelerated dose escalation with a grass pollen allergoid is safe and well-tolerated: A randomised open label Phase II trial. Clin Transl Allergy. 2016;6:4.

39. Allergopharma GmbH & Co. KG.; EU Clinical Trials Register. A multicentre, randomized, open label clinical trial for safety evaluation of an accelerated high dose escalation schedule with one strength for an allergen immunotherapy with an aluminium hydroxide adsorbed allergoid preparation of 6-grasses in patients with moderate to severe seasonal rhinitis or rhinoconjunctivitis with or without asthma. (EudraCT 2017-000754-19). 2017. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-000754-19/DE. Last accessed: 26 June 2018.

40. Kopp M et al. Safety evaluation of an accelerated dose escalation schedule with one strength of a 6-grasses pollen allergoid. Abstract 1214. EAACI Congress, 26-30 May, 2018.

Towards Optimised Management of Cow's Milk Protein Allergy

This symposium took place on 28th May 2018, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Munich, Germany

Chairperson: Philippe Eigenmann[1]

Speakers: José Armando Madrazo-de la Garza,[2] Sophie Nutten,[3] Liam O'Mahony[4]

1. Paediatric Allergy Unit, University Hospitals of Geneva, Geneva, Switzerland
2. National Autonomous University of Mexico, Mexico City, Mexico
3. Nestlé Research Center, Lausanne, Switzerland
4. Swiss Institute of Allergy and Asthma Research, University of Zurich, Davos, Switzerland

Disclosure: Prof Madrazo-de la Garza has acted as a consultant for Abbott, Bayer, Biocodex, Danone, Mayoli-Spindler, Mead-Johnson, Nestlé, and Nestlé Health Science; and as a speaker for Abbott, Bayer, Nestlé, and Nestlé Health Science. Dr Nutten is an employee of Nestec Ltd. Prof O'Mahony has acted as a consultant to Alimentary Health Ltd. and has received research funding from GSK. Prof Eigenmann has declared no conflicts of interest.

Acknowledgements: Medical writing assistance was provided by Amanda Pedder, Ascend, Manchester, UK.

Support: The symposium and the publication of this article was funded by Nestlé Health Science. The views and opinions expressed are those of the authors and not necessarily of Nestlé Health Science.

Citation: EMJ Allergy Immunol. 2018;3[1]:50-59.

Meeting Summary

This symposium took place during the 2018 meeting of the European Academy of Allergy and Clinical Immunology (EAACI). Focussing on the fundamental issues of suboptimal management of patients with cow's milk protein allergy (CMPA), the speakers discussed key themes for optimising management. Prof Madrazo-de la Garza evaluated the challenges of diagnosis and management of CMPA in infants. Nonspecific symptoms, indicative of other conditions, mean that CPMA is often misdiagnosed as lactose intolerance, a rare condition in infants. Increased awareness of CMPA symptoms and a clear distinction from lactose intolerance may facilitate earlier, accurate diagnosis and implementation of appropriate dietary interventions. Dr Nutten followed by exploring variability in the composition of commercialised extensively hydrolysed formulas (eHF) intended for the management of CMPA and the associated potential clinical impact. Large variations in peptide profiles and residual allergenicity reflect a lack of definition for eHF composition. Although the clinical trials required to confirm the efficacy of eHF by demonstrating tolerance in >90% of infants with CMPA are performed, composition analyses for characterisation, quality control, and reproducibility are crucial for ensuring safe and suitable products throughout the product lifecycle. Prof O'Mahony concluded the meeting by focussing on the importance of the gut microbiome in food allergy. The establishment of a stable gut microbial community closely tracks host growth and immune development. Delayed or altered establishment leads to microbiome

immaturity, which has been associated with an increased risk of food allergies. Nutritional strategies, such as the use of eHF containing lactose, to support microbiome development complement existing CMPA treatment.

Knowledge Gaps in Diagnosing and Managing Cow's Milk Protein Allergy

Professor José Armando Madrozo-de la Garza

CMPA is the most common food allergy in infants, yet diagnosis is challenging due to nonspecific symptoms. Limited recognition by healthcare professionals worldwide leads to misdiagnosis and unnecessary nutritional interventions. Accurate dietary advice is important for the effective management of CMPA and the optimal development of affected patients.[1]

Self-overestimation of food allergies is common. Worldwide, 28% of people self-identified with an allergy; however, by skin prick test, only 8% had a confirmed food allergy and by double-blind placebo-controlled challenge test this reduced to only approximately 3%.[2,3] The EuroPrevall birth cohort study followed 12,049 newborn babies from nine European countries over a 5-year period. The results showed variation in the proportions of self-reported food allergy, from 5–8% in Spain to 30% in Germany.[4]

Despite global variation in diet, the three most common food allergens worldwide are cow's milk, egg, and seafood.[5] Among adults, the most common adverse reaction to food is lactose intolerance. Approximately half to two-thirds of the world's adults have primary lactose intolerance, but it is very rare in children aged <5 years.[6] Secondary lactose intolerance can be seen in infants, although still rare, and is usually temporary and caused by transient lactase deficiency as a result of small bowel injury.[7] Low-grade lactose malabsorption is a natural physiological phenomenon in breastfed and formula-fed newborns and young infants, enabling the production of softer and more acidic stools, which promotes gut microbiome development and increases production of short chain fatty acids.[6]

Lactose intolerance is due to an enzymatic deficiency, whereas CMPA is regulated by the immune system, either IgE-mediated or non-IgE-mediated.[8] Symptoms common to both lactose intolerance and CMPA include diarrhoea, perianal rash, and inability to gain weight; however, CMPA is also characterised by vomiting, eczema, and occasionally rectal bleeding.[6,7] A recent European survey uncovered major deficits in the management of CMPA, including limited knowledge of appropriate diagnostic tests, use of elimination diets, and optimal selection of speciality formula for management of non-breastfed infants.[9]

A comprehensive survey has recently been conducted to identify misconceptions about CMPA and lactose intolerance among healthcare practitioners across various international clinical settings.[10] The study aimed to understand the clinical practice of primary healthcare providers treating non-breastfed patients with CMPA or lactose intolerance. Access to local resources and tools for the diagnosis and treatment of CMPA and lactose intolerance were evaluated and knowledge gaps identified.

The survey was conducted in a number of countries worldwide between January and November 2017.[10] Participants were asked 12 multiple-choice questions on CMPA and lactose intolerance, questions relating to two clinical case scenarios, and 10 questions on educational needs. Over 50% of the 1,663 respondents had >10 years' clinical practice experience. Most were general practitioners or general paediatricians working in tertiary care or private practice. Over 60% of the respondents felt confident in distinguishing CMPA from lactose intolerance; however, misconceptions were identified, including overestimation of the prevalence of lactose intolerance among infants. Although 59% of respondents identified eHF as the first-line treatment for managing CMPA, there was uncertainty about the use of lactose-free or lactose-containing eHF, and only 23% correctly identified an amino acid-based formula as an appropriate treatment for cases with anaphylaxis.

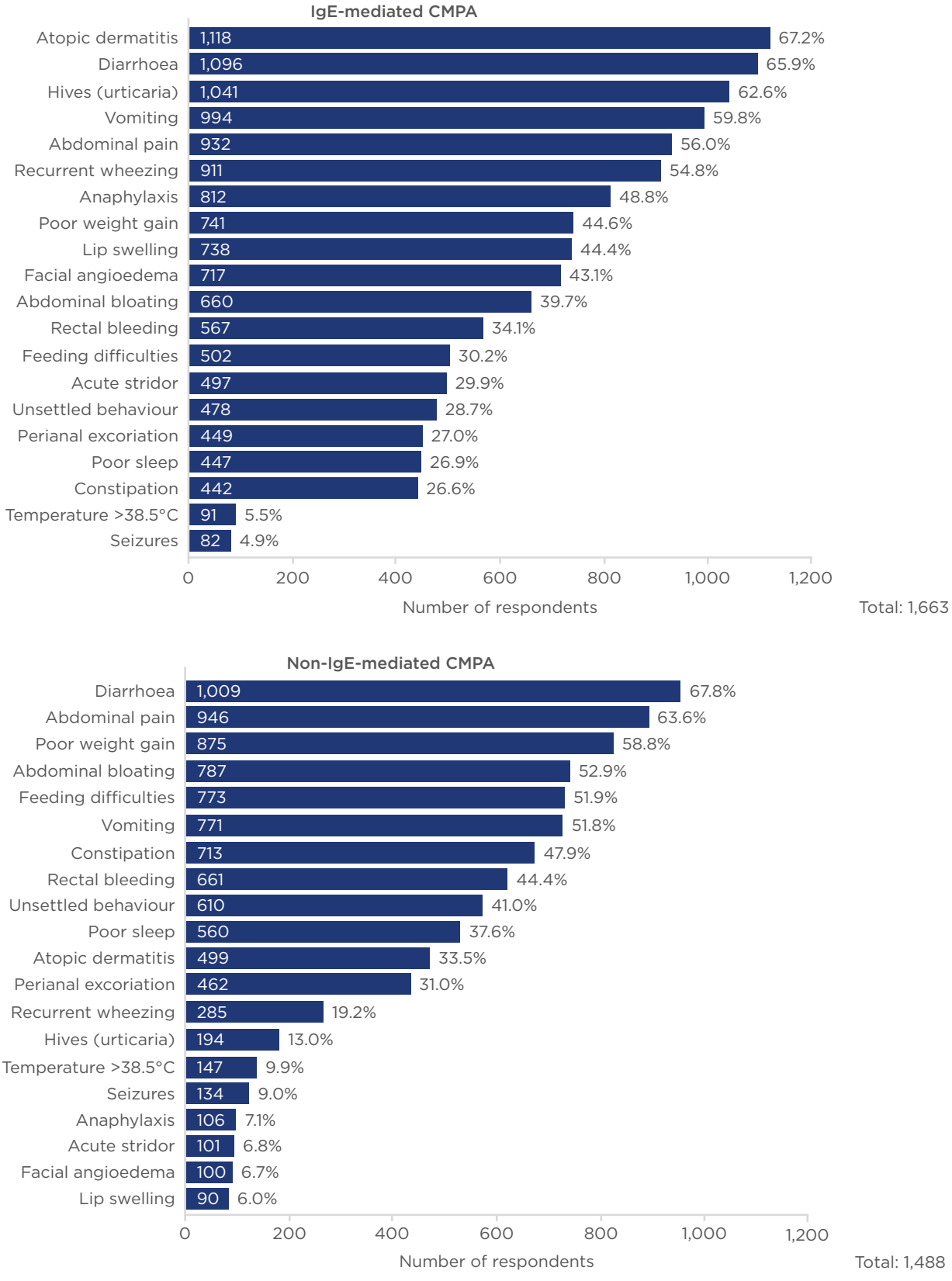


Figure 1: Which clinical signs or symptoms do you consider characteristic of IgE-mediated CMPA and non-IgE-mediated CMPA?

This survey was conducted in >40 countries with 1,663 respondents; multiple answers were permitted from each participant for this question.

CMPA: cow's milk protein allergy.

There were differences in perceived characteristic symptoms of IgE-mediated CMPA, non IgE-mediated CMPA, and lactose intolerance (Figure 1).[10] For IgE-mediated CMPA, the most common symptoms identified were atopic dermatitis, diarrhoea, hives, vomiting, and abdominal pain, although there were some differences in responses by country. For non-IgE-mediated CMPA, the most common symptoms were identified as diarrhoea, abdominal pain, poor weight gain, abdominal bloating, feeding difficulties, and vomiting. Most responses considered only symptoms related to gastrointestinal manifestations rather than wider immune responses, with only a small proportion of respondents considering atopic dermatitis or urticaria as potential symptoms. Although some differences were noted among the countries in identification of primary symptoms, diarrhoea was widely recognised as the leading or second-leading sign or symptom. For lactose intolerance, respondents in all countries identified diarrhoea as the main symptom. Symptoms associated with immune response, such as urticaria, atopic dermatitis, and anaphylaxis, were only mentioned by a small proportion of respondents.

Survey participants were also asked to review two clinical cases and suggest the appropriate formula for management. Clinical Case A was a full-term, vaginal-delivered male infant aged 4 months, exclusively breastfed to 2 months of age, presenting with persistent diarrhoea and eczema. Cow's milk-based formula was introduced at 2 months, followed by development of mild-to-moderate eczema across his body and face, increased regurgitation, loose bowel movements (up to six times a day without visible blood), mild perianal excoriation, and weight loss (from the 25th to the 10th percentile). A total of 40% of survey participants selected initiation of eHF without lactose as the appropriate course of treatment, which was considered the correct choice, as secondary lactose intolerance was indicated by diarrhoea and perianal excoriation.

Clinical Case B was a full-term male infant delivered by caesarean section, aged 5 months and exclusively breastfed, without introduction of solid foods, presenting with generalised urticaria after drinking approximately 60 mL of cow's milk-based formula. He also vomited once, became lethargic and floppy, and was taken to hospital for treatment and observation, where formula was avoided. He had moderate atopic eczema that started from 2 months of age. Most respondents selected an eHF for treatment in this case: 27% selected eHF without lactose and 27% selected eHF with lactose. Amino acid-based formula, the most appropriate option considering the anaphylactic reaction, was selected only by 24% of respondents but was the top choice for physicians in some countries.

The survey also contained questions about diagnostic procedures for identifying IgE-mediated and non-IgE-mediated CMPA, and lactose intolerance. Overall, identification of serum IgE specific to cow's milk and skin prick testing were the most common tools used to diagnose IgE-mediated CMPA. For non-IgE-mediated CMPA, many participants recommended either no diagnostic test or a home challenge to cow's milk.

When asked to consider awareness and education, >60% of respondents felt confident about their skills in diagnosing and managing CMPA, but 82% were interested in receiving further training. Almost half of respondents considered primary lactose intolerance to be common in infancy, despite it being rare in children <5 years old.

Overall, the survey results indicate that clinical recognition and management of CMPA versus lactose intolerance in infancy still poses clinical dilemmas. Significant educational gaps about the diagnosis and treatment of CMPA and lactose intolerance have been identified in several regions globally. There is much room for improvement and a need for targeted education and training to promote evidence-based clinical practice, change perceptions, and prompt physicians to suspect and test for CMPA in infants.

How to Define Extensively Hydrolysed Formula for the Management of Cow's Milk Protein Allergy

Doctor Sophie Nutten

CMPA is the most common food allergy in infants, affecting 2–3% children worldwide.[7] Dr Nutten reported that most children with CMPA have ≥2 symptoms: 50–70% have skin symptoms, 50–60% have gastrointestinal symptoms, and 20-30% have airway symptoms.[11,12] Severe and life-threatening symptoms may occur in 10% of children.[2] Management of these patients focusses on the avoidance of milk proteins and prompt recognition and treatment of allergic reactions resulting from accidental exposure.

Breast milk remains the gold standard for feeding infants with CMPA, with speciality formulas recommended when breastfeeding is not possible. CMPA management guidelines recommend the use of eHF as the first nutritional intervention unless there is an anaphylactic reaction to the original cow's milk product, in cases of eosinophilic oesophagitis, or if the eHF is not tolerated.[2,13-16] eHF contains cow's milk peptides. The key properties of eHF are good safety and tolerability for most babies with CMPA and nutritional completeness to support growth and development. Despite these common goals, eHF have different compositions, with variation observed in carbohydrate sources (lactose versus no lactose), lipid profiles, protein sources, and also in the hydrolysis process. The hydrolysation process involves breaking down larger milk proteins to form small peptides, based on the rationale that peptide size is related to allergenicity. Theoretically, to bind with cell membrane-bound IgE, peptides should be approximately >1,500 Da in size (approximately 15 amino acids), and to crosslink IgE molecules and induce an immune response, they must be >3,000 Da in size (approximately 30 amino acids).[17]

CMPA management guidelines include definitions of eHF composition;[2,13-16] however, there is a lack of consistency between guidelines when it comes to the definition of peptide size. There are some proposed specifications for peptide size, but thresholds are differently defined, with some guidelines stipulating a maximum size of 3,000 Da and others stipulating the largest proportion of peptides should be <1,000 Da.[7,18] Permitted proportions of differently sized peptides (i.e., how much of the protein content must be below the threshold size) are also not consistently defined. This variation is reflected in the heterogeneity of composition of different eHF, which is thought to be the basis of observed tolerability differences.[19]

Providing information to physicians on the degree of hydrolysis and residual allergenicity in eHF would improve selection of optimal formulas.To contribute to this objective, Nestlé Health Science, Vevey, Switzerland, profiled samples of eHF to assess variations in eHF composition around the world (manuscript in preparation).[20] Results presented during this symposium included samples available for sale collected from 10 countries (UK, the Netherlands, Sweden, Finland, Russia, China, Czech Republic, France, Spain, and Mexico), including a variety of manufacturers as well as different batches of the same formula. All formulas tested were eHF intended for the management of CMPA.

A battery of analytical tests were conducted to evaluate the composition of the different formulas. A high degree of variation was observed both in β-lactoglobulin content and in residual casein content (67 samples tested; Figure 2). Importantly, batch-to-batch variation was observed, both across and within countries. Significant variability in peptide molecular weight distribution was also observed (Figure 3), with the proportion of peptides >1,200 Da (critical size for IgE binding) ranging from 1.0–36.0% (66 samples).[20] A large variation (<0.2–7.0%) was observed in the proportion of peptides >3,000 Da (critical size for IgE crosslinking, leading to symptoms). The European Commission Directive 2006/141/EC limited the content of immunoreactive proteins to <1% of total protein in hydrolysates.[21] If we consider peptides >3,000 Da as immunoreactive, in this analysis, the proportion of peptides >3,000 Da was >1% in 3 of the 10 samples analysed. Finally, residual *in vitro* β-lactoglobulin allergenicity[22,23] was found in 60% of the 10 eHF samples analysed, including

7 of the 8 whey-based eHF. Overall, the analyses showed wide variation in the composition of the peptide fraction of commercially available eHF, even between batches of the same brand, indicating that reproducibility is not well controlled during manufacturing.

All guidelines recommend that clinical trials should be conducted to determine suitability and safety of eHF, with a general rule that eHF must be tolerated by >90% of infants.[2,13-16] However, clinical trials generally only provide a snapshot: a single test based on a single production batch. As wide variation has been demonstrated between different batches of the same formula, reproducibility in day-to-day manufacturing is key to ensure product safety and quality, supported by diligent processes.

Figure 2: Residual β-lactoglobulin content (A) and residual casein content (B) in commercially available extensively hydrolysed formula samples.

LoQ: limit of quantitation.

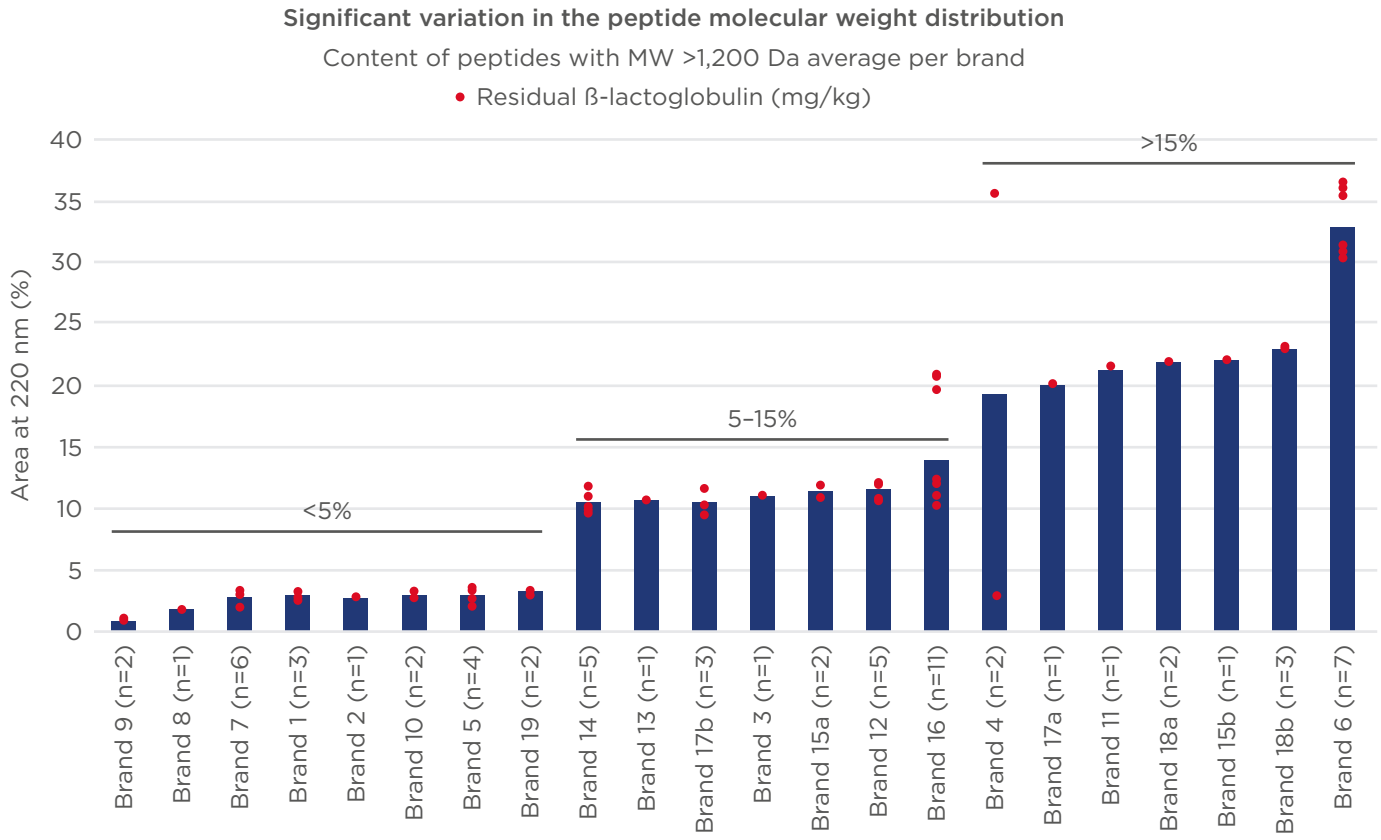


Figure 3: Variation in molecular weight distribution and residual β-lactoglobulin content in different samples of extensively hydrolysed formula.

MW: molecular weight.

Currently, no aligned and actionable definitions are in place to characterise and monitor the quality of eHF, which is reflected in the wide variation observed in the degree of hydrolysis of commercial eHF. More rigorous, globally applied standard definitions of eHF that can be universally applied by manufacturers are necessary. Analytical methods are available to characterise eHF, yet they are not standardised nor proven predictive of a potential allergic reaction. As shown in this study, there is good correlation between peptide molecular weight and allergenicity, which suggests that a high degree of hydrolysis may be preferable. Clinical trials remain important, as a successful, well-designed trial can establish the suitability of formula composition, but a strict quality control of the entire production process is crucial to guarantee consistent composition of the final product and eliminate contamination risks.

The Gut Microbiome and its Role in Early Immune Development and Allergies

Professor Liam O'Mahony

Recent investigations have explored the impact of the gut microbiome on the immune system, including its role in sensitisation to allergens and associated short and long-term effects.[24] Every cell in the mucosal immune system communicates with those around it and responds to the presence of bacteria and secretion of bacterial metabolites.[25] Induction of T regulatory cells by gut microbiota is important for oral tolerance to allergens, as well as wider general tolerance.[26] The beneficial effects of gut microbiota are apparent from the earliest days of life but vary depending on the strain of bacteria. For example, certain Bifidobacteria can induce a T regulatory cell response to

allergens in germ-free mice, reducing the IgE response to a later challenge by allergens.[27] The severity of the allergic response is associated with the types of bacteria present in the gut, with some types associated with a heightened response to allergens.[28]

Children with CMPA have altered microbiome composition and metabolism compared with children who do not have CMPA.[29] In addition, the gut microbiome in children whose CMPA spontaneously resolves is different compared with those in whom allergy persists.[30] Surgery and exposure to antibiotics early in life increase the risk of developing a food allergy, but early administration of probiotics in these patients can reduce the risk of CMPA.[31] Therefore, it may be possible to target probiotics to children at greater risk of gut dysbiosis, and further research in this area is warranted.

Humans are not born with a complete gut microbiome; rather, this is acquired gradually, with development of a mature microbiome requiring 2–3 years.[32] Type of birth, diet, and antibiotic exposure affect microbiome development during this time.[33-35] In particular, babies born vaginally have more complex and varied microbiomes than those delivered by caesarean section, with strain-matching showing direct acquisition of some bacterial strains from the mother.[33] Gut dysbiosis due to type of birth can persist into childhood and may have longer-term consequences,[33] and Prof O'Mahony suggested that inherited strains of gut bacteria may be progressively lost in multiple generations of babies born by caesarean section.

Microbiome development is also heavily influenced by nutrition, including the timing of introduction of yoghurt, fruit, and vegetables into the diet. Balanced nutrition in early life can reduce the risks of developing a food allergy.[36] Short chain fatty acids are immunoregulatory and bind to G-protein-coupled receptors, promoting T regulatory cell and T helper cell responses. These molecules are microbial metabolites from dietary fibre, which is fermented by the gut microbiota, and are also present in some foods such as butter and yoghurt, which contain the short chain fatty acid butyrate. (Roduit and Frei et al. Manuscript submitted.) Children with higher butyrate levels at 1 year of age have fewer allergies by the age of 6 years than those with lower levels. Lactose, which is present in breast milk, is also an important early life prebiotic. It has been shown that children with CMPA consuming an eHF with lactose have significantly higher counts of Bifidobacteria and lactic acid bacteria as well as significantly higher levels of short chain fatty acids than children consuming an eHF not containing lactose.[37] A lack of lactose in specialised formula for children with CMPA could have a detrimental effect on microbiome development and associated response to food allergens.[37] Obesity also affects the microbiome and a higher BMI is associated with greater sensitisation to IgE and food allergies, although the effects are not thought to be direct.[38]

Exposure to antibiotics can significantly delay maturation of the gut microbiome.[33,34] The type of antibiotic, length of exposure, and administration route all influence microbiome development. Although there are conflicting data about the use of antibiotics and the development of food allergies, it is feasible to suggest that antibiotics may modify the microbiome, leading to increased response to, for example, cow's milk protein.[39]

Several opportunities are available for clinical practice interventions to improve food tolerance and reduce allergies via actions on the gut microbiome.[40] Reducing the number of elective caesarean sections would mitigate microbial dysbiosis and potentially reduce the risk of allergic immune responses and inflammation. Reduction in indiscriminate use of antibiotics during infancy and the perinatal period may prevent delayed maturation of the gut microbiome. Minimising inappropriate use of lactose-free formula for infants could have beneficial effects on the microbiome, possibly improving tolerance to food allergens. Finally, an increase in dietary intake of fermentable fibre to increase short chain fatty acids produced by microbial fermentation might reduce allergic responses.

Conclusion

To conclude, the management of patients with CMPA is a challenge but can be optimised.

Three fundamental themes explored during this symposium could be instrumental in improving the management of infants with CMPA. First, targeted education and training is essential to improve early recognition of symptoms and to minimise misdiagnosis, such as lactose intolerance. Second, manufacturers of infant formula should consider focussing on quality control and reproducibility of formula production processes to guarantee consistent composition. Heterogeneity of available eHF is a challenge and the development of standardised global guidelines and definitions for eHF composition is necessary to ensure that physicians can select appropriate formulas for their patients. Finally, the role of the gut microbiome in sensitivity and tolerance to food allergens and the prebiotic role of lactose should be considered. Inappropriate use of lactose-free eHF for infants who are not lactose intolerant could have a detrimental effect on microbiome establishment, particularly in children already at risk of delayed microbiome maturation.

References

1. Heine RG et al. Lactose intolerance and gastrointestinal cow's milk allergy in infants and children – Common misconceptions revisited. World Allergy Organ J. 2017;10(1):41.
2. Fiocchi A et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. World Allergy Organ J. 2010;3(4): 57-161.
3. Bock SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. Pediatrics. 1987;79(5):683-8.
4. McBride D et al. The EuroPrevall birth cohort study on food allergy: Baseline characteristics of 12,000 newborns and their families from nine European countries. Pediatr Allergy Immunol. 2012;23(3):230-9.
5. Rona RJ et al. The prevalence of food allergy: A meta-analysis. J Allergy Clin Immunol. 2007;120(3):638-46.
6. Szilagyi A. Redefining lactose as a conditional prebiotic. Can J Gastroenterol. 2004;18(3):163-7.
7. Luyt D et al. BSACI guideline for the diagnosis and management of cow's milk allergy. Clin Exp Allergy. 2014;44(5):642-72.
8. Johansson SG et al.; EAACI nomenclature task force. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy. 2001;56(9):813-24.
9. Werkstetter K et al. Diagnosis and management of cow's milk protein allergy - How big is the gap between ideal and reality? A quality-of-care survey in Europe. J Pediatr Gastroenterol Nutr. 2018;66(Suppl 2): 399-400.
10. Madrazo J et al. International survey identifies educational needs around the management of infants with cow's milk allergy and lactose intolerance. Thematic Poster Session 09. EAACI Congress, 26-30 May, 2018.
11. Lifschitz C, Szajewska H. Cow's milk allergy: Evidence-based diagnosis and management for the practitioner. Eur J Pediatr. 2015;174(2):141-50.
12. Høst A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. Allergy. 1990;45(8):587-96.
13. Vandenplas Y. Prevention and management of cow's milk allergy in non-exclusively breastfed infants. Nutrients. 2017;9(7).
14. Kemp AS et al.; Australian consensus panel. Guidelines for the use of infant formulas to treat cows milk protein allergy: An Australian consensus panel opinion. Med J Aust. 2008;188(2):109-12.
15. Koletzko S et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr. 2012;55(2):221-9.
16. Muraro A et al. EAACI Food allergy and anaphylaxis guidelines: Diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
17. Huby RD et al. Why are some proteins allergens? Toxicol Sci. 2000;55(2):235-46.
18. Greer FR et al. Effects of early nutritional interventions on the development of atopic disease in infants and children: The role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics. 2008;121(1):183-91.
19. Lambers TT et al. Clustering analyses in peptidomics revealed that peptide profiles of infant formulae are descriptive. Food Sci Nutr. 2015;3(1):81-90.
20. Nutten S et al. Extensively hydrolyzed formulas for the management of cow's milk protein allergy in infants: Is extensive hydrolysis sufficient to guarantee success? Thematic Poster Session 09. EAACI Congress, 26-30 May, 2018.
21. Dupont C et al.; French Society of Paediatrics. Dietary treatment of cows' milk protein allergy in childhood: A commentary by the Committee on Nutrition of the French Society of Paediatrics. Br J Nutr. 2012;107(3):325-38.
22. Knipping K et al. Development of β-lactoglobulin-specific chimeric human IgEκ monoclonal antibodies for in vitro safety assessment of whey hydrolysates. PLoS One. 2014;9(8):e106025.
23. Knipping K et al. Intra- and inter-laboratory validation of an innovative huFcεRIα-RBL-2H3 degranulation assay for in vitro allergenicity assessment of whey hydrolysates. Toxicol In Vitro. 2016;33:29-34.
24. Sampson HA et al. Mechanisms of food allergy. J Allergy Clin Immunol. 2018;141(1):11-9.
25. Mortha A et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science. 2014;343(6178):1249288.
26. Wawrzyniak M et al. Role of regulatory cells in oral tolerance. Allergy Asthma Immunol Res. 2017;9(2):107-15.
27. Morin S et al. Delayed bacterial colonization of the gut alters the host immune response to oral sensitization against cow's milk proteins. Mol Nutr Food Res. 2012;56(12):1838-47.
28. Noval Rivas M et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. J Allergy Clin Immunol. 2013;131(1):201-12.

29. Thompson-Chagoyan OC et al. Faecal microbiota and short-chain fatty acid levels in faeces from infants with cow's milk protein allergy. Int Arch Allergy Immunol. 2011;156(3):325-32.
30. Bunyavanich S et al. Early-life gut microbiome composition and milk allergy resolution. J Allergy Clin Immunol. 2016;138(4):1122-30.
31. Ezaki S et al. Prophylactic probiotics reduce cow's milk protein intolerance in neonates after small intestine surgery and antibiotic treatment presenting symptoms that mimics postoperative infection. Allergol Int. 2012;61(1):107-13.
32. Yatsunenko T et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222-7.
33. Bokulich NA et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 2016;8(343):343ra82.
34. Laursen M et al. Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. mSphere. 2015;1(1): e00069-15.
35. Azad MB et al.; CHILD Study Investigators. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. BJOG. 2016;123(6):983-93.
36. Roduit C et al.; Protection Against Allergy–Study in Rural Environments study group. Development of atopic dermatitis according to age of onset and association with early-life exposures. J Allergy Clin Immunol. 2012;130(1):130-6.e5.
37. Francavilla R et al. Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. Pediatr Allergy Immunol. 2012;23(5):420-7.
38. Visness CM et al. Association of obesity with IgE levels and allergy symptoms in children and adolescents: Results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2009;123(5):1163-9.
39. Hirsch AG et al. Early-life antibiotic use and subsequent diagnosis of food allergy and allergic diseases. Clin Exp Allergy. 2017;47(2):236-44.
40. Huang YJ et al. The microbiome in allergic disease: Current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. J Allergy Clin Immunol. 2017;139(4):1099-110.

Raising the Clinical Bar Beyond Current Biologics in Uncontrolled Persistent Asthma: Translating Emerging Data in Future Clinical Decisions

This symposium took place on 28th May 2018, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Munich, Germany

Chairperson: Klaus F. Rabe[1]

Speakers: Klaus F. Rabe,[1] William Busse,[2] Ian Pavord,[3] Mario Castro[4]

1. Lung Clinic, Grosshansdorf University of Kiel, Kiel, Germany
2. School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA
3. University of Oxford, Oxford, UK
4. School of Medicine, Washington University, St. Louis, Missouri, USA

Disclosure: Prof Rabe has received speaker's honoraria from AstraZeneca, Berlin Chemie, Boehringer Ingelheim, Chiesi Pharmaceuticals, GlaxoSmithKline, Intermune, Novartis, Sanofi Genzyme, Teva, and Takeda; and has been the recipient of a research grant from the Ministry of Education and Science, Germany. Prof Busse is an employee of the University of Wisconsin, Madison, Wisconsin, USA; has received consultancy fees from 3M, AstraZeneca, Genentech, GlaxoSmithKline, Novartis, and Sanofi Genzyme; has been a member of the data monitoring boards and study oversight committees for Boston Scientific, Genentech, and ICON; has received research grants from the National Heart, Lung, and Blood Institute (NHLBI) and National Institute of Allergy and Infectious Diseases (NIAID); and has been a member of the American Academy of Allergy, Asthma, and Immunology (AAAAI), American Association of Immunologists (AAI), American Academy of Pediatrics (AAP), American Thoracic Society (ATS), and Clinical Immunology Society (CIS). Prof Pavord has received speaker's honoraria from Aerocrine, AstraZeneca, Boehringer Ingelheim, Chiesi Pharmaceuticals, GlaxoSmithKline, Novartis, Sanofi Genzyme, and Teva; has been an advisory board participant for Almirall, AstraZeneca, Boehringer Ingelheim, Dey, GlaxoSmithKline, MSD, Napp, Novartis, Regeneron, and Schering-Plough; and has received sponsorship from AstraZeneca, Boehringer Ingelheim, Chiesi Pharmaceuticals, GlaxoSmithKline, and Napp. Prof Castro has been a speaker for AstraZeneca, Boehringer Ingelheim, Boston Scientific, Genentech, and Teva; has been a contracted researcher for AstraZeneca, Boehringer Ingelheim, Chiesi, Mallinckrodt, Sanofi-Aventis, and Vectura; has been a consultant for Aviragen, Genentech, Nuvaira, Sanofi Genzyme, Teva, and Vectura; has received author royalties from Elsevier; and has received research grants from American Laryngological Association (ALA), National Institutes of Health (NIH), and Patient-Centered Outcomes Research Institute (PCORI).

Acknowledgements: Writing assistance was provided by Sarah Stutfield, Ascend, Manchester, UK.

Support: The symposium and the publication of this article was funded by Sanofi-Genzyme-Regeneron. The views and opinions expressed are those of the authors and not necessarily those of Sanofi-Genzyme-Regeneron.

Citation: EMJ Allergy Immunol. 2018;3[1]:60-69.

Meeting Summary

Asthma is a heterogeneous disease with multiple phenotypes, caused by a complex interplay of inflammatory pathways. Up to 70% of patients with asthma have Type 2 inflammation, characterised by the presence of interleukin (IL)-4, IL-5, and IL-13. Uncontrolled persistent asthma represents a considerable disease burden associated with a higher rate of exacerbations, more frequent hospitalisations, greater oral corticosteroid (OCS) use, more impaired lung function, reduced health-related quality of life (QoL), and Type 2 inflammatory comorbidities versus controlled asthma. There remains an unmet need for new therapies for patients with uncontrolled persistent asthma. Several agents targeting mediators of Type 2 inflammation are in clinical development for severe asthma, including prostaglandin D2 receptor 2 (DP2)/chemoattractant receptor-homologous molecule expressed on Th2 (CRTh2) antagonists and monoclonal antibodies (mAb) that specifically bind IL-33, IL-25, thymic stromal lymphopoietin (TSLP), and IL-4 receptor (IL-4Rα). Dupilumab blocks signalling of IL-4 and IL-13 and is under investigation in various diseases driven by Type 2 inflammation. In Phase III clinical trials in patients with uncontrolled, persistent asthma, dupilumab was well tolerated and demonstrated significant efficacy versus placebo in reducing the rate of asthma exacerbations, and improving lung function, asthma symptoms, and QoL. This article summarises the proceedings of a symposium held at the European Academy of Allergy and Clinical Immunology (EAACI) 2018 Congress, which brought together an international faculty of experts to explore current understandings of asthma pathophysiology, with particular focus on Type 2 inflammatory pathways, and to provide an overview of current therapies, unmet medical needs, and the potential role of emerging biologics in the treatment of uncontrolled persistent asthma.

Introduction

Asthma is defined by the Global Initiative for Asthma as a history of respiratory symptoms such as wheeze, dyspnoea, chest tightness, and cough that vary over time and intensity, together with variable expiratory airflow limitation.[1] It is a common chronic respiratory disorder that affects an estimated 358 million people worldwide,[2] and is associated with a substantial socioeconomic burden. It is now acknowledged to be a heterogeneous disease with multiple phenotypes, based on distinct pathophysiological mechanisms.[1] Comorbidities are common, which can have a significant effect on patients' exacerbations, symptom control, and QoL.[3] Importantly, up to 56% of patients continue to have uncontrolled persistent asthma, despite the availability of effective asthma treatments and evidence-based management guidelines.[4-6]

Asthma Heterogeneity is Caused by a Complex Interplay of Inflammatory Pathways

Chronic airway inflammation is the main pathophysiological feature of asthma, together with goblet cell hyperplasia, enhanced mucus production, and smooth muscle contractility abnormalities, as well as airway remodelling. The clinical manifestations of these processes result in airway hyper-responsiveness, airflow obstruction, decreased lung function, and exacerbations.[7] The heterogeneity of asthma is likely the result of a complex interplay of inflammatory pathways that involve multiple cytokines and inflammatory cells.[7] Type 2 inflammation in the airway is characterised by the presence of IL-4, 5, and 13, which are produced by Type 2 helper T (Th2) cells and innate lymphoid cells in response to allergens, infectious agents, irritants, and pollutants.[7]

The interplay between innate and adaptive cells and mediators in Type 2 inflammation underpins asthma pathophysiology,[7] with Type 2 cytokines playing unique but overlapping roles. IL-13 contributes to goblet cell hyperplasia, increases production of MUC5AC (associated with a more adherent type of mucus), induces production of the eosinophil chemoattractant eotaxin, and induces airway smooth muscle contractility and proliferation.[8,9] IL-4 and IL-13 contribute to epithelial barrier disruption by increasing epithelial permeability, and are central to airway remodelling.[10-12]

Type 2 Inflammation is Associated with Multiple Asthma Phenotypes

Asthma driven by Type 2 inflammation (Type-2 high) encompasses allergic (childhood onset) and eosinophilic (adult-onset) phenotypes.[13] These are associated with well-recognised biomarkers such as the presence of blood and sputum eosinophils, elevated fractional exhaled nitric oxide (FeNO; an indirect measure of pulmonary inflammation), serum immunoglobulin (Ig)E, and serum periostin.[14] High levels of sputum and blood eosinophils, and FeNO, are associated with uncontrolled and/or more severe asthma and the risk of exacerbations.[15,16] Approximately 50–70% of patients with asthma have Type 2 inflammation.[17,18] Both IL-4 and IL-13 are key players in Type 2-mediated inflammation in asthma, and have central roles in IgE synthesis, eosinophil recruitment, mucus secretion and airway inflammation, hyper-reactivity, and remodelling.[19] Non-Type 2 (Type-2 low) inflammatory pathways are not well understood, but may include those associated with smooth muscle-mediated factors, obesity, infection, or neutrophilia.[20]

Burden of Uncontrolled Persistent Asthma

Patients with severe, persistent asthma comprise around 5–10% of the total asthma population, but account for >80% of the total direct healthcare costs of asthma.[21,22] Uncontrolled persistent asthma is a complex disease state associated with a high rate of exacerbations, frequent hospitalisations, high oral OCS use, impaired lung function, reduced health-related QoL, and Type 2 inflammatory comorbidities.[1,15,23,24] Exacerbations can be potentially life-threatening, requiring medical intervention in the form of an emergency department visit or admission to hospital,[25] and are thus linked to higher morbidity, greater risk of mortality, and higher treatment costs.[26] Of note, it has been reported that the total cost of managing an exacerbation increases with disease severity and is particularly driven by prior exacerbations.[27] Additionally, there are concerns regarding long-term, regular OCS use, because of potential systemic adverse effects on growth, adrenal function, and bone mass and associations with conditions such as hypertension, Type 2 diabetes mellitus, and gastrointestinal bleeding.[28-30]

The burden of uncontrolled persistent asthma driven by Type 2 inflammation is particularly high. Simultaneous increases in FeNO and blood eosinophil biomarkers are associated with more frequent exacerbations.[15] Other Type 2 inflammatory diseases, notably allergic rhinitis and chronic rhinosinusitis with nasal polyps, are often comorbid with uncontrolled persistent asthma.[31,32] An analysis of data from the UK Optimum Patient Care Research Database showed that both conditions are independent predictors of asthma exacerbations,[33] while data from the Severe Asthma Research Program (SARP) demonstrated that sinusitis is associated with an increased exacerbation rate in patients with severe asthma.[34]

Challenges in the Management of Uncontrolled Persistent Asthma

The Global Initiative for Asthma 2018 guidelines advise a stepwise approach to the use of treatments to achieve asthma control.[1] In patients in whom asthma is severely uncontrolled or with acute exacerbations, a short course of OCS is recommended as an add-on to regular treatment. However, OCS are associated with a variety of significant long-term side effects in patients with severe asthma, which include osteoporosis, hypertension, obesity, Type 2 diabetes, gastrointestinal ulcers/bleeds, fractures, cataracts, muscle weakness, back pain, and bruising.[29,30] Unsurprisingly, long-term OCS use remains a concern and, in a recent large survey in patients with asthma (N=2,003), approximately one-third and one-half of respondents from Europe and Canada, respectively, cited it as a worry.[35] Therefore, the use of steroid-sparing treatments is important, where possible, to minimise side effects and improve patient outcomes.

There is a lack of evidence to support an inhaled corticosteroids dose increase and

other non-biologic controller therapy add-ons (treatment escalation strategies) in patients with uncontrolled asthma. In a USA real-world healthcare claims database study of patients who initiated a treatment escalation strategy, uncontrolled asthma was experienced by 41.5% and 41.0% before and after treatment escalation, respectively.[36] Outcomes before the implementation of an escalation strategy were similar 1 year later. To address the considerable and remaining unmet need for new treatment approaches beyond escalation, a number of biologic therapies targeting the Type 2 inflammatory pathway are approved or in development.

Overview of Current Evidence on Approved and Emerging Biologic Therapies Targeting Type 2 Inflammation

Four biologic therapies are currently licensed in the European Union (EU) and the USA for the treatment of asthma: omalizumab (approved for moderate-to-severe allergic asthma in patients aged ≥6 years), benralizumab and mepolizumab (approved for severe asthma in patients aged ≥12 years with an eosinophilic phenotype), and reslizumab (approved for severe eosinophilic asthma in patients aged ≥18 years).

Omalizumab is a humanised anti-IgE mAb that specifically binds free IgE in serum and can interrupt the allergic cascade by preventing the binding of IgE with its high-affinity FcεRI receptors on mast cells, antigen-presenting cells, basophils, and other inflammatory cells.[37] In clinical and real-world studies involving children, adolescents, and adults, all with moderate-to-severe asthma, omalizumab treatment was well tolerated and was shown to significantly reduce asthma exacerbations, symptoms, and the need for inhaled corticosteroid and rescue medication use. QoL was also improved versus placebo or standard of care.[38-41]

The other three licensed biologics target IL-5 signalling, binding either IL-5 directly (humanised mAb: mepolizumab and reslizumab) or its receptor IL-5R (fully human mAb benralizumab), resulting in a depletion of blood and airway eosinophils and basophils.[42] In children and adults with severe eosinophilic asthma, all three treatments significantly reduced rates of clinically significant asthma exacerbations by ~50% and significantly improved lung function, as shown by increases in mean forced expiratory volume in 1 second (FEV_1) of 0.08–0.11 L versus placebo.[43-49] Patients receiving mepolizumab or benralizumab also significantly reduced their OCS intake (by 50% and 75%, respectively),[43,50] and improvements in asthma control and QoL were seen with mepolizumab or reslizumab treatment versus placebo.[43,44,49] The safety profiles of all three therapies were comparable to that of placebo.[43,46,47,49,50]

Despite demonstrable efficacy, safety, and tolerability in clinical and real-world studies of patients with severe allergic or eosinophilic asthma, currently available biologic therapies have several limitations and are not suitable for, or effective in, many patients with asthma. Their use is often restricted to specific populations, leaving many patients ineligible for treatment. Evidence suggests that between 65% and 76% of patients with severe asthma may be ineligible for any approved biologic therapy, based on the eligibility criteria used.[51] They do not show consistent activity across patients with a broad range of Type 2 biomarkers.[52] A substantial proportion of patients remain suboptimally controlled, and there may be subphenotypes of Type 2-high asthma that do not respond to treatment.[52,53] Current biologic therapies only partially inhibit Type 2 inflammation, and may therefore be less effective than biologics with a broader effect, and no available single biologic therapy can treat the full spectrum of Type 2 comorbid, inflammatory diseases (Table 1).

To address these limitations, a variety of mAb and small molecules that target different mediators of, and pathways involved in, Type 2 inflammation other than IgE and IL-5/IL-5R are under investigation. Agents in Phase II clinical development include the anti-IL-33 mAb, AMG-282/RG6149 (for the treatment of mild atopic asthma), GSK3772847 and SAR44040/REGN3500 (for moderate-to-severe asthma), and ANB020 (for severe eosinophilic asthma). In addition, orally administered ADC3680/ADC3608B is a potent and selective antagonist of the DP2/CRTh2 for inadequately

controlled asthma. Agents undergoing Phase III clinical testing for poorly controlled or uncontrolled, persistent or severe asthma include ABM125 (an anti-IL-25 mAb), fevipiprant (an orally administered competitive and reversible antagonist of DP2/CRTh2), tezepelumab (a human mAb that specifically targets the epithelial cell-derived cytokine, TSLP), and dupilumab (a fully human mAb that specifically binds to the alpha subunit of IL-4Rα).

Dupilumab will be the focus of the rest of this review, because it is being investigated in a broad range of clinical development programmes for diseases driven by Type 2 inflammation. It is already licensed for moderate-to-severe atopic dermatitis in adults, and, in addition to uncontrolled, persistent asthma, clinical studies are being conducted in paediatric atopic dermatitis (Phase III), chronic rhinosinusitis with nasal polyps (Phase III), and eosinophilic oesophagitis (Phase II). Dupilumab blocks signalling of IL-4 and IL-13, both of which bind to IL-4Rα and are key drivers of Type 2 inflammation.[31]

A 24-week, randomised, double-blind, placebo-controlled, parallel-group, pivotal, Phase IIb dose-ranging study[58] evaluated subcutaneous dupilumab (200 mg or 300 mg every 2 [Q2W] or 4 [Q4W] weeks) as add-on therapy in patients aged ≥18 years with uncontrolled, persistent asthma on medium-to-high-dose inhaled corticosteroids plus a long-acting β_2-agonist (N=769). The results showed that dupilumab significantly increased lung function (assessed by FEV_1; p<0.01) and reduced severe exacerbations by ~60–81% versus placebo (p<0.05).[58] Dupilumab treatment also significantly improved asthma control (least squares mean change in 5-item Asthma Control Questionnaire [ACQ-5] score from baseline to Week 24 of -1.49 and -1.45 for 200 and 300 mg dupilumab, respectively, versus -1.14 for placebo) and QoL (measured with the Asthma Quality of Life Questionnaire) and had a favourable safety profile.[58] This study established the optimum dupilumab dosing regimen to be Q2W.

Table 1: Status of currently approved biologics for the treatment of common Type 2 comorbidities associated with uncontrolled persistent asthma.[46-52,54-57]

	Omalizumab	Reslizumab	Mepolizumab	Benralizumab
Asthma	Approved for moderate to severe allergic asthma	Approved for severe eosinophilic asthma	Approved for severe eosinophilic asthma	Approved for severe eosinophilic asthma
Chronic sinusitis with nasal polyps	Reduced polyp size and improved some symptoms (small Phase I; n=24) Phase III trial ongoing	Reduced polyp size in patients with elevated IL-5; no improvement in symptoms (Phase I; n=24) Phase 3 trial ongoing	Reduced polyp size, improved CT scan and symptoms (Phase II; n=105) Phase III trial ongoing	Awaiting Phase II data
Allergic rhinitis	Recommended (not FDA approved)	Not tested	Not tested	Not tested
Atopic dermatitis	No improvement in disease endpoints (Phase II)	Not tested	Discontinued at Phase II	Not tested
Eosinophilic oesophagitis	Histological and clinical improvement in a subset of eosinophilic oesophagitis patients (small Phase I; n=15)	Reduced intraepithelial oesophageal eosinophil counts (Phase II; n=226)	No clinical improvement in adults (Phase II; n=11) Reduced eosinophilic inflammation in children (Phase II; n=59) Reduced intraepithelial oesophageal eosinophil counts (Phase II; n=226)	Not tested

CT: computed tomography; FDA: U.S Food and Drug Administration; IL: interleukin.

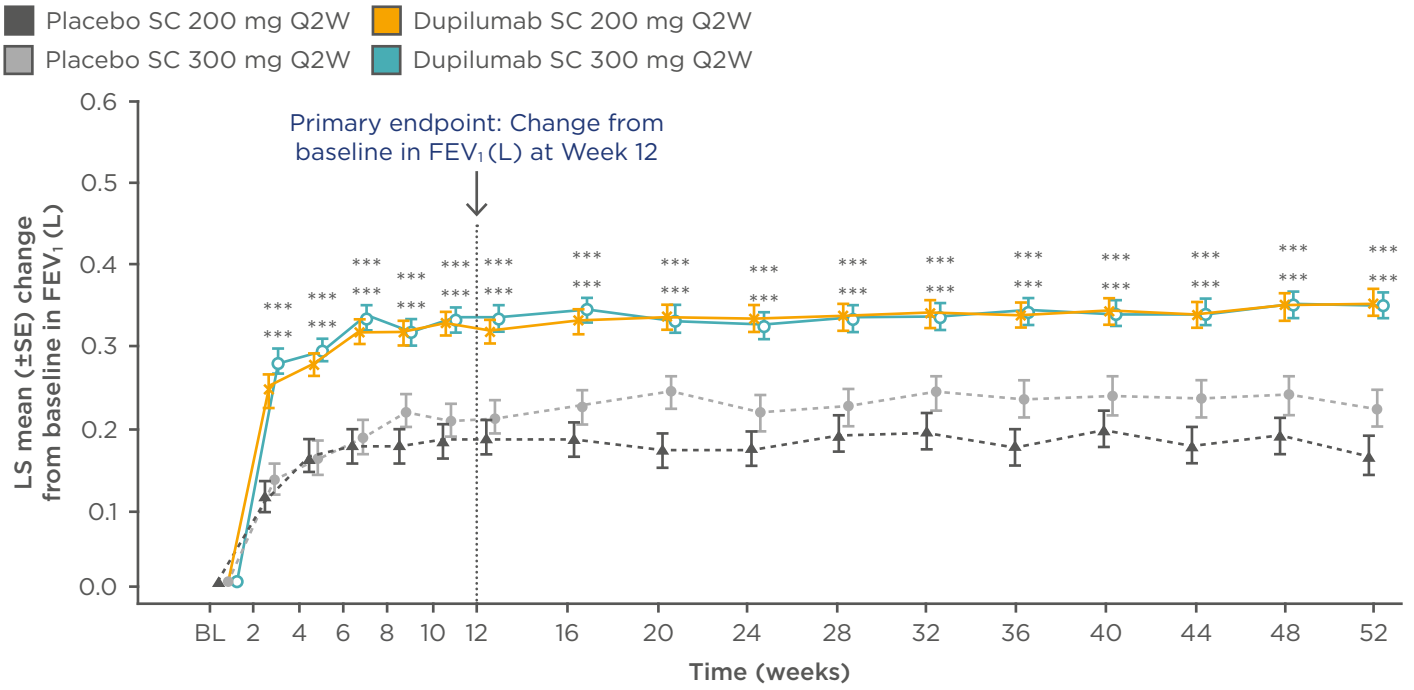


Figure 1: Least squares mean change from baseline in forced expiratory volume in 1 second over 52 weeks of treatment with dupilumab versus placebo.

***p<0.001 versus placebo

BL: baseline; FEV_1: forced expiratory volume in 1 second; LS: least squares; Q2W: every 2 weeks; SC: subcutaneous; SE: standard error.

Adapted from Castro et al.[59]

Phase III Clinical Studies of Dupilumab in Patients with Uncontrolled, Persistent Asthma: LIBERTY ASTHMA QUEST and VENTURE

The 52-week, randomised, double-blind, placebo-controlled, parallel-group, Phase III LIBERTY ASTHMA QUEST study[59] randomised patients aged ≥12 years with uncontrolled, persistent, moderate-to-severe asthma (N=1,902) to subcutaneous dupilumab as add-on therapy (200 mg or 300 mg Q2W) or placebo. Primary endpoints included annualised severe asthma exacerbation rate and absolute change from baseline to Week 12 in FEV_1 before bronchodilator use. Secondary endpoints included exacerbation rate and FEV_1 in patients with a blood eosinophil count of ≥300 cells/mm^3 and stratified by baseline FeNO, a measure of pulmonary inflammation.[59]

In the dupilumab 200 mg Q2W group, the annualised rate of severe asthma exacerbations was 0.46 (95% confidence interval [CI]: 0.39–0.53) versus 0.87 (95% CI: 0.72–1.05) for placebo, equating to a 48% reduction with dupilumab (p<0.001). FEV_1 increased by 0.32 L at 12 weeks (difference versus matched placebo: 0.14 L; p<0.001), and this significant and rapid improvement in lung function was sustained for the remainder of the study (Figure 1). For both primary endpoints, similar results were seen with the 300 mg dose.[59]

Among patients with eosinophilic asthma, the annualised rates of severe asthma exacerbations were 0.37 (95% CI: 0.29–0.48) in the dupilumab 200 mg group versus 1.08 (95% CI: 0.85–1.38) for matched placebo and 0.40 (95% CI: 0.32–0.51) in the dupilumab 300 mg group versus 1.24 (95% CI: 0.97–1.57) for matched placebo. This equated to a reduction in severe asthma exacerbations of 66% and 67% with dupilumab 200 mg and 300 mg, respectively (p<0.001). In patients stratified by baseline FeNO, a greater benefit in exacerbation rate with dupilumab was observed in patients with higher FeNO:

≥50 ppb and ≥25 ppb FeNO levels were associated with significant reductions of 69–70% and 61–65% versus placebo for the 200 mg and 300 mg dupilumab doses (p<0.001), but no significant between-group differences were seen in patients with FeNO <25 ppb (Figure 2). Significantly greater improvements in lung function were also observed in patients with higher baseline eosinophil counts and FeNO levels. Hypereosinophilia was observed in some patients soon after starting treatment (4.1% [n=52] versus 0.6% [n=4] for dupilumab versus placebo, respectively).[59]

In the 36-week, randomised, double-blind, placebo-controlled, Phase III LIBERTY ASTHMA VENTURE study,[62] add-on therapy with dupilumab 300 mg Q2W significantly reduced the use of OCS, while simultaneously reducing severe exacerbations and improving lung function, in patients with corticosteroid-dependent severe asthma (N=210), irrespective of baseline blood eosinophil count. The primary endpoint of the study was percentage reduction in corticosteroid dose at Week 24; key secondary endpoints included proportions of patients at Week 24 with a reduction of ≤50% in corticosteroid dose, and with a reduction to a corticosteroid dose of <5 mg/day.[62]

At Week 24, the percentage change in corticosteroid dose was -70% versus -42% for the dupilumab and placebo groups, respectively (p<0.001), with 80% versus 50% of patients in these respective groups reducing their corticosteroid doses by ≥50% (p<0.001). In total, 69% of patients in the dupilumab group versus 33% in the placebo group had corticosteroid dose reductions to <5 mg/day (p<0.001), with 48% and 25% in the dupilumab and placebo groups, respectively, no longer requiring OCS (p=0.002).

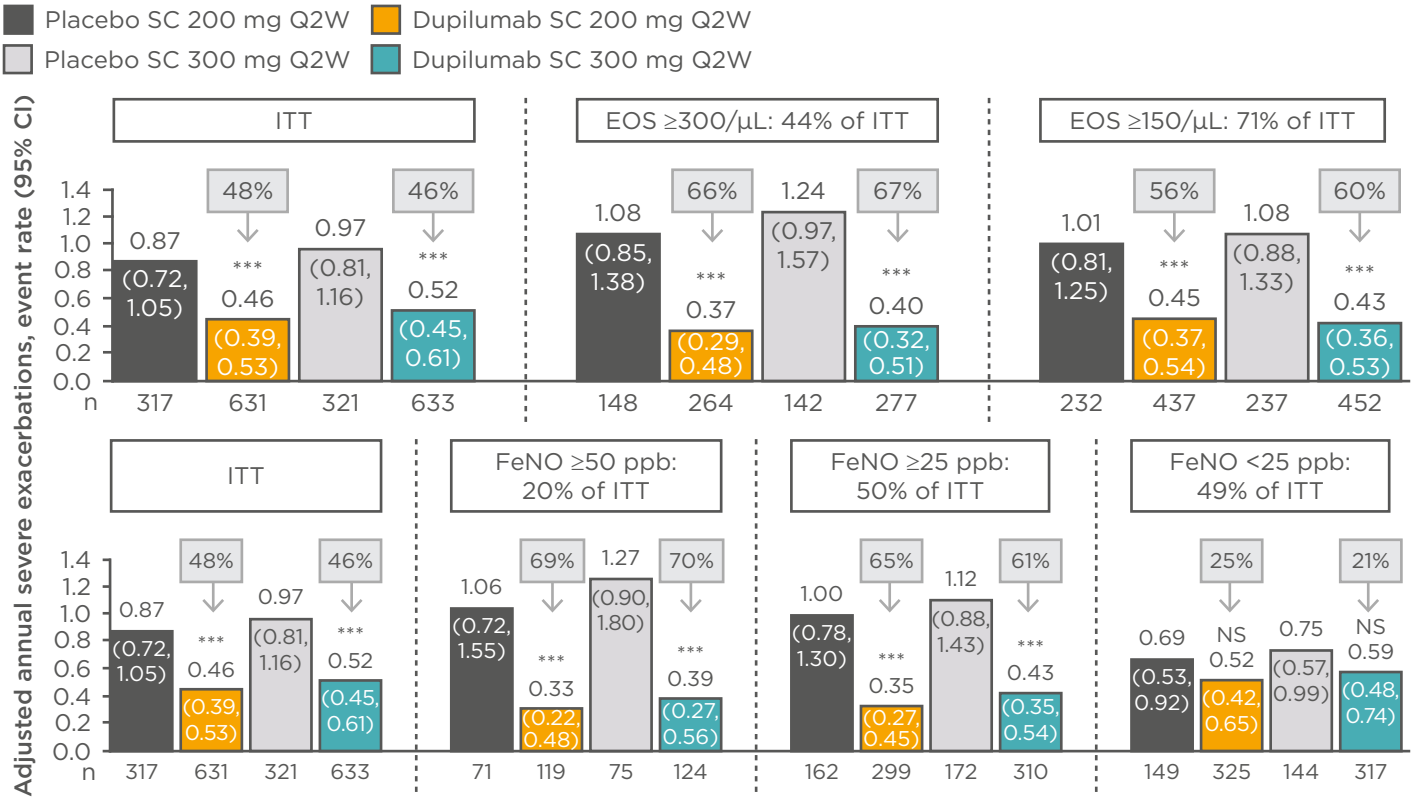


Figure 2: Rate of severe exacerbations in patients stratified by baseline blood eosinophil count or fractional exhaled nitric oxide level treated with dupilumab versus placebo (intent-to-treat population).[59-61]

***p<0.001 versus placebo

CI: confidence interval; EOS: blood eosinophil count; FeNO: fractional exhaled nitric oxide; ITT: intent-to-treat; NS: nonsignificant; Q2Q: every 2 weeks; SC: subcutaneous.

Despite these reductions in corticosteroid dosing, the severe exacerbation rate was 59% lower in the dupilumab group (95% CI: 37–74%), resulting in an FEV_1 that was 0.22 L (95% CI: 0.09–0.34) higher. Compared with placebo, just over twice as many patients on dupilumab experienced injection-site reactions (9% versus 4%), and transient blood eosinophilia was observed in 14% of patients (versus 1% of placebo-treated patients).[62]

Dupilumab Efficacy in Type 2 Inflammatory Diseases

Type 2 inflammation is an important process leading to airway changes in asthma, including airflow obstruction, susceptibility to exacerbations, and persistent airway changes from remodelling. Dupilumab targets IL-4/IL-13 pathway generated-type inflammation. The effects of IL-4/IL-13 on asthma are comprehensive, and consequently the effects of dupilumab in asthma may be more profound than those of current treatments.

Dupilumab has also demonstrated efficacy in patients with comorbid Type 2 inflammatory diseases. In a randomised, double-blind, placebo-controlled, parallel-group, Phase II study[63] in adult patients with symptomatic chronic sinusitis and nasal polyposis refractory to intranasal corticosteroids (N=60; n=35 with comorbid asthma), the addition of subcutaneous dupilumab to mometasone furoate nasal spray reduced endoscopic nasal polyp burden, compared with placebo plus mometasone alone after 16 weeks of treatment (least squares mean change in nasal polyp score: placebo: -0.3 [95% CI: -1.0–0.4]; dupilumab: -1.9 [95% CI: -2.5–-1.2]; p<0.001). Improvements in exploratory endpoints including percent predicted FEV_1 and ACQ-5 score were also seen.[63] The target population of dupilumab therefore appears to be much broader than that of existing IgE/IL-5-targeting biologics.

Conclusions

There remains an unmet need for new therapies to treat patients with persistent, uncontrolled asthma. Currently approved biologics have several limitations, and many patients are ineligible for treatment. A variety of agents that target mediators of Type 2 inflammation are in clinical development for severe asthma, including DP2/CRTh2 antagonists and mAb that specifically bind IL-33, IL-25, TSLP, and IL-4Rα. In Phase III clinical trials, dupilumab, an inhibitor of IL-4 and IL-13, was well tolerated and has demonstrated significant efficacy versus placebo at reducing the rate of asthma exacerbations, and improving lung function, asthma symptoms, and QoL. Due to the complexity of Type 2 inflammation in uncontrolled persistent asthma, assessing a wide panel of biomarkers would help identify pathway involvement and guide the future development of therapeutic interventions.

WATCH THE FULL SYMPOSIUM ONLINE ←

http://mediatheque.cyim.com/mediatheque/media.aspx?mediaId=46022&channel=25007

References

1. Global Initiative for Asthma (GINA). 2018 GINA, Report Strategy for Asthma Management and Prevention. 2018. Available at: https://ginasthma.org/2018-gina-report-global-strategy-for-asthma-management-and-prevention/. Last accessed: 28 June 2018.
2. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5(9):691-706.
3. Tay TR et al. Comorbidities in difficult asthma are independent risk factors for frequent exacerbations, poor control and diminished quality of life. Respirology. 2016;21(8):1384-90.
4. Bateman ED et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med. 2014;170(8):836-44.
5. Hermosa JL et al. Factors associated with the control of severe asthma. J

Asthma. 2010;47(2):124-30.

6. Peters SP et al. Real-world Evaluation of Asthma Control and Treatment (REACT): Findings from a national Web-based survey. J Allergy Clin Immunol. 2007;119(6):1454-61.
7. Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. N Engl J Med. 2017;377(10):965-76.
8. Bonser LR et al. Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. J Clin Invest. 2016;126(6):2367-71.
9. Risse PA et al. Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. Am J Physiol Lung Cell Mol Physiol. 2011;300(6):L958-66.
10. Saatian B et al. Interleukin-4 and interleukin-13 cause barrier dysfunction in human airway epithelial cells. Tissue Barriers. 2013;1(2):e24333.
11. Schleimer RP, Berdnikovs S. Etiology of epithelial barrier dysfunction in patients with type 2 inflammatory diseases. J Allergy Clin Immunol. 2017;139(6):1752-61.
12. Sugita K et al. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. J Allergy Clin Immunol. 2018;141(1):300-10.
13. Wenzel SE. Asthma phenotypes: The evolution from clinical to molecular approaches. Nat Med. 2012;18(5): 716-25.
14. Robinson D et al. Revisiting type 2-high and type 2-low airway inflammation in asthma: Current knowledge and therapeutic implications. Clin Exp Allergy. 2017;47(2):161-75.
15. Malinovschi A et al. Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity. J Allergy Clin Immunol. 2016;138(5):1301-8.
16. Schleich FN et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir. 2014;44(1):97-108.
17. Peters MC et al. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. J Allergy Clin Immunol. 2014;133(2):388-94.
18. Schleich F et al. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). Respir Med. 2014;108(12):1723-32.
19. Bagnasco D et al. A critical evaluation of anti-IL-13 and anti-IL-4 strategies in severe asthma. Int Arch Allergy Immunol. 2016;170(2):122-31.
20. Fahy JV. Type 2 inflammation in asthma--Present in most, absent in many. Nat Rev Immunol. 2015;15(1): 57-65.
21. Chung KF et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43(2):343-73.
22. Rodrigo GJ et al. Acute asthma in adults: A review. Chest. 2004;125(3):1081-102.
23. Gandhi NA et al. Commonality of the IL-4/IL-13 pathway in atopic diseases. Expert Rev Clin Immunol. 2017;13(5):425-37.
24. Matsusaka M et al. Phenotype of asthma related with high serum periostin levels. Allergol Int. 2015;64(2):175-80.
25. Wark PA, Gibson PG. Asthma exacerbations. 3: Pathogenesis. Thorax. 2006;61:909-15.
26. Custovic A et al. EAACI position statement on asthma exacerbations and severe asthma. Allergy. 2013;68(12):1520-31.
27. Suruki RY et al. The frequency of asthma exacerbations and healthcare utilization in patients with asthma from the UK and USA. BMC Pulm Med. 2017;17(1):74.
28. Allen DB. Safety of inhaled corticosteroids in children. Pediatr Pulmonol. 2002;33(3):208-20.
29. Sullivan PW et al. Oral corticosteroid exposure and adverse effects in asthmatic patients. J Allergy Clin Immunol. 2018;141(1):110-6.
30. Walsh LJ et al. Adverse effects of oral corticosteroids in relation to dose in patients with lung disease. Thorax. 2001;56(4):279-84.
31. Gandhi NA et al. Targeting key proximal drivers of type 2 inflammation in disease. Nat Rev Drug Discov. 2016;15(1):35-50.
32. Porsbjerg C, Menzies-Gow A. Co-morbidities in severe asthma: Clinical impact and management. Respirology. 2017;22(4):651-61.
33. Blakey JD et al. Identifying risk of future asthma attacks using uk medical record data: A Respiratory Effectiveness Group Initiative. J Allergy Clin Immunol Pract. 2017;5(4):1015-24.
34. Denlinger LC et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. Am J Respir Crit Care Med. 2017;195(3):302-13.
35. Sastre J et al. Insights, attitudes, and perceptions about asthma and its treatment: A multinational survey of patients from Europe and Canada. World Allergy Organ J. 2016;9:13.
36. Bengtson LGS et al. Inhaled corticosteroid-containing treatment escalation and outcomes for patients with asthma in a U.S. health care organization. J Manag Care Spec Pharm. 2017;23(11):1149-59.
37. Licari A et al. The discovery and development of omalizumab for the treatment of asthma. Expert Opin Drug Discov. 2015;10(9):1033-42.
38. Hanania NA et al. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: A randomized trial. Ann Intern Med. 2011;154(9):573-82.
39. Humbert M et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. Allergy. 2005;60(3):309-16.
40. Niven RM et al. Impact of omalizumab on treatment of severe allergic asthma in UK clinical practice: A UK multicentre observational study (the APEX II study). BMJ Open. 2016;6(8):e011857.
41. Normansell R et al. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev. 2014;13(1):CD003559.
42. Sahota J, Robinson DS. Update on new biologics for intractable eosinophilic asthma: Impact of reslizumab. Drug Des Devel Ther. 2018;12:1173-81.
43. Bel EH et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. N Engl J Med. 2014;371(13):1189-97.
44. Bjermer L et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil levels: A randomized Phase 3 study. Chest. 2016;150(4):789-98.
45. Bleecker ER et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIROCCO): A randomised, multicentre, placebo-controlled Phase 3 trial. Lancet. 2016;388(10056): 2115-27.
46. Castro M et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: Results from two multicentre, parallel, double-blind, randomised, placebo-controlled, Phase 3 trials. Lancet Respir Med. 2015;3(5):355-66.
47. Farne HA et al. Anti-IL5 therapies for asthma. Cochrane Database Syst Rev. 2017;9:CD010834.
48. FitzGerald JM et al. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): A randomised, double-blind, placebo-controlled Phase 3 trial. Lancet. 2016;388(10056):2128-41.

49. Ortega HG et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014;371(13):1198-207.
50. Nair P et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. N Engl J Med. 2017;376:2448-58.
51. Albers FC et al. Biologic treatment eligibility for real-world patients with severe asthma: The IDEAL study. J Asthma. 2018;55(2):152-60.
52. Berry A, Busse WW. Biomarkers in asthmatic patients: Has their time come to direct treatment? J Allergy Clin Immunol. 2016;137(5):1317-24.
53. Woodruff PG et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med. 2009;180(5):388-95.
54. Bachert C et al. Biotherapeutics in chronic rhinosinusitis with and without nasal polyps. J Allergy Clin Immunol Pract. 2017;5(6):1512-16.
55. Bousquet J et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2) LEN and AllerGen). Allergy. 2008;63 Suppl 86:8-160.
56. GlaxoSmithKline. Efficacy and safety study of mepolizumab in subjects with moderate to severe atopic dermatitis. NCT03055195. https://clinicaltrials.gov/ct2/show/NCT03055195.
57. Kyowa Hakko Kirin Co., Ltd. Study of benralizumab (KHK4563) in patients with eosinophilic chronic rhinosinusitis. NCT02772419. https://clinicaltrials.gov/ct2/show/NCT02772419.
58. Wenzel S et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: A randomised double-blind placebo-controlled pivotal Phase 2b dose-ranging trial. Lancet. 2016. 388(10039):31-44.
59. Castro M et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018. [Epub ahead of print].
60. Castro M et al. A randomized, controlled Phase 3 study, LIBERTY ASTHMA QUEST, evaluating the efficacy and safety of dupilumab in uncontrolled, moderate-to-severe asthma. Am J Respir Crit Care Med. 2018;197:A7700.
61. Wenzel SE et al. Type 2 biomarkers associated with dupilumab efficacy in patients with uncontrolled, moderate-to-severe asthma enrolled in the Phase 3 study LIBERTY ASTHMA QUEST. Am J Respir Crit Care Med. 2018;197:A5949.
62. Rabe KF et al. Efficacy and Safety of Dupilumab in Glucocorticoid-Dependent Severe Asthma. N Engl J Med. 2018. [Epub ahead of print].
63. Bachert C et al. Effect of subcutaneous dupilumab on nasal polyp burden in patients with chronic sinusitis and nasal polyposis: A randomized clinical trial. JAMA. 2016;315(5):469-79.

Abstract Reviews

From food allergies to vaccine immunotherapy, discover the latest results from the researchers themselves

Epigenetic Dysregulation of Naïve CD4+ T Cell Activation Genes in Childhood Food Allergy

Authors: David Martino,[1,2] Melanie Neeland,[2] Thanh Dang,[2] Joanna Cobb,[2] Justine Ellis,[2] Alice Barnett,[2] Mimi Tang,[2] Peter Vuillermin,[2,3] *Katrina Allen,[2] Richard Saffery[2]

1. Telethon Kids Institute, Subiaco, Australia
2. Murdoch Children's Research Institute, University of Melbourne, Melbourne, Australia
3. Barwon Health, University Hospital Geelong, Geelong, Australia

*Correspondence to katrina.allen@mcri.edu.au

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors wish to acknowledge the contribution of the HealthNuts investigators and thank the families and study participants involved in the HealthNuts study.

Keywords: Epigenetics, food allergy, T cells.

Citation: EMJ Allergy Immunol. 2018;3[1]:70-71. Abstract Review No. AR1.

Food allergy poses a significant clinical and public health burden, affecting 2–10% of infants, and T cell dysfunction has been previously reported in children with food allergies. Work from this group,[1] and by others,[2] suggests that suboptimal T cell response capacity to mitogens and allergens is an important premorbid factor in the development of food allergies. This group has previously described differences in neonatal total CD4+ T cell activation gene-response capacity and proliferative potential in children who eventually develop food allergies in the first year of life.[1] These differences are apparent at birth, an age that is unrelated to allergen exposure, and therefore of unknown clinical significance.

In the current study, this work is extended to focus on naïve CD4+ T cells, which are mature multipotent precursors with the capacity to adopt a range of different T cell effector and memory phenotypes depending on intracellular signalling factors and extracellular cytokine cues. After activation, naïve CD4+ T cells establish heritable transcriptional programmes that enable progression to short or long-lived effector or memory phenotypes. The initial phase of naïve CD4+ T cell priming involves epigenetic remodelling of chromatin, which is crucial for mounting effective immune responses and influencing T cell lineage decisions.[3]

In this study, we investigated the epigenetic regulation of the naïve CD4+ T cell activation response among children with immunoglobulin E-mediated food allergies. Using integrated DNA methylation and transcriptomic profiling, it was found that food allergy in infancy was associated with dysregulation of T cell activation genes. Reduced expression of cell cycle-related targets of the E2F and MYC transcription factor networks and remodelling of DNA methylation of metabolic (*RPTOR*, *PIK3D*, *MAPK1*, *FOXO1*) and inflammatory (*IL1R*, *IL18RAP*, *CD82*) genes were associated with poorer T lymphoproliferative responses in infancy after polyclonal activation of the T cell receptor (Figure 1). These molecular changes associated with food allergy were revealed post-activation and were not detectable in quiescent cells. Infants who failed to resolve food allergy in later childhood exhibited cumulative increases in epigenetic disruption at T cell activation genes and poorer lymphoproliferative responses compared to children who resolved food allergy.

These data indicate that gene–environment interactions mediated through epigenetic changes associated with food allergy overlap T cell activation pathways.

References

1. Martino DJ et al. T-cell activation genes differentially expressed at birth in CD4+ T-cells from children who develop IgE food allergy. Allergy. 2012;67(2):191-200.
2. Zhang Y et al.; BIS Investigator Group. Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce nonclassic "T_H2-type" immunity associated with development of food allergy. Sci Transl Med. 2016;8(321):321ra8.
3. Powell JD et al. A modified model of T-cell differentiation based on mTOR activity and metabolism. Cold Spring Harb Symp Quant Biol. 2013;78:125-30.

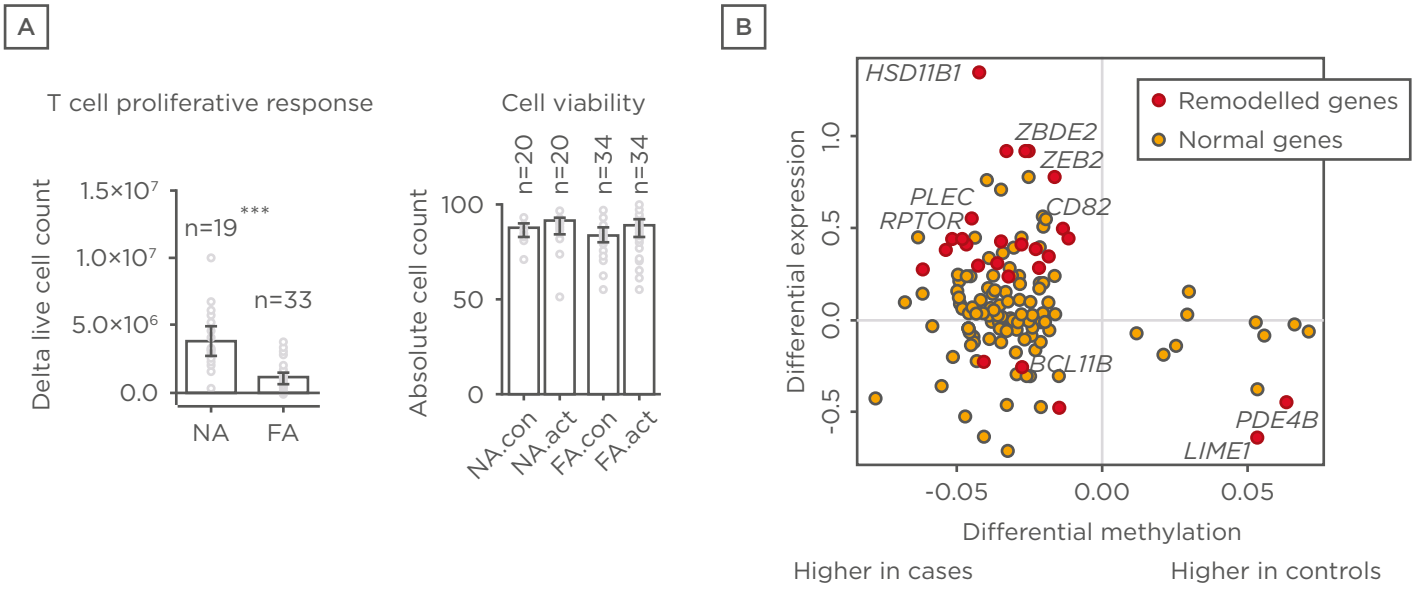


Figure 1: A) Depictions of proliferation responses and T cell activation and B) methylation and gene expressions for individuals with and without food allergy.

A) Proliferative responses and cell viability following T cell activation. Data are expressed as fold-change calculated as post and preactivation cell counts, with bars showing median and interquartile range. Groups were compared using the Mann–Whitney test. B) Relationship between differential methylation and gene expression. X-axis shows delta value expressed as percent methylation (10-2) for the comparison of cases and controls. Y-axis shows the log2 fold-change. Points in red were differentially methylated and expressed (remodelled genes) at the genome-wide level.

FA: food allergy; FA.act: food allergy activated; FA.con: food allergy quiescent; NA: nonallergic; NA.act: nonallergic activated; NA.con: nonallergic quiescent.

***p<0.001.

Evaluation of a Treatment Scheme with the Recombinant Grass Pollen Allergy Vaccine BM32: Yielding High Allergen-Specific Immunoglobulin G Responses Associated with Clinical Efficacy

Authors: *Petra Zieglmayer,[1] Margarete Focke-Tejkl,[2] Milena Weber,[2] René Schmutz,[3] Patrick Lemell,[3] René Zieglmayer,[1] Frank Stolz,[4] Hans Huber,[4] Angela Neubauer,[4] Rainer Henning,[4] Rudolf Valenta[2]

1. Power Project GmbH, Klosterneuburg, Austria
2. Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
3. Vienna Challenge Chamber, Vienna, Austria
4. BIOMAY AG, Vienna, Austria

*Correspondence to petra@zieglmayer.at

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The studies presented were supported by grants F4605 and F4611 from the Austrian Science Fund (FWF), grants 830432 and 835415 from the Austrian Research Promotion Agency (FFG), and by BIOMAY AG (Vienna, Austria).

Keywords: B cell epitope, challenge chamber, grass pollen, immunotherapy, vaccine.

Citation: EMJ Allergy Immunol. 2018;3[1]:72-73. Abstract Review No. AR2.

This European Academy of Allergy and Clinical Immunology (EAACI) presentation discussed the clinical development programme of a new B cell epitope-based allergy vaccine. The compound is based on fusion proteins, consisting of nonallergenic peptides derived from the immunoglobulin (Ig)E-binding sites of disease-causing allergens not binding IgE themselves, and PreS, a hepatitis B surface protein that serves as a nonallergenic carrier protein providing T cell help. The vaccine design and the three-dimensional structure of the Phl p 5 allergen was first described by Focke-Tejkl et al. in 2015.[1] The clinical profile and efficacy of BM32 has been evaluated in four clinical double-blind, placebo-controlled trials.

The absence of IgE and T cell-mediated side effects, which are standard in extract-based immunotherapeutic preparations and an important cause of treatment discontinuation, has been demonstrated in a skin test study by Niederberger et al. in 2015.[2] A total of 60 grass pollen-allergic volunteers exhibited an expected positive skin reaction in skin prick tests with grass pollen extract, but not with the individual BM32 components and the BM32 protein mix. T cell reactivity was investigated using two different concentrations of BM32. In contrast to grass pollen extract, no specific reaction was evident with BM32.

A dose-finding study conducted in an allergen challenge chamber system analysed clinical and immunological responses to three different BM32 doses in 69 grass pollen-allergic subjects.[3] A dose of 20 µg or 40 µg of each BM32 component, compared to 10 µg of placebo, administered three times in monthly intervals, showed a significantly better efficacy compared to placebo in terms of reduction of total nasal symptom score, skin prick test reactivity, and induction of allergen-specific IgG4.

These results were confirmed by a consecutive multicentre, multinational study conducted in 181 allergic subjects over two consecutive grass pollen seasons.[4] In this study, it was shown that the 20 µg dose was the most clinically effective in terms of combined symptom medication score as well as regarding quality of life during the pollen season. The induction of allergen-specific IgG was even more pronounced during the second year of treatment and no increases in IgE levels were recorded.

The dosing regimen was further evaluated in a single-centre study investigating the immunological and clinical response of 3, 4, or 5 monthly administrations of 20 µg of each BM32 component. A significant induction of allergen-specific IgG1 and IgG4 compared to

placebo was achieved with all active dosing regimens in the full analysis set (N=120). The group receiving five preseasonal injections of BM32 developed the highest and most sustained allergen-specific IgG1 and IgG4 responses compared to the other groups (Figure 1). A dose-dependent reduction in total nasal symptom score for the 3, 4, and 5 monthly administrations of 42.9% (BM32 5x), 42.1% (BM32 4x), and 7.3% (BM32 3x), respectively, versus placebo were observed after the grass pollen season and upon exposure in a pollen chamber. The group receiving five preseasonal injections of BM32 showed the best clinical effect, yielding 23.8% lower daily symptom and medication scores during pollen season compared to placebo.

BM32 was safe and well tolerated in all clinical studies. Mostly local injection site reactions were recorded by the patients, and very few and only late-onset systemic reactions, mainly Grade ≤2, were described.

In conclusion, BM32 is therefore a valuable candidate for a high-dose, short-course immunotherapy to treat grass pollen-allergic patients effectively and safely.

References

1. Focke-Tejkl M et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy. J Allergy Clin Immunol. 2015;135(5):1207-7.
2. Niederberger V et al. Skin test evaluation of a novel peptide carrier-based vaccine, BM32, in grass pollen-allergic patients. J Allergy Clin Immunol. 2015;136(4): 1101-10.
3. Zieglmayer P et al. Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy. EBioMedicine. 2016;11:43-57.
4. Niederberger V et al. Safety and efficacy of immunotherapy with the recombinant B-cell epitope-based grass pollen vaccine BM32. J Allergy Clin Immunol. 2018. [Epub ahead of print].

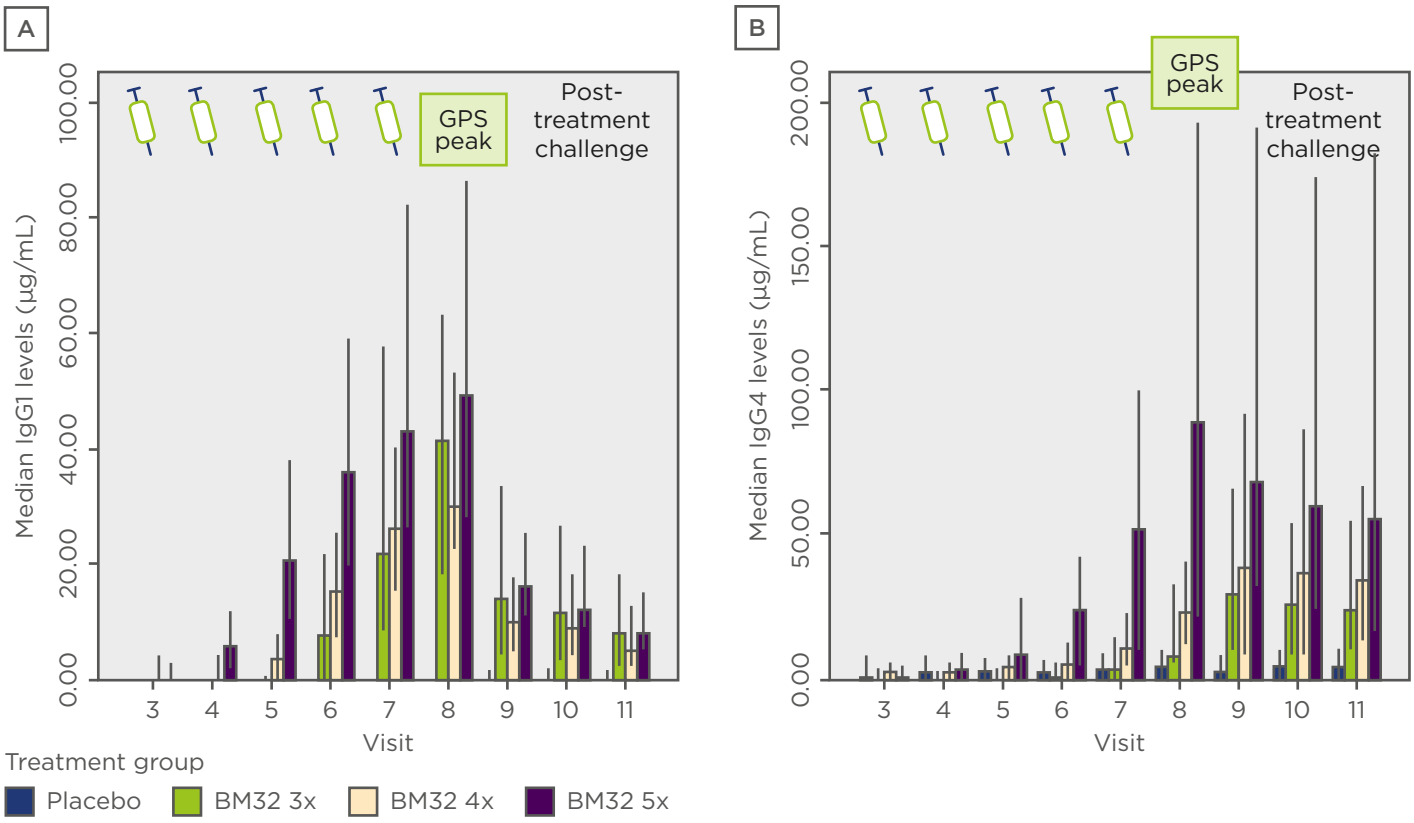


Figure 1: Dose-dependent induction of Phl p 1 and Phl p 5-specific immunoglobulin G1 (A) and immunoglobulin G4 (B) levels.

Error bars indicate the 95% confidence interval range.

GPS: grass pollen season; Ig: immunoglobulin.

Microcrystalline Tyrosine: An Appropriate Alternative to Aluminium as Adjuvant in Vaccines and Allergen Immunotherapy

Authors: Deborah Leuthard, Thomas M. Kündig, *Pål Johansen

Department of Dermatology, University Hospital Zurich, Zurich, Switzerland
*Correspondence to pal.johansen@usz.ch

Disclosure: This study was sponsored in part by Allergy Therapeutics Ltd. (Worthing, UK). Dr Kündig has consultant arrangements with Allergy Therapeutics Ltd. Dr Johansen and Dr Leuthard have received travel and conference grants from Bencard Allergie GmbH (Munich, Germany).

Acknowledgements: The authors are grateful to Matthew Heath at Allergy Therapeutics Ltd. and Matthias Kramer at Bencard Allergie GmbH for the helpful discussions, and to Agathe Duda for technical assistance with the experiments.

Keywords: Adjuvants, allergy immunotherapy (AIT), aluminium (alum), microcrystalline tyrosine (MCT), vaccines.

Citation: EMJ Allergy Immunol. 2018;3[1]:74-75. Abstract Review No. AR3.

ABSTRACT

Adjuvants are compounds added to antigens in vaccines to increase the body's immune response to an antigen.[1] Similarly, adjuvants are added to allergens for use in allergen immunotherapy (AIT), also called desensitisation or hyposensitisation.[2] The most frequently applied adjuvants for human use are gel-forming hydroxide or phosphate salts of aluminium (alum). More recently, MF59, AS03, AS04, MPLA, and virosomes have been approved for use in antiviral vaccines.[1] While being good at stimulating antibody responses, alum is not biocompatible and is difficult to clear from the body; this is important in AIT, which typically requires 50–80 subcutaneous injections. Hence, more biocompatible adjuvant options are subject to research and development.[3] In AIT, microcrystalline tyrosine (MCT) has found its way to some European markets.[4] MCT has a biological half-life of 48 hours but little is known about its adjuvant mechanism of action. Furthermore, head-to-head comparisons with alum have not been published. Hence, we compared alum and MCT in vaccines and in AIT and investigated potential mechanisms of action of MCT in preclinical mouse models.[5]

A single injection of a low antigen dose in mice triggered measurable B cell responses in serum when the antigen was mixed with alum. With MCT, the antigen dose required for triggering IgG responses was approximately 10-fold higher. At higher antigen doses or when the injections were repeated, as typically carried out during vaccination and AIT, alum and MCT stimulated comparable B cell responses, as measured by the amount of antigen-specific immunoglobulin (Ig)G and the subclasses of IgG produced (IgG1, IgG2a, IgG2b, and IgG3). However, MCT-based vaccines stimulated less IgE production than alum-based vaccines; IgE is a pathological factor in allergy and its production upon allergen exposure or AIT is unwanted. When measuring T cell responses, we found that alum-based vaccines triggered more T helper (Th) 2 cell-like responses, characterised by interleukin (IL)-4 and IL-10 secretion from T cells, while MCT-based vaccines stimulated less Th2 cytokines. Of note, IL-4 is required for the Ig switch to IgE, and the result therefore resembles the IgE data. Alum and MCT-based vaccines stimulated comparable secretion of Th1-like cytokines, e.g., IL-2 and interferon-γ. Indeed, one goal of AIT is to suppress Th2-associated immune responses, while triggering protective Th1-associated immune responses.

Alum and MCT were also compared in a mouse model of allergic anaphylaxis. Briefly, mice were made allergic by sensitisation to cat dander allergens and then given AIT with the recombinant major cat dander allergen Feld1 (*Felis domesticus* 1) combined with alum or MCT. Finally, the mice were challenged with a systemic injection of cat dander allergen extract. The challenge caused anaphylactic symptoms and reactions in sensitised mice that had not received AIT. In contrast, the anaphylaxis was

ameliorated in AIT-treated mice, independent of adjuvant used.

Both Alum and MCT were found to activate the inflammasome but this activation was not essential for the stimulation of B and T cell responses. Moreover, B and T cell responses induced with alum or MCT-based vaccines did not depend on signalling through toll-like receptors, which have been the target of many new and experimental adjuvants, e.g., monophosphoryl lipid A, resiquimod, and CpG.[1]

In closing, MCT appear to be an effective, biocompatible, and biodegradable adjuvant and a valid alternative to alum in vaccination and AIT.

References

1. Reed SG et al. Key roles of adjuvants in modern vaccines. Nat Med. 2013;19(12):1597-608.
2. Muraro A et al. EAACI guidelines on allergen immunotherapy: Executive statement. Allergy. 2018;73(4):739-43.
3. Kramer MF, Heath MD. Aluminium in allergen-specific immunotherapy: A German perspective. Vaccine. 2014;32(33):4140-8.
4. Klimek L et al. Clinical use of adjuvants in allergen immunotherapy. Expert Rev Clin Immunol. 2017;13(6): 599-610.
5. Leuthard DS et al. Microcrystalline tyrosine and aluminium as adjuvants in allergen-specific immunotherapy protect from IgE-mediated reactivity in mouse models and act independent of inflammasome and TLR signalling. J Immunol. 2018;200(9):3151-9.

Allergy Prevention by Allergens Depends on Their Ligands

Authors: *Franziska Roth-Walter,[1] Sheriene Moussa Afify,[1] Katharina Kienast,[1] Anton Vidovic,[1] Isabella Pali-Schöll,[1] Luis F. Pacios,[2] Zdenek Dvorak,[3] Erika Jensen-Jarolim[1,4]

1. The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University of Vienna and University of Vienna, Vienna, Austria
2. Center for Plant Biotechnology and Genomics and Department of Biotechnology-Vegetal Biology, ETSIAAB, Technical University of Madrid, Madrid, Spain
3. Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc, Czech Republic
4. Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

*Correspondence to
Franziska.roth-walter@meduniwien.ac.at

Disclosure: Dr Jensen-Jarolim, Dr Pacios, and Dr Roth-Walter are inventors of EP2894478, owned by Biomedical International R+D GmbH, Vienna, Austria. The remaining authors have declared no conflicts of interest.

Keywords: Allergen, beta-lactoglobulin (BLG), Bet v 1, iron, ligands, lipocalin, pathogenesis-related proteins, pathogenesis-related protein 10 (PR-10), siderophore, tolerance.

Citation: EMJ Allergy Immunol. 2018;3[1]:75-76. Abstract Review No. AR4.

The prevalence of allergy in the Western world is increasing, with 35% of women and 24% of men in Germany affected.[1] It is unclear why atopic individuals have a hyper-reactive immune system and an increased risk of developing allergy, but female sex, decreased microbial exposure, and the molecular properties of the allergens are thought to be involved.[2]

Allergens are typically clustered by structure;[3] the major allergens derived from mammals are usually lipocalins, with beta-lactoglobulin (BLG)[4] being a typical example, whereas the major respiratory plant allergens commonly belong to the pathogenesis-related protein 10 (PR-10) family, with the major birch pollen allergen Bet v 1 representing its prototype. Members of both the lipocalin and the PR-10 families have very low sequence homology (often <20%) but very well-conserved structures, which provide a binding pocket for ligands.[5]

In previous studies, we have shown that the binding pocket of allergens can bind to siderophore ligands of the catechol-type,[6] which are high-affinity iron-chelators of plant or

microbial origin. Allergens thereby can act bacteriostatically when binding to these iron complexes and withdraw iron from pathogens. Importantly, their loading state, apo (empty) or holo (filled), affects the immune system. While allergens without a ligand (apoallergens) promoted a T helper 2 cell response *in vitro*,[7,8] the holo forms were immunosuppressive. In subsequent *in vivo* studies with the milk protein BLG, only the apo form generated antigen-specific antibodies and induced an antigen-specific cytokine response in mouse splenocytes. In contrast, the holo form prevented antibody formation and cytokine release, but promoted the generation of regulatory T cells. This also resulted in reduced clinical reactivity upon allergen challenge in holo-BLG pretreated animals but not in apo-BLG pretreated animals. Mechanistically, the immunosuppressive properties of holo-BLG are connected to its iron-chelating ligands, which are known activators of the aryl hydrocarbon receptor (AhR) pathway.[9] BLG has been shown in *in vitro* studies to serve as a shuttle for iron chelators and to enhance AhR activation in a concentration-dependent manner. As such, only allergens with bound ligands prevented the onset of allergy by simultaneously presenting several stimuli to the immune cells: iron and an anti-inflammatory stimulus via AhR.

We conclude that proteins of the lipocalin and PR-10 families, when properly loaded, prevent T helper 2 cell responses and are not compliant with the term 'allergen'. In this setting, the immune cells are not only introduced to the specific antigen, but deliver iron to the AhR, initiating an anti-inflammatory signal that leads to specific tolerance induction.

References

1. Langen U et al. [Prevalence of allergic diseases in Germany: Results of the German health interview and examination survey for adults (DEGS1)]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2013;56(5-6):698-706. (In German).
2. Roth-Walter F et al. Linking iron-deficiency with allergy: Role of molecular allergens and the microbiome. Metallomics. 2017;9(12):166-92.
3. Jensen-Jarolim E et al. Structural similarities of human and mammalian lipocalins, and their function in innate immunity and allergy. Allergy. 2016;71(3):286-94.
4. Roth-Walter F et al. Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. Allergy. 2008;63(7):882-90.
5. Hufnagl K et al. Retinoic acid prevents immunogenicity of milk lipocalin Bos d 5 through binding to its immunodominant T-cell epitope. Sci Rep. 2018;8(1):1598.
6. Gómez-Casado C et al. Modeling iron-catecholates binding to NGAL protein. J Mol Graph Model. 2013;45: 111-21.
7. Roth-Walter F et al. Bet v 1 from birch pollen is a lipocalin-like protein acting as allergen only when devoid of iron by promoting Th2 lymphocytes. J Biol Chem. 2014;289(25):17416-21.
8. Roth-Walter F et al. The major cow milk allergen Bos d 5 manipulates T-helper cells depending on its load with siderophore-bound iron. PloS One. 2014;9(8):e104803.
9. Bialesova L et al. Agonistic effect of selected isoflavones on arylhydrocarbon receptor in a novel AZ-AhR transgenic gene reporter human cell line. Gen Physiol Biophys. 2015;34(3):331-4.

Response to Omalizumab in Urticaria and Asthma: Is it a 'Lifetime Together' Treatment?

Authors: *Ayse Fusun Kalpaklioglu,[1] Ayse Baccioglu,[1] Ahu Cerit[2]

1. Department of Immunology and Allergic Diseases, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
2. Department of Pulmonary Diseases, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey

*Correspondence to afusunk@yahoo.com

Disclosure: The authors have declared no conflicts of interest.

Keywords: Anti-immunoglobulin (Ig)E, asthma, urticaria.

Citation: EMJ Allergy Immunol. 2018;3[1]:76-78. Abstract Review No. AR5.

Omalizumab is a recombinant human monoclonal antibody that blocks the immunoglobulin (Ig)E receptor and is indicated in cases of severe

asthma (SA) and chronic idiopathic urticaria (CIU).[1,2] Clinical responses to omalizumab seem to be different in SA and CIU;[3,4] therefore, the aim of this study was to demonstrate the different effect omalizumab has on SA and CIU.

A pulmonary function test, skin prick test with aeroallergens (ALK, Hørsholm, Denmark), serum specific-IgE (ImmunoCAP™, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), and asthma control test were performed. Follow-up data of 49 patients, who were treated with omalizumab between March 2014 and June 2017, were evaluated 1 year after treatment. Out of the 49 patients, 23 were re-evaluated during this year (11 females). Most of the treated patients had CIU (n=38), while 23.1% (n=11) had SA; these figures almost doubled compared to 1 year ago (20 and 6 patients with CIU and SA, respectively). In patients with SA, omalizumab was given according to the patient's total IgE level and body weight, whereas in CIU a dose of 300 mg/month was used.

The responses of CIU and SA to omalizumab are shown in Table 1. Although the duration of CIU in patients was similar after 1 year, the duration of SA was significantly less 1 year after treatment (25.33±10.38 years and 16.90±3.81 years, respectively). Treatment compliance was significantly better in both groups after 1 year, despite the duration of omalizumab treatment remaining unchanged. Almost half of the CIU patients showed complete remission in both evaluations, while the number of cases of complete remission observed in the SA patients after 1 year (18.2%) was significantly increased compared to the previous year (0.0%; $p<0.05$). The majority of patients with CIU (>80%) had used antihistamine or leukotriene receptor antagonist therapeutics, whereas all patients with SA had used either inhaled corticosteroids or long-acting beta-agonists, six individuals had received additional oral steroids (compared to only one patient 1 year ago). However, 1 year ago, out of a total of 26 patients enrolled, 20 (76.9%) had CIU, with a female predominance of 53.8%.

Treatment failure dropped to almost half of the SA patients, but no change was observed in the CIU cohort. In addition, the recurrence rate following discontinuation of omalizumab was almost half in CIU patients (from 43.8% to 23.7% after 1 year) and improved even more dramatically in SA patients (from 83.3% to 18.2% after 1 year; $p<0.01$), which shows the power of compliance. No variables were related with remission, treatment failure, or recurrence.

Therefore, omalizumab appears to be more effective in CIU patients during the first year of treatment, whereas treatment of SA seems to improve equally in the first and second year.

Table 1: Comparison of response to omalizumab in chronic idiopathic urticaria and severe asthma.

	Chronic idiopathic urticaria				Severe asthma			
	Complete response (n=21)	Partial response (n=16)	No response (n=1)	p value	Uncontrolled (n=2)	Partial control (n=7)	Total control (n=2)	p value
Age (years)	45.19±14.72	34.87±13.96	57.00	0.06	48.00±12.77	38.42±15.44	48.50±7.77	0.57
Female, % (n)	38.09 (8)	75.00 (12)	0.00	0.12	50.00 (1)	42.50 (3)	0.00 (0)	0.32
Disease duration (years)	6.90±1.63	4.87±1.33	2.00	0.55	25.00±8.48	17.00±14.30	8.50±6.36	0.47
Treatment duration (months)	7.38±1.39	7.00±1.53	4.00	0.86	13.50±5.50	12.28±5.10	5.50±0.70	0.75
Patients still on treatment, % (n)	57.14 (12)	15.80 (6)	0.00	0.31	50.00 (1)	42.85 (3)	100.00 (2)	0.35
Incompliant, % (n)	4.80 (1)	62.50 (10)	100.00 (1)	0.008	50.00 (1)	0.00	0.00	0.08
Recurrence rate, % (n)	19.00 (4)	18.75 (3)	100.00 (1)	0.17	100.00 (2)	0.00	0.00	0.41

However, as omalizumab treatment continues, not only the treatment failure rate and the remission rate of the disease improve, as shown by an asthma control test, but, maybe more importantly, recurrence of SA is not seen. In conclusion, although quick relief and response to omalizumab in CIU seems to be an advantage for patients, this leads to less compliance and more disease recurrence.

References

1. Pelaia G et al. Targeted therapy in severe asthma today: Focus on immunoglobulin E. Drug Des Devel Ther. 2017;11:1979-87.
2. Chapman KR et al. The role of omalizumab in the treatment of severe allergic asthma. Can Respir J. 2006;13(Suppl B):1B-9.
3. Vestergaard C, Deleuran M. Chronic spontaneous urticaria: Latest developments in aetiology, diagnosis and therapy. Ther Adv Chronic Dis. 2015;6(6):304-13.
4. Kocatürk E et al. Looking forward to new targeted treatments for chronic spontaneous urticaria. Clin Transl Allergy. 2017;7:1.

Use of Breathomics in the Diagnosis of Paediatric Patients with Persistent Asthma

Authors: *João Cavaleiro Rufo

Basic and Clinical Immunology Laboratory, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal
*Correspondence to jcrufo@gmail.com

Disclosure: The author has declared no conflicts of interest.

Keywords: Asthma, breathomics, diagnosis, volatile organic compounds (VOC).

Citation: EMJ Allergy Immunol. 2018;3[1]:78-79. Abstract Review No. AR6.

In paediatric asthma, symptoms tend to appear during the first 6 years of life and may be associated with different disease phenotypes and endotypes, each responding differently to specific therapy. Therefore, asthma is more commonly used as a concept term comprising a set of non-specific symptoms (wheezing, dyspnoea, and dry cough), while proper identification of the pathophysiological origin of the associated symptoms is considered more important. This identification allows the delivery of a more targeted therapy to the patient, consequently reducing the risk of exacerbations.[1] For instance, persistent eosinophilic asthma with a characteristic Type 2 T helper cell inflammation is usually responsive to inhaled corticosteroid therapy, while neutrophilic phenotypes do not usually respond as well.

However, the currently available diagnostic tools are unable to determine these specific phenotypes at the point of care, and hence there is a need for new and improved asthma biomarkers to be implemented in clinical practice. Breathomics, the measurement of metabolites in the exhaled breath,[2] is currently being hypothesised as a possible technology to solve this problem, and several studies concerning measurement of volatile organic compounds (VOC) in exhaled breath have been published and significantly revised.[3,4] These reviews underlined the promising results of electronic nose (eNose) technologies as fast, portable, and sufficiently sensitive instruments for analysing VOC in exhaled breath samples.

For this reason, eNose breathomics was presented at this year's European Academy of Allergy and Clinical Immunology (EAACI) Congress in Munich, Germany as a technology that can improve asthma diagnosis.[5] In short, exhaled breath condensate samples collected from paediatric patients were processed and analysed using eNose breathomics technology. A multivariate analysis was performed and a hierarchical model was developed to segment different VOC profiles, creating two well-defined clusters. The results showed that individuals with persistent asthma who required corticosteroid therapy were significantly

agglomerated in a single cluster, thus highlighting that breathomics may be useful in identifying Type 2 T helper cell eosinophilic asthma phenotypes. Moreover, the diagnostic values were shown to surpass those from spirometry with bronchodilation, which is currently the most widely used technology to corroborate an asthma diagnosis.

Despite these promising results, external validation studies are still needed to completely understand the effectiveness of breathomics in a real clinical context. Furthermore, an eventual standardisation of the methods and procedures for exhaled breath sample processing is required, among other methodological questions that still need answering. Nevertheless, breathomics may be the solution to achieve one more goal in the gargantuan but honourable mission that has been assigned to researchers and clinicians alike: to improve asthma diagnosis and deliver the best possible treatment for patients.

References

1. Pavord ID et al. After asthma: Redefining airways diseases. Lancet. 2018;391(10118):350-400.
2. Bos LD et al. Breathomics in the setting of asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(4):970-6.
3. Cavaleiro Rufo J et al. Volatile organic compounds in asthma diagnosis: A systematic review and meta-analysis. Allergy. 2016;71(2):175-88.
4. Neerincx AH et al. Breathomics from exhaled volatile organic compounds in pediatric asthma. Pediatr Pulmonol. 2017;52(12):1616-27.
5. Cavaleiro Rufo J et al. Electronic nose analysis of exhaled breath condensate is able to identify paediatric individuals in need of corticosteroid therapy. Abstract 0150. EAACI Congress, 26-30 May, 2018.

Malignancies as the Main Cause of Death in Common Variable Immunodeficiency: An Italian Multicentre Study

Authors: *Federica Pulvirenti, Isabella Quinti

Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
*Correspondence to Federica.pulvirenti@uniroma1.it

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors would like to thank the researchers involved in this study: Prof Carlo Agostini and Dr Francesco Cinetto, University of Padua, Padua, Italy; Prof Antonio Spadaro, Dr Antonio Pecoraro, and Dr Ludovica Crescenzi, University of Naples, Naples, Italy; and Dr Cinzia Milito, Dr Anna Maria Pesce, and Dr Livia Bonanni, Sapienza University of Rome, Rome, Italy.

Keywords: Antibody defect, cancer, cancer immunosurveillance, common variable immunodeficiency (CVID), primary immunodeficiency.

Citation: EMJ Allergy Immunol. 2018;3[1]:79-80. Abstract Review No. AR7.

Cancer immunosurveillance is a major function of the immune system.[1] Strong evidence for the role of the immune system in antitumour surveillance mechanisms comes from the observation of the increased risk of malignancies among immunocompromised patients, including patients with primary immunodeficiencies, a heterogeneous group of diseases caused by >300 gene defects affecting natural and acquired immunity. The knowledge of defective genes and pathways[2] offers the opportunity to dissect the role of cancer immunosurveillance. An increased prevalence of cancer has been observed in patients affected by common variable immunodeficiency (CVID), which is the most commonly diagnosed primary antibody defect.[3,4] In particular, the risk for malignant lymphomas among patients with CVID was found to be 259-times higher in a USA cohort[5] and 30-times higher in a British cohort,[6] while the risk for gastric cancer was 47-times higher than expected in the British study.[5] In the last decade, thanks to immunoglobulin G replacement therapy, CVID patient life expectancy increased due to improvements

in surveillance, prevention, and treatment of recurrent and severe infections; however, the cancer mortality rates of these patients have not changed.

In a large CVID Italian cohort of 462 patients followed-up in three primary immunodeficiency care centres in Italy, we assessed cancer prevalence over a 30-year period. We aimed to estimate the prevalence and mortality rate due to haematological and gastrointestinal malignancies, as well as other cancers. Data on cancers in CVID patients were compared to normative data provided by the Italian Registry for Malignancies (AIRTUM).[7]

We collected data for a cumulative period of 5,326 years across all patients (mean ± standard deviation: 11.7±9.0 years). The prevalence of malignancies was 26.0%. For CVID patients, the risk of developing cancer was 50.0% at 65 years of age, whereas for the general population it was between 33.3% (females) and 50.0% (males) at 85 years of age. In the general population of Italy, breast and prostate cancer were the most frequent cancers diagnosed in females and males, respectively, followed by colorectal cancer. By contrast, in CVID patients, lymphoproliferative malignancies were the most commonly diagnosed cancers in both sexes (10%), followed by gastric cancer (6%). This emphasises the need for specific cancer screening programmes in CVID. The most common haematological malignancies were non-Hodgkin's B cell lymphomas, often involving extra nodal sites; however, T cell lymphomas were also recorded. While cardiovascular diseases were the primary cause of death in the Italian general population, malignancies were the primary cause of death in CVID patients, accounting for 58% of deaths, followed by infections (23%), chronic lung disease complications (13%), and autoimmunity (7%). The overall survival for patients affected by haematological cancers, gastric cancer, and other malignancies was 67%, 54%, and 88% at 1 year, respectively; 61%, 36%, and 80% at 2 years, respectively; and 61%, 27%, and 29% at 20 years, respectively. The treatment of CVID-associated cancers was similar to the treatment of cancers in other clinical settings. We observed a low rate of infection during chemotherapy and a high incidence of severe malabsorption in patients who underwent gastrectomy.

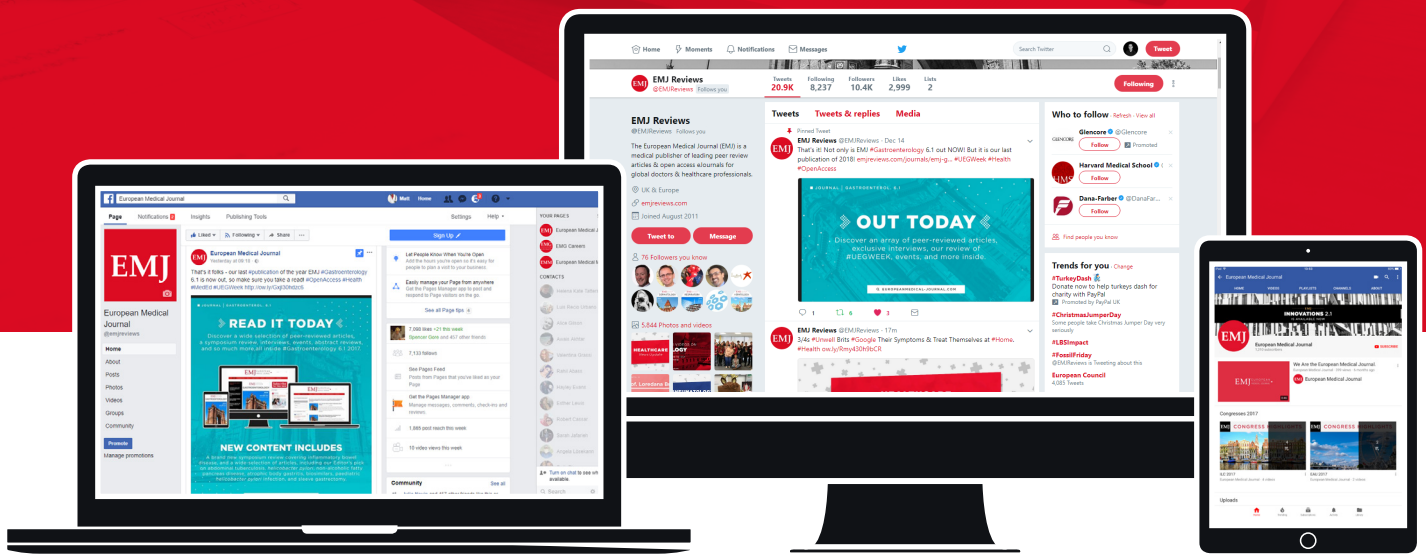
In conclusion, in this Italian cohort, cancer was the main cause of death in CVID patients. Despite immunodeficiency, patients with CVID with cancer might receive a full therapy regimen due to a risk of infection similar to that observed in the non-CVID cancer population. Cancer prevention strategies should be improved to ameliorate survival.

References

1. Dunn GP et al. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004;21(2):137-48.
2. Mayor PC et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol. 2018;141(3):1028-35.
3. Vajdic CM et al. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood. 2010;116(8):1228-34.
4. Quinti I et al. Malignancies are the major cause of death in patients with adult onset common variable immunodeficiency. Blood. 2012;120(9):1953-4.
5. Cunningham-Rundles C et al. Incidence of cancer in 98 patients with common varied immunodeficiency. J Clin Immunol. 1987;7(4):294-9.
6. Kinlen LJ et al. Prospective study of cancer in patients with hypogammaglobulinaemia. Lancet. 1985;1(8423): 263-6.
7. Associazone Italiana Registri Tumori. Available at: http://www.registri-tumori.it/cms/. Last accessed: 8 June 2018.

VIEW MORE ABSTRACTS ONLINE ←

Interact with us on social media.



Join the European Medical Journal community and discover news on the latest healthcare developments.

EUROPEANMEDICAL-JOURNAL.COM

Asthma and Food Allergies

EDITOR'S PICK

The relationship between food allergy and asthma is already well known. This review article looks at this relationship and suggests early intervention strategies in clinical practice. It is important to establish the presence of allergy early by appropriate testing and to start treatment, because the clinical implications for children with both diseases could be significant.

Prof Dr Jacques Bouchard

Laval's University, Canada

Authors: Ru-Xin Foong,[1] Kate Swan,[2] *Adam T. Fox[1]

1. Division of Asthma, Allergy and Lung Biology, Department of Paediatric Allergy, King's College London and Guy's and St. Thomas' Hospitals NHS Foundation Trust, London, UK
2. Paediatric Allergy Department, Guy's and St. Thomas' Hospitals NHS Foundation Trust, London, UK

*Correspondence to adam.fox@gstt.nhs.uk

Disclosure: The authors have declared no conflicts of interest.

Received: 04.03.18

Accepted: 09.05.18

Keywords: Anaphylaxis, asthma, children, food allergies.

Citation: EMJ Allergy Immunol. 2018;3[1]:82-88.

Abstract

There is a close association between various atopic diseases and it is well known that having one atopic disease can increase the risk of further atopy later in life. Research has shown that the development of food allergy in infancy can predispose individuals to the development of respiratory symptoms and subsequent asthma later in childhood. There is also evidence that shows early atopic conditions can be outgrown but may still influence the development of other atopic conditions, such as asthma, in the future. The exact mechanism of how this occurs is not yet fully understood, but the clinical implications for children with both diseases are important because not only are they at greater risk of more severe asthmatic episodes, but also of having respiratory symptoms in food-induced anaphylaxis. This narrative review looks at the relationship between food allergy and asthma and how they are linked to one another. It will also focus on the clinical implications associated with the two atopic conditions and the effect they may have on clinical practice.

BACKGROUND

There is a long-established link between allergic diseases in atopic individuals, whereby having one atopic condition can predispose that individual to others. These diseases include asthma, allergic rhinitis or hayfever, eczema, and food allergies, and they are increasingly common in the paediatric population. There are a multitude of contributing factors, including genetic and environmental, with research providing supportive evidence that there are genes that predispose individuals to atopic conditions.[1,2] A recent systematic review of sibling and twin data found that genetics played a bigger role in predisposing eczema patients to both hayfever and asthma compared to environmental factors, but the link between these atopic conditions was independent of shared early-life environmental factors.[2]

The natural progression in the development of atopy is often referred to as the 'atopic march'.[2,3] The most common pathway along the atopic march is for children to develop eczema during infancy and then, as they get older, they may develop food allergies, followed by allergic rhinitis and asthma. Allergic sensitisation to food early in life has been shown to be associated with the later development of respiratory symptoms and/or asthma. This review article looks more closely at this link between food allergy and asthma.

FOOD ALLERGY

The prevalence of food allergy has increased over the last 20 years,[4,5] which has also led to increased research into food allergies in children. Depending on the country, approximately 4–10% of children have food allergies, which usually develop early in life.[6-8] A food allergy is defined as an adverse immunological reaction that occurs upon exposure to a food and is reproducible following repeat exposure.[9] These immunological reactions are classified as immunoglobulin E (IgE) or non-IgE depending on the clinical history of presenting symptoms and the results of the investigations, such as skin prick tests, specific IgE levels in the blood, and oral food challenges. Food allergies often present during infancy; in fact, research has shown that in some locations >10% of children aged 1 year old have a food allergy.[10] Common foods that children are allergic to include eggs, cow's milk, wheat, and peanuts.[8] There are various routes by which allergen exposure can occur; for example, it can occur orally via the gastrointestinal tract, cutaneously via the skin barrier, or via inhalation through the respiratory tract. Allergy develops following exposure of an allergen to the immune system, namely antigen-presenting cells that engulf the allergen and activate naïve CD4 T-helper lymphocytes, which results in the production of antigen-specific antibodies to the allergen by mature B cells. These antibodies bind to mast cell surface receptors in various tissues of the body, as well as to cell-surface receptors on basophils in the bloodstream. Thus, on repeat exposure, the allergen binds and crosslinks these specific IgE antibodies triggering degranulation and release of inflammatory mediators, thus causing an allergic reaction.[11] IgE-mediated allergic reactions to food have a rapid onset after exposure to the allergen (usually <2 hours) and can present with various symptoms, such as respiratory (wheeze, shortness of breath, difficulty in breathing), gastrointestinal (vomiting, diarrhoea), and skin (urticaria, rash), or, if severe, anaphylaxis. Lower respiratory symptoms are commonly seen in food-allergic reactions in asthma patients, although asthma is rarely seen as the sole manifestation of food allergy presentations.[12] Non-IgE-mediated food allergy has a slower onset of symptoms, which can be chronic in nature due to ongoing allergen exposure and can occur if the association between the allergen and the symptoms is not recognised. Symptoms of non-IgE-mediated allergy mimic many common childhood conditions, such as eczema, gastro-oesophageal reflux, and constipation, but can also present with chronic mucousy stools.

Children with food allergies are at risk of developing other allergic conditions, but there are little data available on long-term outcomes of food allergy in infancy and childhood in terms of the future development of other allergic conditions. A recent study by Peters et al.[13] found that 40–50% of the children diagnosed with challenge-confirmed food allergy at 1 year of age had symptoms of an allergic disease, such as wheeze, itchy rash, and/or nose symptoms, in the first 4 years of their life.

ASTHMA

Asthma is a chronic disease affecting approximately 9% of children around the world and is characterised by inflammation of the airways and bronchial hyper-reactivity causing recurrent symptoms of cough, wheeze, shortness of breath, and difficulty breathing.[2,14] The pathophysiology in acute episodes of airway narrowing is the result of a combination of factors, including an increase in populations of inflammatory cells (i.e., mast cells, eosinophils, macrophages, lymphocytes, dendritic cells), which, when triggered, produce mediators that result in airway hyper-responsiveness and narrowing.[15] However, with recurrent episodes and disease progression, airway changes can continue to progress and eventually result in airway remodelling, such as increased smooth muscle, thickening of the basement membrane, and a loss of normal distensibility of the airway.[15] Traditionally, there has been a focus on controlling the inflammation with inhaled corticosteroids and relieving the bronchial constriction with bronchodilators such as salbutamol. This occurs in conjunction with anticipation of the impact of physical triggers such as exercise, pollution, and cold air. While this is still important, there is an increasing recognition that there may be an allergic component in the development of asthma with aeroallergens (i.e., house dust mite, cat and dog dander, grass and tree pollens) being predominant triggers. These children often have positive skin prick tests and/or specific IgE blood tests to the common allergens, which makes it relatively straightforward to identify the triggers and, in some cases, make efforts to try and avoid them.[16] There have also been studies that show that children as young as 6 months of age with high levels of IgE and reactivity to aeroallergens have an increased risk of developing asthma.[17,18]

Various atopic phenotypes have been reported in the literature that describe how the presence of different risk factors (i.e., sensitisation to specific allergens) are linked to the risk of asthma progression.[19] The phenotypes include sensitisation based on type of allergen (e.g., dust mite compared to non-dust mite) or early or late sensitisation.[19] One study found that the risk of new-onset bronchial hyper-responsiveness was highest in children who had early sensitisation to outdoor allergens (including *Alternaria* mould) and later sensitisation to indoor allergens (including *Aspergillus* mould).[20] Other studies have looked at sensitisation in birth cohorts to various allergens, and these studies resulted in the finding that the development of asthma at 6 years of age was associated with earlier sensitisation to dog and cat allergens.[21,22]

There is also evidence to suggest that aerosolised food proteins can induce food-triggered asthmatic episodes as the inhalation of allergenic food proteins stimulates an inflammatory reaction of the mast cells in the airways causing wheeze and shortness of breath.[14] Occupational asthma has been described extensively in adults because of chronic inhalation of food allergens in a work environment. The wheeze of a baker with asthma occurs due to inhaled flour proteins triggering a localised IgE-mediated reaction.[23] Chronic exposure to aerosolised fish can cause the same problem and these aerosolised proteins have been detected in fish markets.[24] In a study of a paediatric cohort with proven IgE-mediated food allergy and asthma, the researchers found that even with dietary avoidance of the food the children were allergic to (i.e., fish, milk, eggs, chickpeas, buckwheat), if the families continued to cook the allergenic food in the home environment, the children's asthma symptoms were worsened due to environmental exposure to the food. However, if the families stopped cooking the allergenic foods in the home environment, there was a reduction in the child's asthma symptoms and also in their need for inhaled corticosteroid treatment.[25]

There have also been studies that have looked at respiratory symptoms as a result of exposure to airborne food particles on flights.[26] In a study on adults who self-reported an allergic reaction to peanuts, tree nuts, or seeds during a flight, 9% reported a reaction on planes, with the most commonly reported mode of exposure being inhalation of airborne particles.[26,27] However, in a study of children with severe or reported inhalational reactions to peanut who had blind inhalational peanut challenges (i.e., peanut butter was held 12 inches from the face for 10 minutes), they did not exhibit any allergic symptoms.[28] Furthermore, a study conducted by Brough et al.[29] looked at the distribution of

peanut protein in the home environment by measuring airborne peanut protein levels in a number of simulated scenarios and found that peanut protein was unlikely to be transferred into the environment by aerosolisation.

THE LINK BETWEEN ASTHMA AND FOOD ALLERGY

Food allergy and asthma are known to coexist, but the extent to which they may impact one another is still not fully established. Approximately half of children with food allergies have reactions that involve respiratory symptoms[30] and of children who have asthma, 4–8% have food allergy.[31]

There is indirect evidence that food allergic infants have an increased risk of developing asthma later in life.[32-34] Illi et al.[35] found that a strong predictor of asthma development by school age was food sensitisation early in life (i.e., before 2 years of age) either with or without concurrent inhalant sensitisation. Another recent large retrospective birth cohort study showed that food allergy was associated with the development of asthma and rhinitis, and rates were approximately double in those children with food allergies compared to children in the general population.[36] More specifically, the researchers found that the children with allergies to peanuts, milk, and eggs, as well as those with multiple food allergies, had a significant increased risk of developing respiratory allergy (i.e., rhinitis and/or asthma).

Studies have been performed that looked at specific foods and their potential link to asthma. Priftis et al.[34] found that children who were allergic to egg or fish in infancy were at a greater risk of having wheeze and hyper-reactive airways at school age. A study of a Danish birth cohort of 562 children resulted in the finding that both transient and persistent early-life sensitisation to egg was associated with asthma and rhinoconjunctivitis at 14 years of age.[37] This was also supported by evidence from an Isle of Wight birth cohort study[38] that showed egg allergy in infancy was associated with the development of respiratory symptoms and aeroallergen sensitisation by 4 years of age. Furthermore, the authors reported a positive predictive value for asthma of 40% if the child had an egg allergy.[38] Another study by Rhodes et al.[39] found that for infants at a higher risk of developing atopic disease due to a parental family history of atopy, sensitisation to egg and milk in the first year of life was a predictive feature of developing asthma in adulthood.

Research looking at allergen molecules through microarrays provides supporting evidence that sensitisation to allergen molecules (both food and aeroallergens) in early childhood can precede asthma and rhinitis in adolescence.[40,41] More recently, Vermeulen et al.[42] conducted a population-based study that showed children with oral challenge-proven food allergy in infancy have an increased risk of asthma at 4 years of age irrespective of whether their food allergy resolves. They reported that children with ≥2 food allergies and coexistent eczema were also three-times more likely to develop asthma compared to children without food allergies.

MORBIDITY AND MORTALITY

The impact of this link between asthma and food allergy in terms of morbidity and mortality has also been researched, with asthma being a risk factor for fatal or near-fatal anaphylaxis to foods.[12] In a study of asthmatic adults, those who also had an allergy to >1 food were found to have a higher risk of lifetime hospitalisations and visits to the emergency department for asthma as well as increased use of oral corticosteroids.[43] In a study conducted by Simpson et al.[44] including children aged 3 months to 14 years old, those who had milk and peanut allergies had a significantly increased number of hospitalisations due to asthma. Other studies have been performed looking at patients with near-fatal asthma (i.e., requiring ventilation in an intensive care unit) and have found that they were more likely to have a food allergy and/or have had anaphylaxis.[45] Roberts and Lack[31] performed a study that recruited children aged between 1 and 16 years old who had been ventilated for an acute asthma exacerbation in paediatric intensive care and compared these children to matched controls. The researchers found that food allergy was an independent risk factor for life-threatening asthma. It is likely that asthma is a risk factor for anaphylaxis and may be

associated with poorer outcomes for children with food allergy. A study found that children with cow's milk allergy had a 10-fold greater risk of a severe reaction if they also had asthma.[46] More specifically, with regard to actual fatalities, in a cohort of children with peanut allergy, 9% (4/46) of the children died of asthma exacerbation.[47,48] Bock et al.[47] also performed a series looking at food-related anaphylactic fatalities and reported that the majority of the children had a diagnosis of asthma and, for the most severe reactions, respiratory symptoms were most predominant.

CLINICAL IMPLICATIONS AND THE EFFECT ON PRACTICE

This close relationship between asthma and food allergies has, therefore, influenced the way in which clinicians approach children with atopy. As in any consultation, clinical history becomes vital in managing these children, especially if there is a clear account of food exposure causing respiratory symptoms in a child diagnosed with asthma. Alongside this, the use of skin prick testing and specific food and aeroallergen IgE-testing may be useful to confirm allergies and identify triggers. The diagnosis of asthma can be complicated because various asthma phenotypes exist;[49] however, chronic asthma is largely managed with the use of inhaled short and long-acting beta agonists, inhaled corticosteroids, leukotriene receptor antagonists, and systemic corticosteroids. This takes place alongside regular assessment of symptoms, lung function tests if appropriate, and regular reviews for adherence and compliance to treatment.[50]

If an immediate food allergy is identified, the advice is to avoid the allergen. Patients should be equipped with personalised emergency action plans, which should include the administration of an adrenaline autoinjector in the presence of any signs of anaphylaxis (airway, respiratory, or cardiovascular compromise) in patients with both food allergy and asthma or in those patients who have previously experienced anaphylaxis to any food allergen.[51,52] The use of antihistamines in accidental exposure is advised for mild-to-moderate reactions. In an acute emergency presentation of what appears to be life-threatening asthma, in children with both asthma and food allergies, it is also important that the diagnosis of anaphylaxis is considered along with the use of intramuscular adrenaline (in conjunction with ongoing medical management of the presentation of an acute exacerbation of asthma). In most circumstances of anaphylaxis there will also be other features of an immediate allergic reaction, such as cutaneous signs that will guide the use of adrenaline if a history of allergen exposure was not forthcoming.

FUTURE IMPLICATIONS AND AREAS OF RESEARCH

There has been increasing research on the use of immunomodulators in the treatment of allergic diseases, such as the use of monoclonal antibodies. Omalizumab is a monoclonal antibody that selectively binds IgE and has been used in the treatment of allergic asthma.[53] With regard to asthma, omalizumab has been shown to help reduce exacerbation rates, the number of hospitalisations and missed school days, daily rescue medication use, and symptom days.[54] It has also been used in conjunction with allergen immunotherapy, as well as for the treatment of food allergies (i.e., milk, peanut, and egg) in the research setting, with studies showing it may be able to aid in desensitisation to allergic foods but also decrease basophil activation.[55-57] Further research into the therapeutic role of immunomodulators in children with both food allergy and asthma is required to better evaluate the safety of use but also the long-term maintenance of tolerance.

CONCLUSION

There is increasing evidence to suggest that food allergies and asthma in children are linked. Children who develop food allergies are at greater risk of developing asthma, and even those infants who outgrow their allergies may have respiratory symptoms that persist and develop into asthma.[13,42] Those that have both atopic conditions are at increased risk of severe asthmatic episodes, allergen-triggered asthma episodes, and food-induced anaphylaxis. Therefore, clinicians need to be mindful of this associated link in acute presentations of asthma exacerbations or food-induced anaphylaxis to ensure appropriate treatment is delivered.

References

1. Tamari M et al. Genome-wide association studies of allergic diseases. Allergol Int. 2013;62(1):21-8.
2. Khan SJ et al. Is the atopic march related to confounding by genetics and early-life environment? A systematic review of sibship and twin data. Allergy. 2018;73(1):17-28.
3. Shaker M. New insights into the allergic march. Curr Opin Pediatr. 2014;26(4):516-20.
4. Anagnostou K et al. The rapidly changing world of food allergy in children. F1000Prime Rep. 2015;7:35.
5. Prescott SL et al. A global survey of changing patterns of food allergy burden in children. World Allergy Organ J. 2013;6(1):21.
6. Sampson HA et al. Food allergy: A practice parameter update-2014. J Allergy Clin Immunol. 2014;134(5):1016-25.e43.
7. Lee S. IgE-mediated food allergies in children: Prevalence, triggers, and management. Korean J Pediatr. 2017;60(4):99-105.
8. Turnbull JL et al. Review article: The diagnosis and management of food allergy and food intolerances. Aliment Pharmacol Ther. 2015;41(1):3-25.
9. Sampson HA. Update on food allergy. J Allergy Clin Immunol. 2004;113(5):805-19; quiz 820.
10. Osborne NJ et al.; HealthNuts Investigators. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. J Allergy Clin Immunol. 2011;127(3):668-76.
11. Foong RX, Brough H. The role of environmental exposure to peanut in the development of clinical allergy to peanut. Clin Exp Allergy. 2017;47(10):1232-8.
12. Kewalramani A, Bollinger ME. The impact of food allergy on asthma. J Asthma Allergy. 2010;3:65-74.
13. Peters RL et al.; HealthNuts Study. The prevalence of food allergy and other allergic diseases in early childhood in a population-based study: HealthNuts age 4-year follow-up. J Allergy Clin Immunol. 2017;140(1):145-53.e8.
14. Caffarelli C et al. Asthma and food allergy in children: Is there a connection or interaction? Front Pediatr. 2016;4:34.
15. Papadopoulos NG et al. International consensus on (ICON) pediatric asthma. Allergy. 2012;67(8):976-97.
16. Milgrom H et al. Treatment of childhood asthma with anti-immunoglobulin E antibody (omalizumab). Pediatrics. 2001;108(2):E36.
17. Burrows B et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med. 1989;320(5):271-7.
18. Mrazek DA et al. Prediction of early-onset asthma in genetically at-risk children. Pediatr Pulmonol. 1999;27(2):85-94.
19. Oksel C, Custovic A. Development of allergic sensitization and its relevance to paediatric asthma. Curr Opin Allergy Clin Immunol. 2018; 18(2):109-16.
20. Lee E et al. Association of atopy phenotypes with new development of asthma and bronchial hyperresponsiveness in school-aged children. Ann Allergy Asthma Immunol. 2017;118(5):542-50.
21. Schoos AM et al. Sensitization trajectories in childhood revealed by using a cluster analysis. J Allergy Clin Immunol. 2017;140(6):1693-9.
22. Stoltz DJ et al. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. Clin Exp Allergy. 2013;43(2):233-41.
23. Quirce S, Diaz-Perales A. Diagnosis and management of grain-induced asthma. Allergy Asthma Immunol Res. 2013;5(6):348-56.
24. Taylor AV et al. Detection and quantitation of raw fish aeroallergens from an open-air fish market. J Allergy Clin Immunol. 2000; 105(1 Pt 1):166-9.
25. Roberts G et al. Bronchial challenges with aerosolized food in asthmatic, food-allergic children. Allergy. 2002;57(8):713-7.
26. Comstock SS et al. Allergic reactions to peanuts, tree nuts, and seeds aboard commercial airliners. Ann Allergy Asthma Immunol. 2008;101(1):51-6.
27. Sicherer SH et al. Self-reported allergic reactions to peanut on commercial airliners. J Allergy Clin Immunol. 1999;104(1):186-9.
28. Simonte SJ et al. Relevance of casual contact with peanut butter in children with peanut allergy. J Allergy Clin Immunol. 2003;112(1):180-2.
29. Brough H et al. Distribution of peanut protein in the home environment. J Allergy Clin Immunol. 2013;132(3):623-9.
30. James JM. Respiratory manifestations of food allergy. Pediatrics. 2003; 111(6 Pt 3):1625-30.
31. Roberts G, Lack G. Food allergy and asthma--What is the link? Paediatr Respir Rev. 2003;4(3):205-12.
32. Alduraywish SA et al. The march from early life food sensitisation to allergic disease: A systematic review and meta-analyses of birth cohort studies. Allergy. 2016;71(1):77-89.
33. Saarinen KM et al. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. J Allergy Clin Immunol. 2005;116(4):869-75.
34. Priftis KN et al. Asthma symptoms and bronchial reactivity in school children sensitized to food allergens in infancy. J Asthma. 2008;45(7): 590-5.
35. Illi S et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. J Allergy Clin Immunol. 2001;108(5):709-14.
36. Hill DA et al. The epidemiologic characteristics of healthcare provider-diagnosed eczema, asthma, allergic rhinitis, and food allergy in children: A retrospective cohort study. BMC Pediatr. 2016;16:133.
37. Christiansen ES et al. Early-life sensitization to hen's egg predicts asthma and rhinoconjunctivitis at 14 years of age. Pediatr Allergy Immunol. 2017;28(8):776-83.
38. Tariq SM et al. Egg allergy in infancy predicts respiratory allergic disease by 4 years of age. Pediatr Allergy Immunol. 2000;11(3):162-7.
39. Rhodes HL et al. Early life risk factors for adult asthma: A birth cohort study of subjects at risk. J Allergy Clin Immunol. 2001;108(5):720-5.
40. Wickman M et al. Detection of IgE reactivity to a handful of allergen molecules in early childhood predicts respiratory allergy in adolescence. EBioMedicine. 2017;26:91-9.
41. Westman M et al.; Mechanisms for the Development of Allergies Consortium. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. J Allergy Clin Immunol. 2015;135(5):1199-2016.
42. Vermeulen EM et al. Food allergy is an important risk factor for childhood asthma, irrespective of whether it resolves. J Allergy Clin Immunol Pract. 2017. [Epub ahead of print].
43. Berns SH et al. Food allergy as a risk factor for asthma morbidity in adults. J Asthma. 2007;44(5):377-81.
44. Simpson AB et al. Food allergy and asthma morbidity in children. Pediatr Pulmonol. 2007;42(6):489-95.
45. Mitchell I et al. Near-fatal asthma: A population-based study of risk factors. Chest. 2002;121(5):1407-13.
46. Boyano-Martínez T et al. Accidental allergic reactions in children allergic to cow's milk proteins. J Allergy Clin Immunol. 2009;123(4):883-8.
47. Bock SA et al. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol. 2001; 107(1):191-3.
48. Bock SA et al. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol. 2007;119(4):1016-8.
49. Brand PL et al. Definition, assessment and treatment of wheezing disorders in preschool children: An evidence-based approach. Eur Respir J. 2008;32(4):1096-110.
50. British Thoracic Society (BTS)/ Scottish Intercollegiate Guidelines Network (SIGN). British guideline

on the management of asthma: Quick reference guide. 2016. Available at: https://www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-quick-reference-guide-2016/. Last accessed: 10 May 2018.

51. Muraro A et al.; EAACI Food Allergy and Anaphylaxis Guidelines Group. Anaphylaxis: Guidelines from the European Academy of Allergy and Clinical Immunology. Allergy. 2014;69(8):1026-45.
52. European Academy of Allergy and Clinical Immunology (EAACI). EAACI Food Allergy and Anaphylaxis Public Declaration. 2015. Available at: http://www.eaaci.org/outreach/public-declarations/2648-food-allergy-anaphylaxis-2012.html. Last accessed: 10 May 2018.
53. Dantzer JA, Wood RA. The use of omalizumab in allergen immunotherapy. Clin Exp Allergy. 2018;48(3):232-40.
54. Anderson WC III et al. Approaching current and new drug therapies for pediatric asthma. Pediatr Clin North Am. 2017;64(6):1197-207.
55. Stokes J. Anti-IgE treatment for disorders other than asthma. Front Med (Lausanne). 2017;4:152.
56. Nadeau KC et al. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. J Allergy Clin Immunol. 2011;127(6):1622-4.
57. Frischmeyer-Guerrerio PA et al. Mechanistic correlates of clinical responses to omalizumab in the setting of oral immunotherapy for milk allergy. J Allergy Clin Immunol. 2017;140(4):1043-53.

The Oligosaccharide Galactose-α-1,3-Galactose and the α-Gal Syndrome: Insights from an Epitope that is Causal in Immunoglobulin E-Mediated Immediate and Delayed Anaphylaxis

Authors: *Jeffrey M. Wilson, Thomas A.E. Platts-Mills

Division of Allergy & Clinical Immunology, University of Virginia, Charlottesville, Virginia, USA
*Correspondence to jmw2gc@virginia.edu

Disclosure: Dr Platts-Mills has a patent on an α-Gal IgE assay and has received material support from Thermo Fisher/Phadia and grants from the National Institutes of Health (NIH). Dr Wilson has declared no conflicts of interest.

Acknowledgements: We thank Dr Ira Schulman and Dr Kevin Lynch for critically reviewing the manuscript.

Received: 12.02.18

Accepted: 16.04.18

Keywords: Allergen, delayed anaphylaxis to red meat, food allergy, galactose-α-1,3-galactose (α-Gal), glycolipid.

Citation: EMJ Allergy Immunol. 2018;3[1]:89-98.

Abstract

Galactose-α-1,3-galactose (α-Gal) is an oligosaccharide that was first described as a cause of immunoglobulin E-mediated anaphylaxis in cases of first-in-man reactions to the monoclonal antibody cetuximab. Soon thereafter, immunoglobulin E antibodies to this epitope were linked with anaphylactic episodes to mammalian meat, which had a characteristic delay of ~3-6 hours. The 'α-Gal syndrome' is now recognised globally as a significant form of food allergy, albeit with regional variation, which reflects that sensitisation relates to bites from certain species of hard tick. The α-Gal epitope is present in organs and muscles from most mammals (with the exception of humans, apes, and Old World monkeys) as a glycan conjugated to both proteins and lipids. There are a number of unusual features that distinguish α-Gal from other traditional food allergies, including the fact that the oligosaccharide can be causal in both immediate and delayed allergic responses, and that co-factors, such as alcohol or exercise, often relate to the instigation and/or severity of clinical reactions. In this narrative review, the authors focus on the novelty of α-Gal's intrinsic lipid form; consider aspects of glycolipid digestion, absorption, and processing; and explain how this 'glycolipid hypothesis' may explain several of the clinical features of α-Gal syndrome. This review draws on pioneering studies of the biochemistry of α-Gal, contemporary understanding of lipid metabolism, and comparisons to other clinically important oligosaccharides.

INTRODUCTION

It has been 10 years since the blood group antigen galactose-α-1,3-galactose (α-Gal), an oligosaccharide produced by non-primate mammals, was identified as an important allergen epitope in immediate anaphylaxis to the monoclonal antibody cetuximab.[1,2] While carbohydrates had been previously recognised as targets of immunoglobulin (Ig)E, this was the first example of a carbohydrate epitope that commonly contributed to severe clinical symptoms: i.e., anaphylaxis. In 2009, it was reported that α-Gal was an important allergen in episodes of delayed anaphylaxis to mammalian meat.[3] As a result of reactions occurring to a variety of mammalian products, including meat, innards, and gelatin, this allergy is now commonly referred to as the 'α-Gal syndrome'. To date, the authors are aware of published reports of α-Gal syndrome from North and Central America, Europe, Asia, Australia, Africa, and, anecdotally, from South America.[4,5]

When considering the immune response to α-Gal, it is important to realise that this is the same antigen that was initially described by Landsteiner and Miller[6] as 'B-like'. The relevance is that immunocompetent humans produce abundant natural antibodies (i.e., IgM, IgG2, and IgA, specific to α-Gal)[7,8] presumably related to immune recognition of α-Gal-laden enteric Gram-negative bowel flora.[9] An important feature of α-Gal is that it can be present as both glycoproteins and glycolipids,[10,11] and that there can be significant diversity in the complexity of the oligosaccharide. For example, some glycoconjugates have a single terminal α-Gal epitope, but upwards of eight branches with terminal α-Gal have been reported.[12,13] It is currently unclear whether this complexity affects epitope recognition in hypersensitivity reactions.

Remarkably, it has become clear that bites from certain hard ticks are important agents for the induction of IgE specific for α-Gal.[14] In the USA, the lone star tick (*Amblyomma americanum*) is the major contributor, but, in other parts of the world where there are no lone star ticks, other hard ticks have been implicated. A likely scenario is that factors in tick saliva favour the class-switch of pre-existing natural antibody-producing α-Gal-specific B cells. Indeed, it is well established that saliva from hard ticks can favour the production of host T helper 2 cell (Th2)-related cytokines.[15,16] Two groups have now identified the presence of a carbohydrate with a terminal α-Gal moiety in hard ticks. Hamsten et al.[17] reported α-Gal in the gut of *Ixodes ricinus* and, more recently, α-Gal was described in the saliva of *Amblyomma sculptum*.[18] The latter report by Araujo et al.[18] went on to demonstrate that tick saliva was sufficient to induce specific IgE to α-Gal using a humanised mouse model (i.e., an α-Gal knock-out mouse), further bolstering the argument that ticks are an important cause of IgE sensitisation to α-Gal. While these experiments suggest that some hard ticks intrinsically produce α-Gal, an alternative possibility is that ticks could harbour bacterial symbionts that are the source of the α-Gal. The latter possibility is interesting in light of the fact that α-Gal syndrome appears to be uncommon in some areas of the south-eastern USA that are reported to have established lone star tick populations;[19] however, an alternative explanation may be that tick populations have been dynamic, and existing tick maps rely on historical data.[20] In any event, the mechanism whereby tick bites favour IgE induction remains an important but open question, as well as the possibility that there are other mechanisms of sensitisation. Taken together, the α-Gal syndrome has a number of features that are unusual for an IgE-mediated allergy (Box 1A). This article reviews the evidence that both immediate and delayed forms of anaphylaxis to mammalian products can be mediated by IgE specific to α-Gal and speculates on the mechanisms that explain this discrepancy.

EVIDENCE THAT IMMUNOGLOBULIN E IS CAUSAL IN DELAYED ANAPHYLAXIS TO RED MEAT

It is well established that the mammalian meat allergy related to α-Gal typically has a delayed response: i.e., the reactions occur >2 hours after exposure.[21] This was apparent from the initial cases that were identified in south-eastern USA and was confirmed in prospective controlled food challenges. Ten of twelve adult subjects with detectable specific IgE to α-Gal and self-reported history of reactions to

meat experienced a hypersensitivity reaction following a monitored challenge with 150 g of pork or beef sausage.[22] None of the reactions were evident before 2.5 hours and the mean time to reaction was 4 hours and 12 minutes. Moreover, clinical reactions were largely mirrored by the time course of *ex vivo* basophil activation, which peaked at 4 or 5 hours post challenge.[22] The time course of reactions to mammalian meat clearly differs from those that occur to cetuximab, which typically occur within the first 30 minutes of the first antibody infusion.[1,23] Notably, very few patients who have had reactions to cetuximab attempted a second dose.

There are several lines of evidence that support specific IgE as being causal in the delayed reaction that occurs in α-Gal syndrome (Box 1B). Perhaps the most compelling is that *in vitro* stimulation of α-Gal sensitised basophils, with cetuximab or α-Gal-laden glycoproteins, elicits rapid stimulation as judged by CD63 expression (i.e., within 30 minutes).[22,24] This is in contrast to the delayed kinetics of basophil activation examined *ex vivo* from peripheral blood mononuclear cells drawn following oral meat challenge. The working hypothesis to explain how IgE sensitisation to α-Gal could mediate delayed reactions to mammalian meat relates to the time required for digestion, absorption, processing, and presentation of glycoconjugates in a form that could be recognised by IgE bound to the surface of tissue mast cells or circulating basophils. An explanation that seems likely is that the delay specifically involves the glycolipid forms of α-Gal. The idea that the causal mechanism involves immune pathways other than IgE cannot be excluded, although to date this has not been supported by direct evidence. For example, activation of CD4+ T cells by an oligosaccharide would be unexpected via traditional major histocompatibility complex II, except in the case of zwitterions. Non-canonical presentation via CD1 molecules to T cells or natural killer T cells remains a possibility, but has been little explored.[25,26] Another possibility is that specific IgG1, an immunoglobulin subtype that increases in parallel with α-Gal specific IgE, could play a role by activating FcγR-expressing haematopoietic cells.[27,28] Taken together, existing data strongly supports a role for specific IgE to α-Gal as causal in delayed reactions to mammalian meat, but further research may uncover additional immune cells and molecular mediators that are also important.

Box 1: A) Ways that α-Gal syndrome differs from traditional immunoglobulin E-mediated food allergies. B) Evidence that immunoglobulin E to galactose-α-1,3-galactose is causal in delayed reactions to ingested mammalian products.

A: Ways that α-Gal syndrome differs from traditional immunoglobulin E-mediated food allergies.
1. The allergen epitope is an oligosaccharide, not a protein.
2. Anaphylactic reactions to mammalian meat are delayed, usually ~3–6 hours.
3. Primary sensitisation is mediated via tick bites, not oral or inhalant exposure.
4. Allergy onset is most often in adults.
5. Lipids are an important source of the allergen epitope in meat.
6. Skin prick tests with meat extract are often not adequate for diagnosis.
B: Evidence that immunoglobulin E to galactose-α-1,3-galactose is causal in delayed reactions to ingested mammalian products.
1. Skin reactions with intradermal testing occur rapidly; i.e., within 15 minutes.
2. *In vitro* basophil activation occurs rapidly upon stimulation with galactose-α-1,3-galactose glycoconjugates.
2. Upon mammalian meat ingestion, activation of basophils occurs with a delay as assessed by *ex vivo* analysis.
3. Parenteral administration of a molecule with galactose-α-1,3-galactose (cetuximab) elicits rapid reactions.

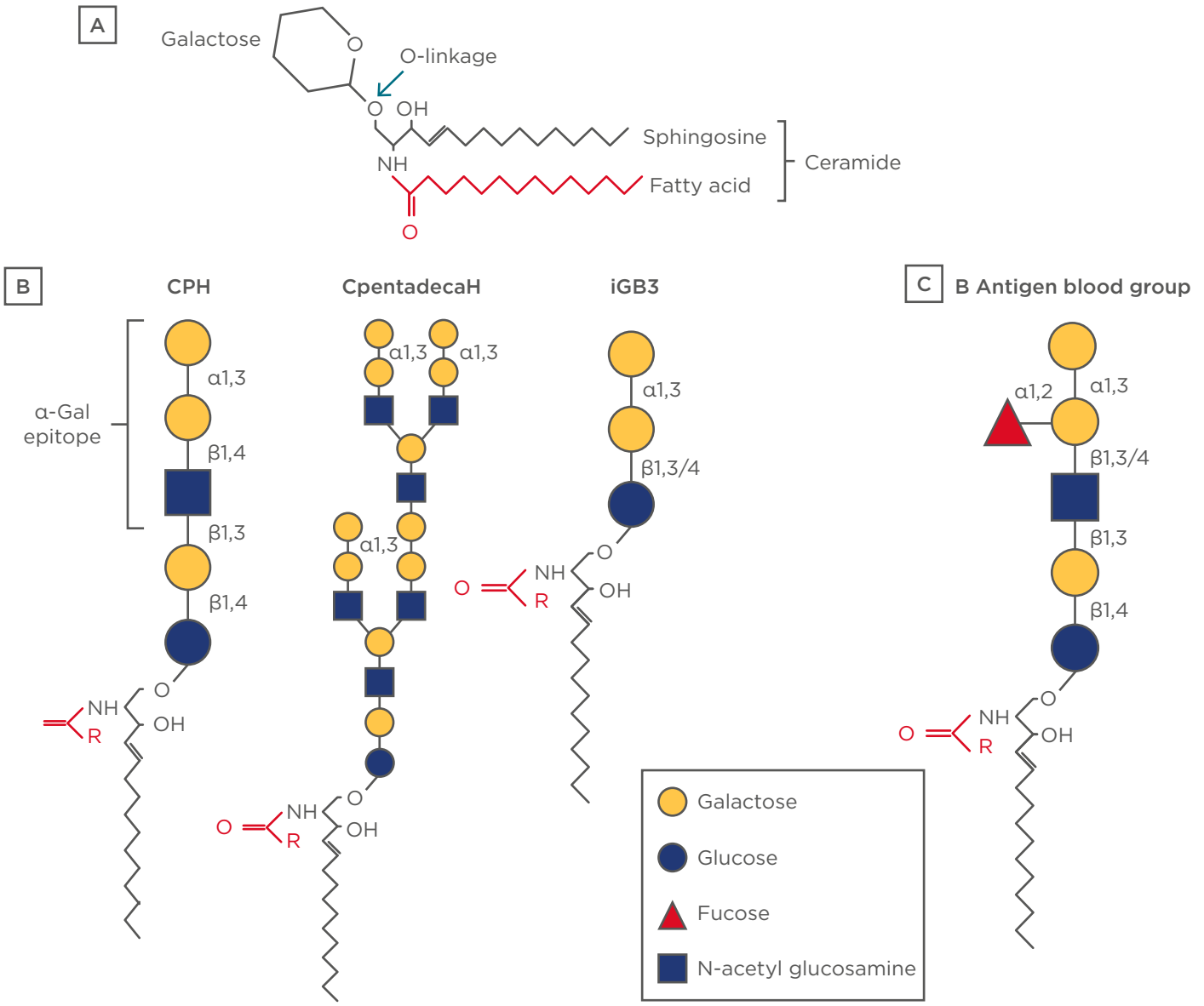


Figure 1: Schematic diagram of representative glycosphingolipids.

A) The backbone of the molecule is sphingosine (shown in black), which is amide-linked to a fatty acid (shown in red), which together forms a ceramide. The fatty acid tail can vary in length (14–36 carbon atoms) and degree of saturation. A glycosphingolipid consists of a ceramide coupled to a carbohydrate headgroup via an O-linked glycosidic bond. There are a large number of subspecies of sphingolipid and glycosphingolipids, which reflects a diversity of biologic functions.[33] B) Examples of α-Gal-linked glycosphingolipids that have been characterised in mammalian (nonhuman) cells and tissues. Note that iGB3 has a terminal galactose-α-1,3-galactose motif but lacks N-acetylglucosamine. The full α-Gal epitope is often considered to be the trisaccharide form, including N-acetylglucosamine; however, many anti-α-Gal antibodies can recognise the disaccharide.[26] C) The blood group B antigen is a glycosphingolipid that is structurally homologous to CPH.

α-Gal: galactose-α-1,3-galactose; CpentadecaH: ceramide pentadecahexoside; CPH: ceramide pentahexoside; iGB3: isogloboside 3.

THE GLYCOLIPID HYPOTHESIS

The fact that α-Gal is an oligosaccharide is often considered the most important feature of this allergen. However, it could be argued that an equally distinguishing feature of the α-Gal allergen is that it exists in the form of a glycolipid. Indeed, while there are many examples of carbohydrate allergens and of lipids modulating Th2 cell immune responses, the authors are

unaware of any other common food allergen that is intrinsically part of a lipid.[29,30] Thus, it is important to consider the glycolipid content of mammalian meat and organs, as well as the biochemical pathways involved in glycolipid digestion and metabolism, when considering the pathophysiology of α-Gal allergenicity.

The first report of lipids with the terminal α-Gal epitope date back to studies of rabbit red blood cells in 1968.[31] Subsequent experiments established that glycosphingolipids from almost all non-primate mammals were a rich source of α-Gal antigen.[32] Neutral glycosphingolipids are the major form of glycolipids that have been reported to have terminal α-Gal epitopes, although gangliosides (negatively charged glycosphingolipids) can also have the epitope (Figure 1).[10,33] Collectively, glycosphingolipids constitute a diverse family of membrane-bound lipids with a number of biological functions. The authors are unaware of studies that have characterised the amount of α-Gal linked glycosphingolipids in red meat, but it is clear that erythrocytes, which are present in the highly vascularised muscle tissue, are an abundant source.[10] Bovine and porcine kidneys have also been shown to have glycosphingolipids with α-Gal.[33]

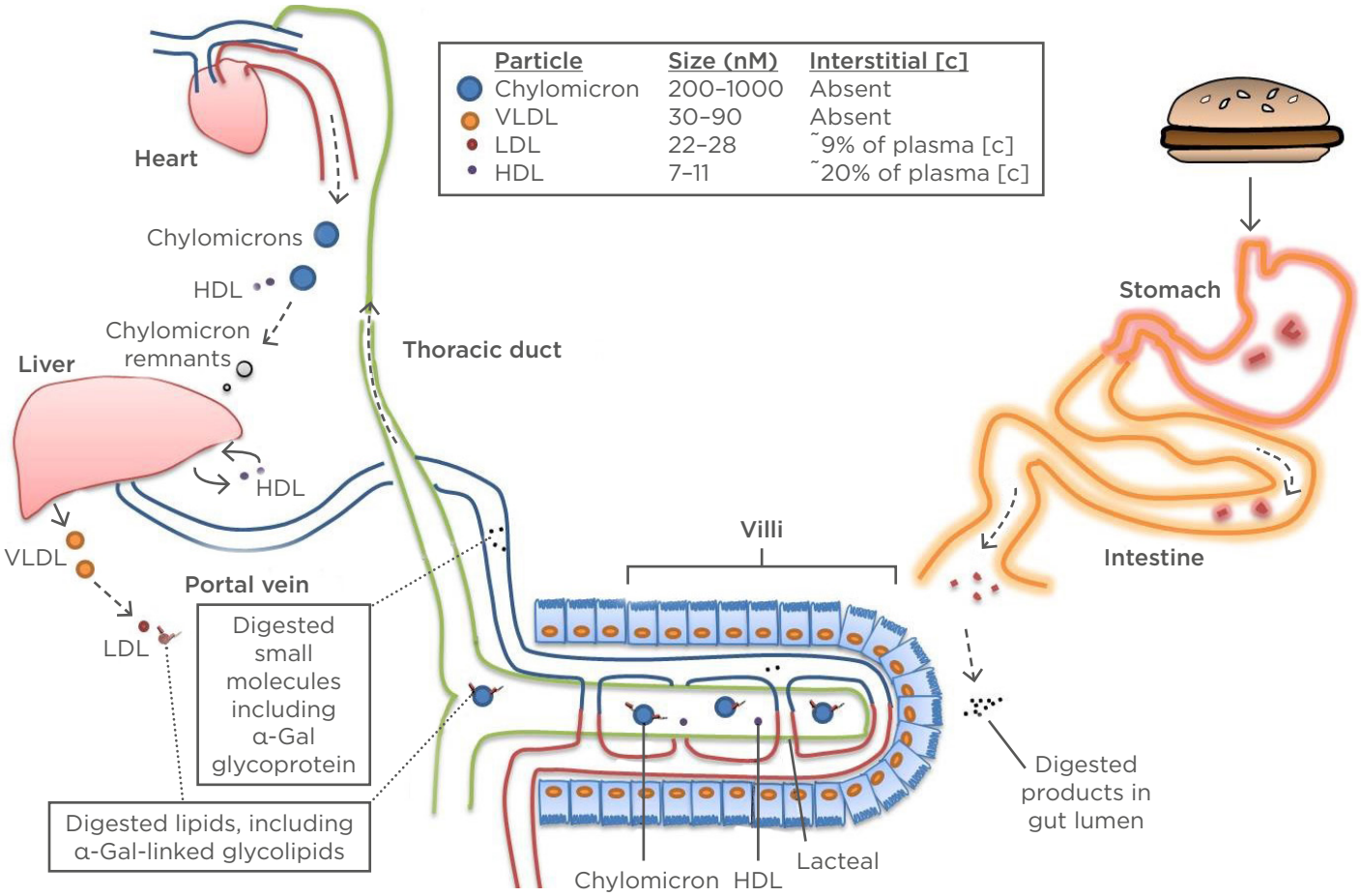


Figure 2: Model of delayed anaphylaxis to red meat.

The α-Gal syndrome and the 'glycolipid hypothesis'. Epitopes containing α-Gal are consumed as glycoproteins and glycolipids in mammalian products. Neutral glycosphingolipids account for most of the α-Gal-bearing lipids.[10] The mechanism and efficiency of transit through the epithelial barrier is unclear and may involve passive or active processes. Glycolipids are packaged into chylomicron lipoprotein particles, although incorporation into HDL within the intestine is also possible.[34] These lipoprotein particles transit via the lacteals into the thoracic duct before entering the systemic circulation at the left subclavian vein. Lipids can only filter into interstitial tissue after passing to the relatively smaller LDL or HDL particles.[34] Peak levels of lipids emerge from the thoracic duct ˜4 hours post-prandial.[35]

α-Gal: galactose-α-1,3-galactose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low density lipoprotein.

Specific information about dietary glycosphingolipid digestion and systemic absorption is limited and is largely derived from studies using animal models. For major dietary lipids (i.e., triglycerides) hydrolysis by pancreatic enzymes in the intestinal lumen generates fatty acids and glyceride metabolites. Subsequently these enter intestinal epithelial cells, facilitated by bile salts, in the form of micelles. The lipid constituents are then metabolised and packaged into chylomicrons. These large lipoprotein particles are then released into the lacteals where they subsequently traffic to the systemic circulation via the thoracic duct.[35] Once in the bloodstream, lipids pass to other lipoprotein particles, including very low-density lipoproteins, low-density lipoproteins (LDL), and high-density lipoproteins (HDL), and ultimately to end-organs, such as the liver, and to adipose or muscle tissue. The key point is that the time frame for lipids to pass from the gut to lipoprotein particles small enough to penetrate the microvasculature or tissue (i.e., LDL or HDL), and thus be recognised by specific-IgE bound to the surface of basophils or mast cells, is expected to occur on the order of hours (Figure 2). Indeed, Labbé et al.[36] reported that ingestion of a radiolabelled lipid in human volunteers with positron emission tomography/computed tomography (PET/CT) imaging revealed that peak levels were achieved in the distal thoracic duct at 4 hours and in muscle at 5 hours. Thus, this provides a rational mechanism that explains why ingested forms of α-Gal-linked glycolipid could lead to an IgE-mediated reaction with delayed onset. While this remains a favoured explanation, there are additional aspects to this 'glycolipid hypothesis' that warrant consideration.

A fundamental question remains: how do α-Gal linked glycosphingolipids access the systemic circulation? Current evidence suggests that dietary glycosphingolipids cannot pass directly from the lumen of the intestine to the systemic circulation with the carbohydrate linkage intact. In experiments published in 1969 that used radiolabelled cerebrosidase and a rat feeding model, Nilsson[37] concluded that dietary glycosphingolipids are metabolised in the small intestine and are not transported intact into the thoracic duct lymph. However, the α-Gal syndrome offers clear evidence that, at least in some subjects, ingested α-Gal epitopes can pass through the intestinal epithelium. Moreover, the barrier problem is not unique to α-Gal. Most ingested protein or carbohydrate macromolecules require digestion before the metabolites can transit into and through the epithelial barrier, but in the case of all IgE-mediated food allergies, allergens with intact epitopes are clearly penetrating the epithelial barrier. Possible explanations include transcellular or paracellular transit, which could involve antibody-mediated processes. For example, there is evidence that the low affinity IgE receptor, FcεRII (also known as CD23), on apical enterocytes can facilitate the transit of specific allergens from the lumen to the lamina propria.[39] The mechanism that explains how α-Gal glycolipids are packaged into lipoprotein particles within the intestine is unknown. While chylomicrons are the dominant particles that shuttle dietary lipid to the systemic circulation, direct incorporation into HDL in the intestine is also possible.[35,40] Intersubject variability in epithelial transit could be a factor that impacts which of the sensitised individuals develop allergic symptoms (many subjects who are sensitised can tolerate red meat) and/or the severity of allergic symptoms, a possibility that fits with the premise that allergy is an epithelial barrier disease.[41] Galili et al.[13] suggested that a typical hamburger may contain up to or exceeding 100 billion α-Gal epitopes, even a small fraction of this total could yield a significant antigenic load.[13] Intersubject differences in lipid metabolism could also impact the time taken for the allergen to access peripheral tissue and also the severity of reactions. For example, fatty acids have been shown to transit to the liver and muscle more rapidly following a meal in subjects with Type 2 diabetes mellitus than in healthy controls.[42]

LESSONS FROM OTHER OLIGOSACCHARIDES

Recent reports have highlighted other oligosaccharides as the target of IgE-mediated food allergy.[43] Galacto-oligosaccharides (GOS) represent a mixture of unconjugated oligosaccharides (i.e., those with no protein or lipid backbone) with a series of terminal galactose residues that are commonly used as prebiotics.

The mechanism of sensitisation is unclear, but several cases of anaphylaxis to GOS have been identified in Asian children.[44] Importantly, the relevant epitopes do not appear to have the α-1,3 linkage and thus the antibodies to GOS, despite being specific to galactose residues, are distinct from those in α-Gal. A key difference in the clinical response to GOS and α-Gal is that the former has been reported to occur much more rapidly. In the initial 2012 GOS report, all the cases occurred within 30 minutes.[44] Thus, this example provides further evidence that the backbone of the α-Gal glycoconjugate is the key to understanding the characteristic delay.

N-glycolylneuraminic acid (Neu5Gc) represents a glycan with xeno-antigen characteristics that are similar to α-Gal. Neu5Gc is a sialic acid that is widely expressed in mammals but is not endogenously produced in humans owing to a mutation in the gene that encodes the enzyme cytidine monophospho-*N*-acetylneuraminic acid hydroxylase. This mutation, estimated to have occurred 2–3 million years ago in ancestral humans, prevents Neu5Gc generation from the precursor sialic acid *N*-acetylneuraminic acid.[45] Similar to α-Gal, many humans develop IgG antibodies specific for Neu5Gc, and, interestingly, a role for the immune response to Neu5Gc in the pathophysiology of carcinogenesis and inflammatory diseases has been suggested.[46] However, a key difference between the two xeno-antigens is that Neu5Gc has been shown to be metabolically incorporated into human cells and tissues following dietary intake.[47] There is no simple explanation that would allow for a similar process in relation to α-Gal. The difference relates to the fact that in the case of Neu5Gc, the enzyme defect is at the level of sialic production, whereas with α-Gal it is with the carbohydrate linkage. Additionally, IgE specific for Neu5Gc has not been reported.[48]

GALACTOSE-α-1,3-GALACTOSE–BEARING GLYCOPROTEINS

Glycolipids have been little studied in relation to the α-Gal syndrome, though multiple investigators have reported on glycoprotein sources of α-Gal in mammalian meat or organs. Takahashi et al.[49] studied Japanese subjects who reported anaphylaxis to mammalian meat and identified laminin-γ1 and collagen α1 as α-Gal-containing glycoproteins. Apostolovic et al.[38] investigated a Swedish cohort and found seven novel α-Gal-containing beef allergens. Of these glycoproteins, creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase were heat-stable. A more recent report identified α-Gal on angiotensin-I-converting enzyme and aminopeptidase N in porcine kidneys, and additionally showed these glycoproteins could trigger rapid *in vitro* basophil activation on cells collected from subjects with α-Gal syndrome.[24]

ADDITIONAL FACTORS THAT MAY MODULATE ALLERGIC REACTIONS TO GALACTOSE-α-1,3-GALACTOSE

Several groups have described co-factors that are associated with clinical reactions to α-Gal. Two of the studies that focussed on subjects with reactions to porcine kidney identified co-factors in >70% of the cases, with alcohol consumption being the most common, followed by exercise.[50,51] These data suggest that co-factors, especially alcohol, may be more important in α-Gal syndrome than in other forms of food allergy.[52] The mechanisms whereby alcohol or exercise contribute to a lower threshold of allergic reactions are not entirely clear, but could relate to decreased epithelial barrier function or to sensitisation of the calcium ion channel that facilitates histamine-mediated reactions (i.e., transient receptor potential vanilloid 1 receptor).[53] A particularly interesting possibility is that alcohol is important in α-Gal syndrome because of a relationship between alcohol and lipid metabolism. Indeed, a century ago, the effects of acute alcohol ingestion were described by Feigl[54] as lipaemia of intoxication and more recent studies have demonstrated that alcohol raises post-prandial levels of plasma triglyceride and HDL.[55,56]

The form of mammalian product ingested may also affect the likelihood and/or severity of allergic reactions related to α-Gal. Porcine kidney seems to elicit reactions with more rapid kinetics than mammalian meat. For example, Morisset et al.[50] described 14 subjects with IgE to α-Gal who had a history of anaphylaxis or urticaria to porcine kidney, and 64% of

the reactions were reported within 2 hours. A similar result was reported by Fischer et al.,[51] where 13 of 21 subjects with a history of reactions to porcine kidney relating to α-Gal occurred within 2 hours. There are multiple possible explanations to consider, one of which relates to the relative content of glycoprotein versus glycolipid containing α-Gal epitopes. However, it is clear that α-Gal containing glycolipids are present in porcine kidney,[33] and the favoured explanation is that the content of α-Gal glycoconjugates is higher in kidneys than meat (i.e., muscle tissue). Using an inhibition enzyme-linked immunosorbent assay (ELISA), Morisset et al.[50] showed that porcine kidney was a much stronger inhibitor of monoclonal antibody binding (specific to α-Gal) than either pork or beef. Two other reports showed a similar result using prick-to-prick testing, where porcine kidney led to more frequent positive results and/or larger wheals than beef or pork.[24,51]

BEYOND ALLERGY

Because α-Gal represents an epitope that is regularly consumed in the form of mammalian glycolipids and a subset of the population has a strikingly different type of immune response to the antigen, it is possible that IgE to α-Gal, or concomitant specific IgG1 or T cell production, has implications beyond traditional allergic disease.[27,28,57,58] It is intriguing that red meat and high fat dairy are also risk factors for another inflammatory disease with geographic variability, and that has been associated with elevated total IgE levels and mast cells: i.e., in atherosclerosis.[59-62] Consistent with this possibility, the authors have recently reported that IgE to α-Gal was associated with the severity of coronary artery disease in a cohort of 'at-risk' adults from Virginia, USA, whose recruitment was unrelated to allergic disease.[63] Prior associations of IgG antibody response to α-Gal have been described for thyroid disease and inflammatory bowel disease.[13]

CONCLUSION

One of the striking aspects of α-Gal, an oligosaccharide that can be present on both mammalian glycoproteins and glycolipids, is that it contributes to two forms of anaphylaxis with markedly different kinetics. Multiple lines of evidence point to a role for IgE in mediating these reactions. It is possible that the glycolipid form of α-Gal, which is unique for an allergen, is critical to understanding the characteristic delay. Investigation into α-Gal-bearing glycolipids may shed further insight into aspects of this atypical food allergy but may additionally illuminate connections with inflammatory diseases not traditionally associated with allergy.

References

1. Chung CH et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med. 2008;358(11):1109-17.
2. Steinke JW et al. The alpha-gal story: Lessons learned from connecting the dots. J Allergy Clin Immunol. 2015;135(3):589-96.
3. Commins SP et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. J Allergy Clin Immunol. 2009;123(2):426-33.
4. Wilson JM et al. Galactose-alpha-1,3-galactose: Atypical food allergen or model IgE hypersensitivity? Curr Allergy Asthma Rep. 2017;17(1):8.
5. Kaloga M et al. Allergy to red meat: A diagnosis made by the patient and confirmed by an assay for Ige antibodies specific for alpha-1,3-galactose. Case Rep Dermatol. 2016;8(1):10-3.
6. Landsteiner K, Miller CP. Serological studies on the blood of the primates: III. Distribution of serological factors related to human isoagglutinogens in the blood of lower monkeys. J Exp Med. 1925;42(6):863-72.
7. Hamadeh RM et al. Anti-alpha-galactosyl immunoglobulin A (IgA), IgG, and IgM in human secretions. Clin Diagn Lab Immunol. 1995;2(2):125-31.
8. Galili U et al. A unique natural human IgG antibody with anti-alpha-galactosyl specificity. J Exp Med. 1984;160(5):1519-31.
9. Galili U et al. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. Infect Immun. 1988;56(7):1730-7.
10. Galili U et al. Identification of erythrocyte Gal alpha 1-3Gal glycosphingolipids with a mouse monoclonal antibody, Gal-13. J Biol Chem. 1987;262(10):4683-8.
11. Thall A, Galili U. Distribution of Gal alpha 1–3Gal beta 1–4GlcNAc residues on secreted mammalian glycoproteins (thyroglobulin, fibrinogen, and immunoglobulin G) as measured by a sensitive solid-phase radioimmunoassay. Biochemistry. 1990;29(16):3959-65.
12. Lammerts van Bueren JJ et al. Anti-galactose-alpha-1,3-galactose IgE from allergic patients does not bind alpha-galactosylated glycans on intact therapeutic antibody Fc domains. Nat Biotechnol. 2011;29(7):574-6.
13. Galili U, The Natural Anti-Gal

Antibody as Foe Turned Friend in Medicine (2017), 1st edition, Cambridge, MA: Elsevier.

14. Commins SP et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. J Allergy Clin Immunol. 2011;127(5):1286-93.e6.
15. Skallová A et al. Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. J Immunol. 2008;180(9):6186-92.
16. Ferreira BR, Silva JS. Successive tick infestations selectively promote a T-helper 2 cytokine profile in mice. Immunology. 1999;96(3):434-9.
17. Hamsten C et al. Identification of galactose-alpha-1,3-galactose in the gastrointestinal tract of the tick Ixodes ricinus; possible relationship with red meat allergy. Allergy. 2013;68(4):549-52.
18. Araujo RN et al. Amblyomma sculptum tick saliva: Alpha-Gal identification, antibody response and possible association with red meat allergy in Brazil. Int J Parasitol. 2016;46(3):213-20.
19. Platts-Mills TAE et al. Characterizing the geographic distribution of the alpha-gal syndrome: Relevance to Lone Star Ticks (Amblyomma americanum) and Rickettsia. J Allergy Clin Immun. 2018;141(2). Abstract 467.
20. Dahlgren FS et al. Expanding range of amblyomma americanum and simultaneous changes in the epidemiology of spotted fever group rickettsiosis in the United States. Am J Trop Med Hyg. 2016;94(1):35-42.
21. Burks AW et al. ICON: Food allergy. J Allergy Clin Immunol. 2012;129(4):906-20.
22. Commins SP et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. J Allergy Clin Immunol. 2014;134(1): 108-15.
23. O'Neil BH et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. J Clin Oncol. 2007;25(24):3644-8.
24. Hilger C et al. Two galactose-alpha-1,3-galactose carrying peptidases from pork kidney mediate anaphylactogenic responses in delayed meat allergy. Allergy. 2016;71(5):711-9.
25. Cohen NR et al. Antigen presentation by CD1 lipids, T Cells, and NKT cells in microbial immunity. Adv Immunol. 2009;102:1-94.
26. Iweala O et al. Serum IgE specific for alpha-gal sugar moiety can bind glycolipid. J Allergy Clin Immun. 2017;139(2 Suppl):Ab88.
27. Rispens T et al. IgE production to alpha-gal is accompanied by elevated levels of specific IgG1 antibodies and low amounts of IgE to blood group B. PLoS One. 2013;8(2):e55566.
28. Kollmann D et al. The quantity and quality of alpha-gal-specific antibodies differ in individuals with and without delayed red meat allergy. Allergy. 2017;72(2):266-73.
29. del Moral MG, Martinez-Naves E. The role of lipids in development of allergic responses. Immune Netw. 2017;17(3):133-43.
30. Homann A et al. Glycans and glycan-specific IgE in clinical and molecular allergology: Sensitization, diagnostics, and clinical symptoms. J Allergy Clin Immunol. 2017;140(2):356-68.
31. Eto T et al. Chemistry of lipid of the posthemyolytic residue or stroma of erythrocytes. XVI. Occurrence of ceramide pentasaccharide in the membrane of erythrocytes and reticulocytes of rabbit. J Biochem. 1968;64(2):205-13.
32. Galili U et al. Evolutionary relationship between the natural anti-Gal antibody and the Gal alpha 1–3Gal epitope in primates. Proc Natl Acad Sci U S A. 1987;84(5):1369-73.
33. Hendricks SP et al. Regulation of the expression of Gal alpha 1-3Gal beta 1-4GlcNAc glycosphingolipids in kidney. J Biol Chem. 1990;265(29):17621-6.
34. Merrill AH Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chem Rev. 2011;111(10):6387-422.
35. Randolph GJ, Miller NE. Lymphatic transport of high-density lipoproteins and chylomicrons. J Clin Invest. 2014;124(3):929-35.
36. Labbé SM et al. Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. Am J Physiol Endocrinol Metab. 2011;300(3):E445-53.
37. Nilsson A. Metabolism of cerebroside in the intestinal tract of the rat. Biochim Biophys Acta. 1969;187(1): 113-21.
38. Apostolovic D et al. Immunoproteomics of processed beef proteins reveal novel galactose-alpha-1,3-galactose-containing allergens. Allergy. 2014;69(10):1308-15.
39. Yu LC. The epithelial gatekeeper against food allergy. Pediatr Neonatol. 2009;50(6):247-54.
40. Iqbal J et al. Sphingolipids and lipoproteins in health and metabolic disorders. Trends Endocrinol Metab. 2017;28(7):506-18.
41. Mattila P et al. Allergy as an epithelial barrier disease. Clin Transl Allergy. 2011;1(1):5.
42. Ravikumar B et al. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. Am J Physiol Endocrinol Metab. 2005;288(4):E789-97.
43. Soh JY et al. Anaphylaxis to galacto-oligosaccharides–An evaluation in an atopic population in Singapore. Allergy. 2015;70(8):1020-3.
44. Chiang WC et al. Anaphylaxis to cow's milk formula containing short-chain galacto-oligosaccharide. J Allergy Clin Immunol. 2012;130(6):1361-7.
45. Chou HH et al. Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution. Proc Natl Acad Sci U S A. 2002;99(18):11736-41.
46. Samraj AN et al. A red meat-derived glycan promotes inflammation and cancer progression. Proc Natl Acad Sci U S A. 2015;112(2):542-7.
47. Tangvoranuntakul P et al. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc Natl Acad Sci U S A. 2003;100(21):12045-50.
48. Apostolovic D et al. Red meat allergic patients have a selective IgE response to the alpha-Gal glycan. Allergy. 2015;70(11):1497-500.
49. Takahashi H et al. Laminin gamma-1 and collagen alpha-1 (VI) chain are galactose-alpha-1,3-galactose-bound allergens in beef. Allergy. 2014;69(2):199-207.
50. Morisset M et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose-alpha-1,3-galactose. Allergy. 2012;67(5): 699-704.
51. Fischer J et al. Galactose-alpha-1,3-galactose sensitization is a prerequisite for pork-kidney allergy and cofactor-related mammalian meat anaphylaxis. J Allergy Clin Immunol. 2014;134(3):755-9.e1.
52. Versluis A et al. Cofactors in allergic reactions to food: Physical exercise and alcohol are the most important. Immun Inflamm Dis. 2016;4(4):392-400.
53. Smith PK et al. Risk multipliers for severe food anaphylaxis. World Allergy Organ J. 2015;8(1):81.
54. Feigl, J. Beiträge zur chemischen physiologie und pathologie. Biochem. Ztschr. 1918;92:282. 1906-23. [In German].
55. Di Luzio NR. Effect of acute ethanol intoxication on liver and plasma lipid fractions of the rat. Am J Physiol. 1958;194(3):453-6.
56. Hendriks HF et al. Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition. Alcohol Alcohol. 1998;33(4):403-10.

57. Apostolovic D et al. Immunoprofile of alpha-Gal- and B-antigen-specific responses differentiate red meat allergic patients from healthy individuals. Allergy. 2018. [Epub ahead of print].
58. Cretin N et al. The role of T cell help in the production of antibodies specific for Gal alpha 1-3Gal. J Immunol. 2002;168(3):1479-83.
59. Keys A et al. Epidemiological studies related to coronary heart disease: Characteristics of men aged 40-59 in seven countries. Acta Med Scand Suppl. 1966;460:1-392.
60. Roth GA et al. Trends and patterns of geographic variation in cardiovascular mortality among US counties, 1980-2014. JAMA. 2017;317(19):1976-92.
61. Sun J et al. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. Nat Med. 2007;13(6):719-24.
62. Kounis NG, Hahalis G. Serum IgE levels in coronary artery disease. Atherosclerosis. 2016;251:498-500.
63. Wilson JM et al. IgE to the mammalian oligosaccharide galactose-α-1,3-galactose is associated with increased atheroma volume and plaques with unstable characteristics. Arterioscler Thromb Vasc Biol. 2018;In Press.

Allergy to Stinging Insects: Diagnosis and Management

Authors: Jessica B. Perkins, *Anne B. Yates

Allergy and Immunology, University of Mississippi Medical Center, Jackson, Mississippi, USA
*Correspondence to ayates@umc.edu

Disclosure: The authors have declared no conflicts of interest.

Received: 26.02.18

Accepted: 08.05.18

Keywords: Anaphylaxis, basal serum tryptase (BSL), *Hymenoptera* venom allergy, insect sting, venom immunotherapy (VIT), venom skin testing.

Citation: EMJ Allergy Immunol. 2018;3[1]:99-105.

Abstract

Stinging insects that cause allergic reactions belong to the order *Hymenoptera*, which includes wasps, hornets, bees, yellow jackets, true hornets, and stinging ants. Individuals stung by these insects can have different clinical outcomes, from common local reactions to severe systemic reactions. Anaphylaxis as a result of insect stings can result in death; therefore, individuals with a history of systemic reaction to stings should be further evaluated and treated. A history of systemic reaction to insect stings and immunoglobulin E sensitivity to specific insect venoms, determined by blood or skin testing, are criteria for venom immunotherapy administration. Venom immunotherapy modulates the immune system to make the recipient less sensitive to venom and can be curative. All individuals with a history of systemic reaction to insect stings should be provided with an adrenaline auto-injector and educated in avoidance measures to prevent future stings. This review will discuss the diagnosis of venom allergy, the management of venom allergic individuals with venom immunotherapy, and identification of risk factors for severe anaphylaxis to insect stings. This review will also aid clinicians in discussing avoidance measures with patients.

STINGING INSECT CHARACTERISTICS

Stinging insects that cause allergic reactions belong to the order *Hymenoptera*, which contains three medically important families: *Apidae*, *Vespidae*, and *Formicidae*. The family *Apidae* includes honey bees, bumblebees, and sweat bees; the family *Vespidae* includes yellow jackets, yellow hornets, bald-faced hornets, true hornets, and paper wasps; and the family *Formicidae* includes the fire ant, jack jumper ant, and harvester ant. Most individuals are not able to properly identify the culprit insect after a sting; therefore, knowing certain characteristics of these stinging insects can aid in the identification. For instance, the worker honey bee has a barbed stinger that usually remains in the skin after a sting, although it should be noted that sometimes the stinger from other insects remains in the skin, especially if swatted. Yellow jackets usually nest underground, while hornet nests are found in bushes, trees, and overhangs. Yellow jackets are scavengers and tend to fly around rubbish bins and food. Paper wasps build small, open celled nests that hang from a pedicle on eaves or in shrubs. Wasps are attracted to open bodies of water, e.g., swimming pools and streams.

Table 1: *Hymenoptera* characteristics and avoidance measures.

Insect (Genus)	Nesting habits	Activities that increase risk of sting	Level of aggression	Avoidance strategies
Honey bee (*Apis*).	Domestic hives, waxy comb nest in hollow cavities.	Walking barefoot outside, beekeeping, being in close proximity to nectar, pollen, and sweets.	Nonaggressive.	Keep feet covered, wear white clothing covering most of the body, avoid flowering plants.
Bumblebee (*Bombus*).	Bunch of grapes, waxy nest, underground, or under structures.	Walking barefoot outside, being in close proximity to nectar and pollen.	Nonaggressive.	Keep feet covered, wear white clothing covering most of the body, avoid flowering plants.
Aerial yellow jackets: yellow hornet and bald-faced hornet (*Dolichovespula*).	Large grey paper nests with a thick, scalloped exterior, found in trees, shrubs, or on buildings.	Disturbing nests; being sensitive to vibration, yellow jackets swarm aggressively if their nest is disturbed.	Aggressive when their nest is disturbed.	Avoid yard work if these insects are seen in the vicinity. Professionally exterminate nests.
Yellow jacket (*Vespula*).	Multilayered brittle paper nests in the ground or in landscape materials.	Close proximity to outdoor foods and rubbish bins, and conducting yard work.	Very aggressive.	Avoid open food sources and rubbish bins, avoid fruit orchards, remove pet faeces.
True hornets. These are larger than the American 'hornets' (*Vespa*).	Large, brittle, brown aerial paper nests with or without envelope, found in hollow trees or building cavities.	Outdoor activities, especially in wooded areas.	Aggressive when their nest is disturbed.	Avoid yard work if these insects are seen in the vicinity, avoid bright lights at night.
Paper wasps (*Polistes*).	Single level paper nests without an exterior cover, found under eaves or overhangs.	Gardening and lawn maintenance.	Aggressive when their nest is disturbed.	Avoid disturbing nests in shrubs or on eaves.
Fire ant (*Solenopsis*).	Large mounds in the ground, prefer sandy soil.	Outdoor activities, sitting on the ground.	Aggressive when their nest is disturbed.	Wear socks and closed shoes, wear gloves for gardening, look for and avoid mounds.

Fire ants are prevalent in the south eastern USA, Central and South America,[1,2] and have spread to Hong Kong and Japan.[3] Fire ants build their nests as mounds in the ground and use their mandibles to hold onto their prey as they administer multiple stings. The jack jumper ant, *Myrmecia pilosula*, is prevalent in Tasmania and mainland Australia.[4] Features such as these can aid the clinician in history taking and insect identification, as well as educating patients on avoidance measures, which will be discussed later in the review (Table 1).

CLINICAL SYMPTOMS

In general, reactions to insect stings fall into two categories: local and systemic.

Local Reactions

Most stings will result in an immediate-type local reaction that lasts for a few hours. Local reactions are raised, erythematous, and pruritic. About 5–25% of individuals will experience large local reactions (LLR).[5] LLR usually occur hours after the sting and manifest clinically as an area of erythematous induration surrounding the sting site.[6] LLR can measure >10 cm in diameter and can cross joint lines.[6,7] This area is usually painful and pruritic, which can last for several days after the sting. LLR are usually an immunoglobulin (Ig)E dependent late-phase reaction, and patients who experience a LLR have about a 4–10% risk of a systemic reaction to a future sting.[8] The European Academy of Allergy

and Clinical Immunology (EAACI) Task Force on Venom Immunotherapy reports a 0.8–7.0% risk of systemic reaction to future stings in those with a previous LRR.[7] Fire ant stings cause a sterile pruritic pustule at the sting site(s), which typically develop about 24 hours after the sting.

Systemic Reactions

Stinging insects can cause IgE-mediated systemic reactions that are cutaneous-only or life threatening.[9] Life threatening systemic reactions that involve one or more organ systems are referred to as anaphylaxis. Anaphylaxis can have several different manifestations: respiratory compromise resulting in stridor, wheezing, coughing, or shortness of breath; cardiovascular involvement resulting in hypotension, lightheadedness, syncope, or arrhythmia; gastrointestinal upset, including nausea, vomiting, abdominal cramp and pain, or diarrhoea; skin findings, such as flushing, pruritus, urticaria, and angioedema; and neurological symptoms, including seizures, or a sense of impending doom. All of the aforementioned are signs and symptoms of this condition. In about 60% of children with allergic reactions to *Hymenoptera* stings, skin symptoms are the sole manifestation, but this is found in only 15% of adults.[10] In the USA, 3% of adults and 1% of children are reported to have had a systemic reaction to an insect sting.[5] In Europe, the prevalence of systemic reactions due to stinging insects is 0.3–7.5%.[6] At least 40 fatal sting reactions occur in the USA[10,11] and about 20 fatal reactions from stings occur in the UK annually.[12] Patients with a prior systemic reaction to a sting have about a 30–65% risk for a subsequent systemic reaction to future stings, even to the same species.[10] The majority of deaths occur in adults as a result of circulatory collapse. Turner et al.[13] report a summary of 535 stinging insect fatalities that showed 80–90% of fatal cases occurred in men with an average age of 50–60 years. Half of the fatal reactions that occur with stinging insect allergy have no history of sting reaction.[10] There appears to be a higher risk of having a systemic reaction if there are multiple stings at the same time, or if repeated stings in a single season occur.[14]

In addition to IgE-mediated reactions, stinging insects can cause adverse clinical outcomes due to envenomation from a large number of stings. Envenomation causes systemic toxicity due to the increased load of venom in the body. Toxic compounds found in venom such as phospholipase A1, hyaluronidase, and antigen 5 can be toxic to tissues in the body.[15] Venom can also trigger proinflammatory cytokines such as IL-1β, TNF-α, and IL-6, which has been demonstrated in mouse models.[16] Acute renal failure, hypotension, haemolysis, and rhabdomyolysis have all been described in the literature as causing adverse reactions due to envenomation.[17] Fire ant venom contains alkaloids that are capable of causing cardiorespiratory depression and seizures.[18]

Other Findings

Mast cell disease is reported in 1–8% of *Hymenoptera* venom allergic patients in Europe,[19] and is reported in 2% of *Hymenoptera* venom allergic patients in the USA.[8,19] These patients usually have an elevated basal serum tryptase (BST) level (>11.4 ng/mL) and may develop more severe anaphylaxis to *Hymenoptera* stings, even with the first sting. They may have severe hypotension and loss of consciousness without cutaneous findings and have a higher risk for mortality from *Hymenoptera* stings.[10] Other uncommon reactions reported after *Hymenoptera* stings include serum sickness-like reactions and chronic urticaria.[5,10]

DIAGNOSIS

Diagnosis of venom allergy is based on the clinical history and identification of venom specific IgE to the culprit insect by skin testing or *in vitro* tests. Only patients with a history of anaphylaxis to stings or individuals >16 years of age with diffuse cutaneous reactions need to undergo skin testing or *in vitro* tests.[8] In the USA, five *Hymenoptera* venoms are available for testing: honey bee, yellow jacket, yellow hornet, bald-faced hornet, and paper wasp. Skin testing is usually done with skin prick tests at 1-100 µg/mL concentration. A German article advocated use of up to 300 µcg/mL venom for skin prick testing.[20] If prick tests are negative, then intradermal skin tests are done at progressively increasing concentrations from 0.001–0.010 µg/mL (increase by 10-fold increments), until either a positive skin test occurs or the 1 µg/mL concentration is reached.[8]

Quirt et al.[21] advocate using a single step protocol with intradermal skin testing with 1 µg/mL of the five commercially available venoms without preceding skin prick testing or having a stepwise approach, but this has not been adopted by North American or British guidelines.[8,22] For imported fire ants, a whole body extract is used for testing. Skin prick testing with 1:1000 weight per volume (w/v) with an imported fire ant body extract is carried out, then, if negative, intradermal skin tests starting with 1:1 million w/v should proceed and increase at 10-fold increments until there is a positive test, or the 1:1000 w/v concentration is reached. A positive skin prick test response is usually defined as a wheal of 3 mm or greater than the negative control, whereas a positive intradermal skin test is a wheal 5 mm or greater than the negative control. Venom skin testing is positive in 70–90% of patients with a clinical history of *Hymenoptera* sting allergy, but about 25% of patients react only to the 1 µg/mL intradermal skin test.[10] If skin testing is negative, then *in vitro* testing should be carried out that, if positive, confirms the diagnosis. The level of specific IgE antibody to venom does not predict the severity of the allergic reaction to a sting.[23] If both skin testing and *in vitro* testing are negative in a patient with a history of sting anaphylaxis, the skin testing should be repeated after 6–12 weeks.

There are some patients for whom testing to whole venom extracts will not identify the cause of the allergy. About 4–6% of patients with a history of systemic reaction to *Hymenoptera* stings have negative skin tests and *in vitro* tests to whole *Hymenoptera* venom.[24] Another problem is that some patients are sensitive to multiple *Hymenoptera* venoms.[7] *In vitro* testing with recombinant venom proteins may provide information as to whether allergy to multiple different venoms is due to cross-reactivity of IgE to similar venom proteins, or if there is true double sensitisation to unique proteins in different types of venom. Molecular component tests are available in Europe to recombinant proteins for honey bee (*Apis*), paper wasp (*Polistes),* and vespid (*Vespula*) venoms. The most commonly used are the antigen 5 proteins (Pol d 5 and Ves v 5), the phospholipases (Api m 1, Pol d 1, and Ves v 1) and bee acid phosphatase (Api m 3), melittin (Api m 4), and icarapin (Api m 10).[25] Use of these recombinant venom proteins allows the determination of which patients are sensitised to multiple venoms versus those who demonstrate cross-reactivity.[25] For instance, component testing may be helpful in evaluating those individuals who have positive specific IgE against both honey bee and yellow jacket venom. Those patients who are positive to Api m1, Api m3, and/or Api m10 should receive honey bee venom immunotherapy (VIT); patients who are positive to Ves v1 and/or Ves v5 should receive yellow jacket VIT; and individuals positive for both Api and Ves components should receive honey bee and yellow jacket VIT.[26] Studies have shown that double positive IgE to component proteins occurs in about 50% of vespid allergic cases.[24] Bumblebee venom is available for *in vitro* testing but is not available for skin testing or treatment. Bumblebee venom has limited cross-reactivity to honey bee venom.[8]

MANAGEMENT AND THERAPEUTICS

Supportive Care Techniques

Uncomplicated local reactions to stings are treated with cold compresses and oral antihistamines. Urticaria is treated with oral antihistamines. Cold compresses, oral antihistamines, high potency topical steroids, nonsteroidal anti-inflammatory drugs for pain, and oral steroids for more extensive and persistent swelling are all suitable treatments for LLR.

Treatment of Anaphylaxis

Anaphylaxis is initially managed with intramuscular adrenaline, which is usually injected into the anterolateral thigh and additional doses may be required. Full emergency medical care should be given. The patient should be placed in a supine position with the legs elevated to promote blood return to the heart.[10] Hypotensive patients should be given isotonic intravenous fluid. Oxygen is given if oxygen saturation is low, and a bronchodilator is administered for persistent bronchospasm; intubation and mechanical ventilation may be required. Patients should be monitored for prolonged or recurrent anaphylaxis. Glucagon should be prescribed to patients taking a beta-adrenergic blocker who have hypotension.[10]

Venom Immunotherapy

It is important to know that only those that have experienced a systemic allergic reaction to an insect sting should receive further testing to see if they are a candidate for VIT. Patients with positive results should be counselled about VIT, and it should be initiated if possible. VIT is highly effective and may be curative therapy for many with IgE mediated venom allergy.[5,7] The principle of VIT is to introduce small amounts of venom to the patient, and, by gradually increasing increments of venom administration, modulate the immune system, which makes the individual less sensitive. For flying *Hymenoptera* insects, VIT is made from pure insect venom. It is difficult to obtain enough pure venom from fire ants to offer it commercially; as a result, the whole body of the fire ant is crushed to create extracts for diagnosis and treatment.[27] Fire ant whole-body extracts contain sufficient venom proteins for therapeutic use but are inferior to purified ant venom for testing and treatment.[28] Mixed vespid VIT is 95–100% effective at preventing systemic reactions to future stings, but bee venom is less effective, about 77%.[5,7] VIT has a similar safety profile to aeroallergen immunotherapy. Systemic symptoms to VIT occur in 5–15% of the population during their first weeks of treatment and are usually mild.[5]

Vespa crabro is a prevalent stinging insect capable of causing anaphylaxis and is prevalent in the Mediterreanean area of southern Europe. Most proteins in *Vespa* and *Vespula* families are highly cross-reactive; however, the antigen 5 from *Vespa crabro* and *Vespula* are unique and not cross-reactive, so treatment with *Vespula* VIT will not confer complete protection.[29] *Vespa crabro* venom is available for testing and treatment in some European countries.

VIT schedules can vary but typically an individual will receive subcutaneous injections weekly until a maintenance dose is achieved on a standard schedule. Once a maintenance dose is achieved, patients receive monthly therapy for at least 1 year. Following this, the maintenance interval is extended to every 6–8 weeks.[5] VIT is usually administered for 3–5 years.[7] Patients needing maintenance therapy for >5 years may be given VIT at 12 week intervals.[8] Certain scenarios would cause an individual to be on injections indefinitely, such as those with a honey bee allergy. Patients with a mast cell disorder or those who had a systemic reaction to a sting while actually on VIT may also need treatment >3–5 years.[5] This is best addressed on a case by case basis. There are schedules available to help reach maintenance dosing faster such as rush and ultra-rush, in which maintenance dosing can be achieved in days. It is reported that ultra-rush scheduling is associated with a higher risk of systemic reaction to VIT.[8,7]

The specific venom used for treatment depends on the history of the culprit insect. However, as many patients are unable to identify the specific type of insect causing the sting, there is debate as to whether treatment with VIT should include all venoms with positive tests or only the most likely culprit insect venom. Use of *in vitro* recombinant venom component testing may help to resolve this issue in the future.

Although this assay is not yet standardised, basophil activation tests (BAT) may be useful in determining if VIT has been successful at achieving a protective immune response.[7] BAT use basophils from a patient with suspected *Hymenoptera* allergy, and these basophils are exposed to defined concentrations of *Hymenoptera* venom. Activation is measured based on the percentage of basophils that express the activation marker CD63 on basophils.[8] EAACI states that performing sting challenges is the most reliable and gold standard for monitoring the effectiveness of VIT. Sting challenges are used to identify those individuals who are not protected at the maintenance dose of 100 µg.[7] Sting challenges are not routinely performed in the USA.

Contraindications to Venom Immunotherapy

The EAACI Task Force on Contraindications to Allergy Immunotherapy recommends several absolute contraindications to VIT: poorly controlled asthma, active autoimmune disorders, active malignant neoplasias, children <2 years of age, and acquired immune deficiency syndrome (AIDS).[30] Use of angiotensin-converting-enzyme (ACE) inhibitors and beta-blockers are not a contraindication for VIT.[7,30] Pre-existing cardiovascular disease, even in elderly patients, is not a contraindication to VIT.[7]

Identifying Risk Factors for Anaphylaxis

Certain risk factors can put an individual at increased risk of severe anaphylaxis or death as a result of an insect sting. Experiencing anaphylaxis without cutaneous symptoms (such as urticaria or angioedema) is a risk factor for severe reaction with future sting.[31] Older age (>45 years), chronic cardiovascular disease, and having a history of a previous severe anaphylactic reaction are also risk factors for further anaphylaxis.[5] There is controversy as to whether patients taking beta-blockers or ACE inhibitors may have some increased risk of anaphylaxis, because they may be less responsive to adrenaline treatment and require repeat dosing.[8] However, currently the United States Joint Task Force practice parameters advise that patients requiring beta blockers or ACE inhibitors should continue VIT, as the benefits of VIT outweigh the risk of severe reactions to *Hymenoptera* stings.[8] Honey bee venom is associated with a higher risk for reaction during the build-up phase of VIT and has a lower rate of protection from future sting reactions (85% for honey bee versus 96% for mixed vespid venom).[32] Patients who were not protected at the usual maintenance dose of 100 µg of honey bee or vespid venom were protected when the maintenance dose was increased to 200 µg.[32]

Individuals with a clonal mast cell disorder or elevated baseline tryptase may also be at an increased risk for severe reactions to VIT and *Hymenoptera* stings.[7,8] BST level should be measured after the reaction has fully resolved in all patients with anaphylaxis to *Hymenoptera* stings, typically about 4–6 weeks after the reaction. Patients with a BST level >11.4 ng/mL should undergo an evaluation for an underlying mast cell disease; BST levels of 20 ng/mL or higher are a minor criterion for diagnosis of clonal mast cell disease. Patients with elevated BST levels also have a higher risk of systemic reactions to VIT injections, and increased risk of treatment failure with VIT.[10] About 15% of patients with mast cell disease will have systemic reactions to *Hymenoptera* stings but negative tests for IgE to venom.[24] However, patients with mastocytosis and evidence of allergy to *Hymenoptera* stings should receive VIT.[7]

Avoidance Measures

Avoiding cooking and eating outdoors, walking outside barefoot, and exposure to flowering plants can help decrease the risk of encountering stinging insects.[8] If an individual encounters a flying *Hymenoptera* insect, they should slowly walk away from the area and avoid swatting at the insect or making sudden movements; furthermore, stinging insects are attracted to an individual's breath, so covering the mouth and nose is recommended. Seeking shelter indoors or in an enclosed vehicle should be pursued if possible. Fire ants may sting multiple times and should be quickly removed if found on the skin. Fire ant mounds should be treated with pesticides when seen. *Hymenoptera* are most defensive near their nests, so observing the area for flying insects or visible nests prior to starting outdoor activities can also aid in avoidance. (Table 1)

SUMMARY

Stinging insect allergy can result in different clinical manifestations. Those with systemic reactions require prompt treatment with adrenaline and the clinician should educate these patients on avoidance measures to prevent future stings. Individuals with systemic reactions to insect stings should undergo further testing so the diagnosis of stinging insect allergy can be made. If so, these patients could be candidates for VIT, which can be curative.

References

1. Kemp SF et al. Expanding habitat of the imported fire ant: A public health concern. J Allergy Clin Immunol. 2000;105(4):683-91.
2. deShazo RD et al. Reactions to the stings of the imported fire ant. N Engl J Med. 1990;323(7):462-6.
3. Potiwat R, Sitcharungsi R. Ant allergens and hypersensitivity reactions in response to ant stings. Asian Pac J Allergy Immunol. 2015;33(4):267-75.
4. Brown SGA et al. Causes of ant sting anaphylaxis in Australia: The Australian Ant Venom Allergy Study.

Med J Aust. 2011;195(2):69-73.

5. Golden DBK, "Insect allergy," Adkinson NF et al. (eds.), Middleton's allergy: Principles and practice (2014), Philadelphia: Elsevier Saunders, pp.1260-73.
6. Bilo MB, Bonifazi F. The natural history and epidemiology of insect venom allergy: Clinical implications. Clin Exp Allergy. 2009;39(10):1467-76.
7. Sturm G et al. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. Allergy. 2018;73(4):744-64.
8. Golden DBK et al. Stinging insect hypersensitivity: A practice parameter update 2016. Ann Allergy Asthma Immunol. 2017;118:28-54.
9. Brown SGA et al. "Anaphylaxis," Adkinson NF et al. (eds.), Middleton's allergy: Principles and practice (2014), Philadelphia: Elsevier Saunders, pp.1237-59.
10. Golden DB. Anaphylaxis to insect stings. Immunol Allergy Clin N Am. 2015;35(2):287-302.
11. Jerschow E et al. Fatal anaphylaxis in the United States, 1999-2010: Temporal patterns and demographic associations. J Allergy Clin Immunol. 2014;134(6):1318-28.
12. Pumphrey R. Anaphylaxis: Can we tell who is at risk of a fatal reaction? Curr Opin Allergy Clin Immunol. 2004;4(4):285-90.
13. Turner P et al. Fatal anaphylaxis: Mortality rate and risk factors. JACI In Practice. 2017;5(5):1169-78.
14. Pucci S et al. Shortness of interval between two stings as risk factor for developing Hymenoptera venom allergy. Allergy. 1994;49(10):894-6.
15. Ittyachen AM et al. Multi-organ dysfunction secondary to severe wasp envenomation. Int J Emerg Med. 2015;8(6):1-5.
16. Prado M et al. Acute physiopathological effects of honeybee (Apis mellifera) envenoming by subcutaneous route in a mouse model. Toxicon. 2010;56(6):1007-17.
17. Perkins JB, Yates AB, "Pathologic findings in hymenoptera sting fatalities," Freeman T, Tracy J (eds.), Stinging insect allergy (2017), Cham: Springer, pp.101-8.
18. Howell G et al. Cardiodepressant and neurologic actions of *Solenopsis invicta* (imported red fire ant) venom alkaloids. Ann Allergy Asthma Immunol. 2005;94(3):380-6.
19. Bonadonna P et al. Mastocytosis and insect venom allergy. Curr Opin Allergy Clin Immunol. 2010;10(4): 347-53.
20. Przybilla B et al. Diagnosis and therapy of bee and wasp venom allergy. Allergo J. 2011;20:318-39.
21. Quirt J et al. Venom allergy testing: Is a graded approach necessary? Ann Allergy Asthma Immunol. 2016;116(1):49-51.
22. Krishna M et al. Diagnosis and management of hymenoptera venom allergy: British Society for Allergy and Clinical Immunology (BSACI) guidelines. Clin Exp Allergy. 2011;41:1201-20.
23. Hoffman DR. Fatal reactions to hymenoptera stings. Allergy and Asthma Proc. 2003;24(2):123-7.
24. Alfaya AT et al. Key issues in hymenoptera venom allergy: An update. J Investig Allergol Clin Immunol. 2017;27(1):19-31.
25. Jakob T et al. Diagnostics in hymenoptera venom allergy: Current concepts and developments with special focus on molecular allergy diagnostics. Allergo J Int. 2017;26(3):93-105.
26. Tomsitz D, Brockow K. Component resolved diagnosis in hymenoptera anaphylaxis. Curr Allergy Asthma Rep. 2017;17(6):38.
27. Freeman TM et al. Imported fire ant immunotherapy: Effectiveness of whole body extracts. J Allergy Clin Immunol. 1992;90(2):210-5.
28. Tankersley MS, Ledford DK. Stinging insect allergy: State of the art 2015. J Allergy Clin Immunol Pract. 2015;3(3):315-22.
29. Macchia D et al. Vespa crabro immunotherapy versus vespula-venom immunotherapy in vespa crabro allergy: A comparison study in field re-stings. World Allergy Organ J. 2018;11(1):3-8.
30. Pitsios C et al. Clinical contraindications to allergen immunotherapy: An EAACI position paper. Allergy. 2015;70(8):897-909.
31. Stoevesandt J et al. Over and underestimated parameters in severe hymenoptera venom-induced anaphylaxis: Cardiovascular medication and absence of urticaria/angioedema. J Allergy Clin Immunol. 2012;130(3):698-704.
32. Rueff F et al. Patients still reacting to a sting challenge while receiving conventional hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy Clin Immunol. 2001;108(6):1027-32.

Autoinflammatory Diseases: Consequences of Uncontrolled Inflammasome Activation

Authors: Sinéad Kenealy, *Emma M. Creagh

School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Dublin, Ireland
*Correspondence to ecreagh@tcd.ie

Disclosure: The authors have declared no conflicts of interest.

Received: 07.02.18

Accepted: 16.05.18

Keywords: Autoinflammatory diseases (AD), caspase-1, interleukin (IL)-1β, inflammasome, NLRP3.

Citation: EMJ Allergy Immunol. 2018;3[1]:106-113.

Abstract

Inflammasomes are sensors within the innate immune system that are responsible for the regulation of caspase-1 activation and the initiation of inflammatory responses following cellular infection or damage. A significant number of chronic inflammatory and metabolic diseases have recently been identified to have inflammasome-mediated inflammation as a key driver of their pathogenesis; this area of research is under intense investigation at present. This review focusses on autoinflammatory diseases (AD), a rapidly expanding group of debilitating diseases that are associated with severe systemic inflammation. AD commonly arise as a result of mutations to genes that encode inflammasome components. Monogenic AD are relatively rare because they require fully penetrating mutations; however, they often present at birth and last a lifetime. Clinical awareness of AD is lacking and it is believed that, at present, many cases go undiagnosed. This review specifically discusses a number of inflammasome-associated AD and metabolic disorders that provide significant insight into our understanding of inflammasome signalling pathways. These AD highlight the potency of inflammasomes in their ability to initiate and sustain systemic inflammation. The debilitating symptoms of AD also reveal the extensive consequences of uncontrolled inflammasome activity. Clinical therapies that target the inflammasome and interleukin-1β, a product of its activation, in the successful management of AD and certain metabolic diseases will also be discussed.

INTRODUCTION

The immune system is an evolutionarily conserved system that has evolved to protect the host from invading pathogens and cellular damage. While the immune system is crucial in protecting the host from a variety of insults, the dysregulation of immune components is strongly linked to the development of both autoinflammatory and autoimmune diseases. Autoinflammatory diseases (AD) are a relatively new category of immunological disease. The clinical term AD was proposed in 1999, when only two genes (*MEFV* and *NLRP3*) had been genetically associated with this disease category. Today, 30 genes have been linked to AD, which is the term still used to describe this expanding group of diseases, caused by the overactivation of the innate immune system. As this is a relatively new group of diseases, with new clinical subtypes being identified on an ongoing basis, there are limited statistical analyses

available on AD. In 2013, a study[1] estimated the incidence of AD to be 2.83 patients per million people in Sweden. Owing to their relatively recent identification and their low incidence rates, it is believed that clinical cases of AD are currently underdiagnosed and increased clinical awareness of AD is required.

Although both autoinflammatory and autoimmune diseases result from the immune system attacking the body's own tissues, AD are characterised by intense episodes of inflammation, driven by innate immune cells, and are caused by mutations in genes that regulate innate immunity.[2] The classical symptoms of autoinflammation are recurrent fever attacks, skin rash, and abdominal pain. However, AD symptoms vary greatly across clinical subtypes, and patients can present with a range of physical manifestations, including mouth ulcers, pyogenic skin or bone lesions, joint swelling, serositis, and granulomatous lesions.[2] The number of gene mutations associated with AD is rapidly increasing. Examples of AD include cryopyrin-associated periodic syndrome (CAPS), Blau syndrome, familial mediterranean fever (FMF), tumour necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), and inherited hyperimmunoglobulinaemia D with periodic fever syndrome (HIDS). The prevalence of a given disease can vary from 1:1,000 people (Sweet's syndrome) to 1:1,000,000 (Marshall's syndrome, also known as periodic fever adenitis pharyngitis aphthous [PFAPA] ulcers), and vary between populations. McGonagle and McDermott[3] proposed a 'continuum model' for immunological diseases in 2006, integrating AD into a spectrum ranging from monogenic autoinflammatory disorders to monogenic autoimmune diseases, with polygenic autoinflammatory/autoimmune diseases and other diseases that may have both an autoinflammatory and an autoimmune component included within this spectrum. The majority of mutated genes identified to date linked to monogenic AD represent critical innate sensor or receptor proteins involved in inflammatory responses, such as *NLRP3* for CAPS, *NOD2* for Blau, and *TNFR1* for TRAPS. Both NLRP3 and NOD2 proteins belong to the Nod-like Receptor (NLR) protein family, a group of cytosolic sensor proteins that are capable of detecting intracellular infection or damage. A number of the NLR proteins initiate inflammatory pathways by their formation of multiprotein complexes, termed inflammasomes. The majority of AD identified to date are in fact linked to mutations in inflammasome components.

INFLAMMASOMES: CRITICAL MEDIATORS OF INFLAMMATION

Inflammasomes are large complexes of proteins that form to mediate the activation of an inflammatory enzyme, termed caspase-1. Caspase-1 is transcribed as the inactive precursor protein pro-caspase-1, which requires proteolytic processing before the generation of its active form.[4] Once active, caspase-1 is responsible for the maturation and secretion of interleukin (IL)-1β and IL-18, two potent proinflammatory cytokines that induce fever and interferon (IFN)γ secretion, respectively.[5,6] In addition to the activation of the cytokines IL-1β and IL-18, inflammasome activation also results in a type of cell death, termed pyroptosis.[7] Pyroptosis is an inflammatory form of cell death, mediated by caspase-1-dependent cleavage of an executioner protein, Gasdermin D (GSDMD).[8] Cleaved GSDMD is responsible for forming pores in the cell membrane, mediating the release of proinflammatory cytokines IL-1β and IL-18.[9] Thus, the ultimate outcome of inflammasome activation in cells is acute inflammation, driven by the secretion of potent inflammatory mediators IL-1β and IL-18, and pyroptotic cell death, which also contributes to local tissue inflammation in addition to eliminating damaged and infected cells.[10]

Inflammasomes are composed of a sensor protein, such as certain NLR proteins; an adaptor protein, usually the ASC protein; and the enzyme caspase-1. The sensor protein is responsible for recognising intracellular pathogens, such as bacteria or viruses, and/or intracellular danger or stress signals, such as detection of nuclear factors (e.g., DNA or high motility group box 1 protein [HMGB1]) in the cytosol. Once activated following the recognition of a pathogen or danger signal, the sensor protein oligomerises and triggers the formation of an inflammasome.[11,12] Inflammasomes generally require a priming step before they can become activated, which is termed Signal 1. This priming step is mediated by NFκB signalling, which occurs following

extracellular pathogen recognition by a toll-like receptor (TLR) or intracellular recognition by certain types of NLR, such as NOD1/2. Activation of NFκB during Signal 1 results in the transcriptional upregulation of inflammasome components, such as NLRP3 and pro-IL-1β, the inactive precursor form of IL-1β. Signal 2 involves activation and formation of the inflammasome complex via ligand binding to a sensor protein (Figure 1). An alternative method of NLRP3 inflammasome activation, known as the noncanonical inflammasome, requires a Signal 3, mediated by inflammatory caspases-4/5 in humans (caspase-11 in mice).[13] Caspase-4, 5, and 11 are responsible for direct recognition of intracellular lipopolysaccharides, which results in their subsequent cleavage and activation.[13] In addition to regulation of the noncanonical inflammasome,[14] active caspases-4, 5, and 11 can also initiate pyroptosis, because they are capable of directly processing GSDMD.[9] Several inflammasome complexes have been identified to date, including those that consist of NLR sensor proteins (NLRP1, NLRP3, NLRC4, NLRP6, and NLRP12) and other sensors, such as AIM2 and IFI16, which are members of the PYHIN protein family.[15]

There have been >23 distinct NLR genes identified in the human genome, several of which have been implicated in the regulation and activation of inflammasome complexes, which subsequently lead to the activation and secretion of the proinflammatory cytokines IL-1β and IL-18.

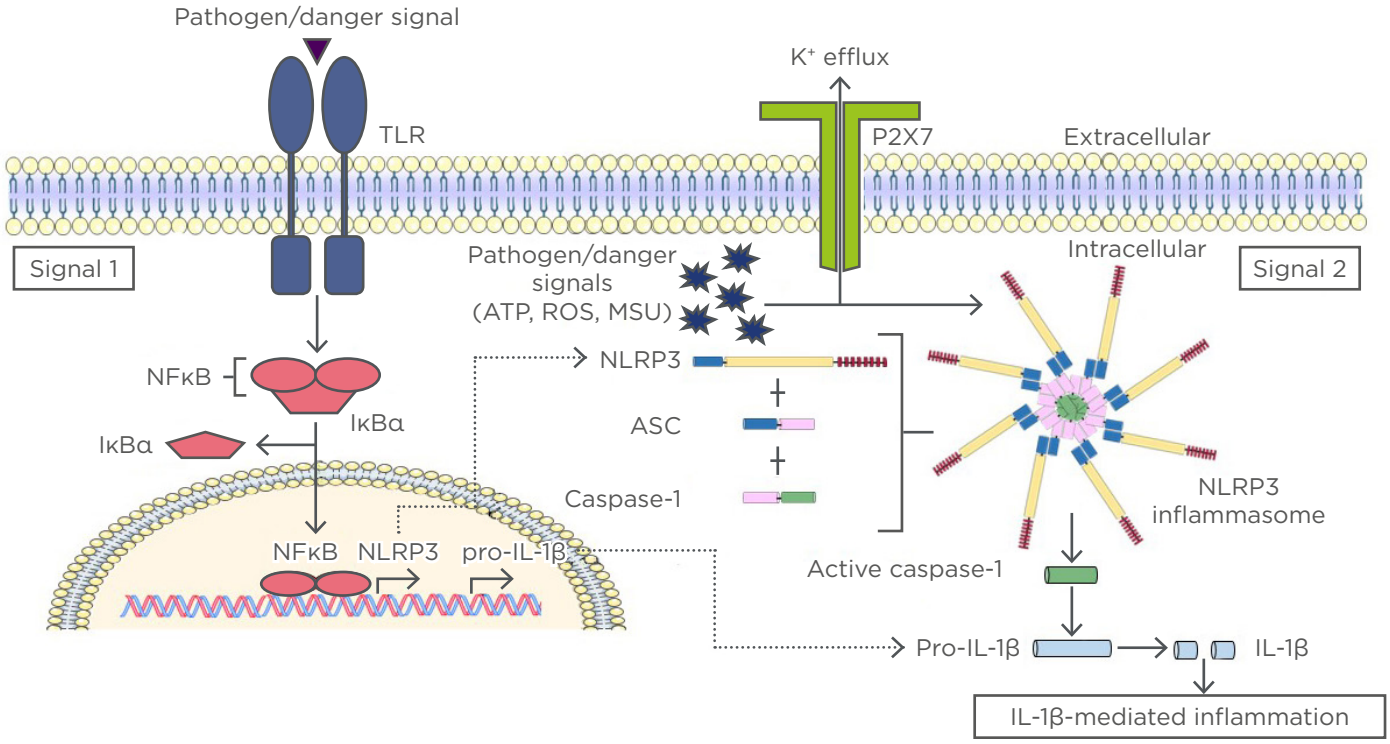


Figure 1: Inflammasome activation results in IL-1β mediated inflammation.

The initial priming step of inflammasome activation is mediated by pathogen recognition receptors, such as TLR, which recognise pathogen or danger signals during infection or injury. TLR activation results in translocation of NFκB into the nucleus to promote transcription and translation of inflammasome components and their targets, including IL-1β and NLR proteins (Signal 1). There are a number of mechanisms proposed to mediate Signal 2 activation; however, most occur via potassium (K+) efflux induced by pathogen or danger signals (e.g., ATP, ROS, MSU) and subsequent activation of NLRP3. NLRP3 oligomerisation initiates the formation of a multimeric inflammasome complex. The inflammasome complex facilitates the proteolytic cleavage and activation of caspase-1, allowing it to cleave pro-IL-1β into its mature and active form, which is secreted from the cell to mediate inflammation.

ATP: adenosine triphosphate; IL: interleukin; IκBα: inhibitor of NFκB; MSU: monosodium urate; NFκB: nuclear factor kappa B; NLRP3: NACHT, LRR, and PYD domains-containing protein 3; ROS: reactive oxygen species; TLR: toll-like receptor.

The most widely studied and best characterised of all inflammasomes is that of NLRP3. Extensive research has elucidated a range of microbial and nonmicrobial activators of the NLRP3 inflammasome. NLRP3 has been implicated in detecting a plethora of microbial pathogens, including the Influenza A virus, *Vesicular stomatitis* virus, bacterial *Staphylococcus aureus*, *Escherichia coli*, fungal *Candida albicans*, *Aspergillus fumigatus*, and parasitic *Schistosoma mansoni* and *Dermatophagoides pteronyssinus*.[16-22] Additionally, phagocytosis of particulates, such as monosodium urate (MSU) crystals, amyloid-β, silica, calcium pyrophosphate dehydrate, asbestos, and alum have all been reported to activate NLRP3.[23-27] These nonmicrobial agonists induce potassium efflux that results in subsequent NLRP3 activation. Recent studies[28,29] have reported NEK7 as a novel NLRP3 inflammasome regulator. NEK7, a member of the NIMA-related kinase family, was originally found to be responsible for regulating mitotic progression and response to DNA damage but has since been reported to control NLRP3 oligomerisation, formation of an ASC speck, and subsequent caspase-1 activation downstream of potassium efflux and reactive oxygen species (ROS).[28,29] Following their phagocytosis by innate immune cells, intracellular particulates are thought to damage the lysosomal membrane, resulting in the release of the lysosomal enzyme, Cathepsin B, into the cytosol, resulting in NLRP3 activation.[30]

INFLAMMASOME-MEDIATED AUTOINFLAMMATORY DISEASES

While inflammasome activation is a key mechanism responsible for mediating the host innate response following infection and injury, inappropriate inflammasome activity can lead to AD. As outlined previously, some of the well-characterised AD occur as a result of mutations in inflammasome-associated genes.

Mutations in *NLRP3* have been linked to a group of disorders collectively known as CAPS, including familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome, and neonatal-onset multisystem inflammatory disorder (also known as chronic infantile neurologic, cutaneous, and articular syndrome).[31-33] All three CAPS subphenotypes occur as a result of dominantly inherited gain-of-function mutations in the *NLRP3* gene, which result in systemic inflammation with blood neutrophilia and fever.[32] Localised neutrophilic inflammation is also observed in various tissues, such as skin, muscles, joints, and cerebrospinal fluid. Symptoms common to all CAPS patients are rash, periodic fevers, headaches, joint pain, conjunctivitis, and general malaise. FCAS is the least severe of the CAPS and symptoms, which occur from early infancy, are triggered within 2 hours after cold exposure and generally subside within 24 hours.[34] FCAS is distinct from cold urticaria, which is caused by an allergic response to cold and generally develops later in life. Symptoms in Muckle–Wells syndrome patients, triggered by cold, stress, or other unknown factors, are similar to those of FCAS but may also be accompanied by progressive hearing loss and the development of amyloidosis, due to excessive serum amyloid production.[34] Neonatal-onset multisystem inflammatory disorder has the highest degree of chronic inflammation of all CAPS, with symptoms including aseptic meningitis, papilloedema, joint problems, hearing loss, and often mental and physical developmental delays.

Both *in vitro* and *in vivo* data support the hypothesis that CAPS-associated *NLRP3* mutations result in enhanced responsiveness of the NLRP3 sensor protein, leading to inappropriate inflammasome activation and subsequent secretion of the potent inflammatory mediators, IL-1β and IL-18.[35-38] Downstream markers of inflammation, such as IL-6, are also consistently elevated in patients with FCAS after a mild cold even when significant increases in IL-1β and IL-18 are undetectable.[39] Additionally, pretreatment with anti-IL-1 therapy can prevent the FCAS response to a mild cold, suggesting a causative role for IL-1β in mediating the response.[39] Approved and effective treatment options for CAPS patients now exist, as blocking the action of IL-1β using anakinra, rilonacept, or canakinumab are effective therapies for all CAPS patients.[40] Therefore, the prognosis for all CAPS patients is greatly improved if the AD is diagnosed early and treated with the appropriate therapy before the damage caused by chronic inflammation has any permanent effect on the body. Mutations in the *LPIN2* gene, encoding the lipin-2 protein,

result in another NLRP3-associated AD, termed Majeed syndrome. Lipin-2 has been shown to regulate both the priming and activation steps of the NLRP3 inflammasome, and Majeed-associated *LPIN2* mutations result in elevated pro-IL-1β and enhanced potassium efflux in macrophages, leading to aberrant NLRP3 activation.[41]

The most common AD is FMF, which occurs as a result of mutations in the *MEFV* gene, encoding the pyrin protein. Patients with FMF display longer periods of fever and can have a range of other symptoms, including skin rash, arthritis, and serositis.[42] As the name suggests, FMF affects populations of Mediterranean descent, particularly Armenian, Turkish, Arabic, and some Jewish-Israeli populations, in which the carrier rates can be as high as 1:5.[43-45] The high frequency carrier rates suggest that a selective advantage may exist, and previous reports have suggested that the mutated pyrin protein could provide increased protection against infection, asthma, or allergy.[46-49]

The pyrin protein, named due to the presence of a PYRIN domain in its protein structure, is thought to be responsible for protein–protein interactions. NLRP3 and other NLRP proteins are also characterised by the presence of a PYRIN domain, which is crucial for its ability to recruit ASC and other adaptor proteins into inflammasome complexes. *In vitro* pyrin overexpression studies reveal that, similar to NLRP3, pyrin oligomerises with ASC resulting in subsequent caspase-1 activation and release of IL-1β.[50,51] To identify the impact of the *MEFV* Met694Val mutation, the most commonly found mutation in FMF patients, the Kastner group engineered a transgenic mouse strain that harboured the equivalent mutation in the murine *Mefv* gene.[52] The genetically altered mice displayed FMF-like symptoms and also secreted high levels of IL-1β in an ASC-dependent manner, but *Mefv* deficient mice did not, suggesting that the FMF-associated mutations are gain-of-function, and cause enhanced and inappropriate inflammasome activation.[52] Additional support for this hypothesis comes from a more recent study, which suggested that the pyrin inflammasome is negatively regulated by its phosphorylation of the pyrin protein, which is mediated by the RhoA signalling pathway.[53] Under normal circumstances, the pyrin inflammasome is proposed to be selectively activated following RhoA GTPase inhibition by bacterial toxins;[54] however, in FMF patients, mutated pyrin proteins are not efficiently phosphorylated by RhoA-dependent kinases, resulting in a lowered threshold for the activation of the pyrin inflammasome.[53] This hypothesis is further supported by the fact that the antimitotic drug, colchicine, which inhibits microtubule polymerisation and activates RhoA, is an effective prophylactic treatment for FMF patients.

Inappropriate activation of the pyrin inflammasome is also linked to another unrelated AD: HIDS, also known as mevalonate kinase deficiency. HIDS is caused by mutations in the *MVK* gene, which encodes for the mevalonate kinase enzyme, an enzyme responsible for an early step in the isoprenoid synthesis pathway, catalysing the phosphorylation of mevalonic acid.[55] RhoA signalling is dependent upon its translocation to the plasma membrane, which is regulated by the isoprenylation of RhoA.[56] Defective isoprenoid synthesis occurs in the presence of mutations in the mevalonate kinase enzyme, resulting in loss of RhoA activity. Therefore, the molecular mechanism underlying the inflammatory symptoms of HIDS is also proposed to be mediated via the pyrin inflammasome.[53] In contrast to FMF patients, colchicine is ineffective at preventing HIDS flares, most likely due to its inability to activate RhoA, which is not tethered to the membrane due to the absence of isoprenylation. Anti-IL-1β therapies are the main treatment option for HIDS patients, although not all patients respond. Other treatment options include nonsteroidal anti-inflammatory drugs (NSAID), glucocorticoids, and other biologics, such as TNF-α or IL-6 blocking agents.[57]

INFLAMMASOME ACTIVATION IN THE PATHOGENESIS OF METABOLIC DISEASE

The pathogenesis of many metabolic disorders, including atherosclerosis, Type 2 diabetes mellitus, obesity, and gout, is strongly associated with chronic inflammation. The inflammasome, and products of inflammasome activation (active IL-1β and IL-18), have recently been identified as key mediators of this inflammation, and thus are being intensively studied for

their ability to modulate the pathogenesis and progression of metabolic disease. For example, results from the recent CANTOS trial[58] reveal that targeting IL-1β-mediated inflammation reduces the risk of adverse cardiac events in patients with a previous history of myocardial infarction and high sensitivity C-reactive protein level (>2 mg/L). Additional analysis from the CANTOS study[59] suggests that inhibition of IL-1β in these patients is also associated with reduced incidences of lung cancer. This suggests that further investigation into the use of anti-IL-1β and inflammasome targeting therapies for cancers with an established inflammatory component is warranted. However, the potential adverse effects that may arise when blocking such a potent inflammatory mediator must also be considered, as patients receiving canakinumab during the CANTOS trial had an increased occurrence of potentially fatal infections and sepsis.[58]

The contribution of the NLRP3 inflammasome to metabolic disease has been reviewed in great detail elsewhere;[60,61] however, the proposed role of the inflammasome in the molecular pathogenesis of gout is summarised here as an example. Gout is a chronic inflammatory disease characterised by deposition of MSU crystals in joints, which form when high concentrations of urate are present. The clinical symptoms of gout arise as a result of the inflammatory response that occurs following recognition of the MSU crystals. Gout is believed to be the most common cause of inflammatory arthritis, with an increasing prevalence in both developing and developed countries.[62] Activation of the NLRP3 inflammasome in gout has been well investigated and it is believed that TLR activation most likely acts as the first priming signal in the response to MSU crystals.[63] Phagocytosis of MSU crystals by macrophages, which causes lysosomal damage and subsequent activation of the NLRP3 inflammasome constitutes Signal 2 (Figure 1). IL-1β has been implicated as a key inflammatory mediator responsible for driving the development of gout by promoting an influx of neutrophils into the synovium and joint fluid, which is a typical hallmark of an inflammatory bout in this disease.[64] Anti-inflammatory therapies such as NSAID and glucocorticoids are effective in reducing the symptoms of gout. Colchicine is also prescribed as a prophylactic treatment or to relieve gouty flares. As described previously, colchicine inhibits microtubule polymerisation and, in contrast to the pyrin inflammasome, has been shown to disrupt NLRP3 inflammasome activation. Colchicine also inhibits microtubule-based inflammatory cell chemotaxis, secretion of chemokines and cytokines, and phagocytosis. Many of these cellular processes can be found in other diseases involving chronic inflammation, suggesting the potential efficacy of low-dose colchicine in other comorbid conditions associated with gout, such as osteoarthritis and cardiovascular disease.[65]

CONCLUSION

Anti-IL-1β and inflammasome targeting therapies are emerging as important clinical treatments for the management of AD, metabolic diseases, and certain cancers. Although inflammasome activation may not be the primary cause or major pathogenic factor for many metabolic diseases, recent evidence suggests that targeting the inflammatory contribution to these diseases may limit their progression. In contrast, certain monogenic AD, including CAPS, FMF, and HIDS, have been reported to arise directly as a result of defective and uncontrolled inflammasome activation. The fact that many AD are effectively treated by IL-1β blockade and drugs that target inflammasome activity highlights the potency of inflammasomes in driving chronic inflammation. As the mechanisms governing inflammasome regulation continue to evolve, so too will additional targets and therapies to regulate inflammasome activity during disease. However, the importance of controlled, functional inflammation for homeostasis cannot be ignored. Thus, therapeutic inflammasome inhibition needs to be balanced against the beneficial contribution of inflammasomes to innate immunity.

References

1. Hemminki K et al. Incidence of hereditary amyloidosis and autoinflammatory diseases in Sweden: Endemic and imported diseases. BMC Med Genet. 2013;14(88):1471-2350.
2. Brydges S, Kastner DL. The systemic autoinflammatory diseases: Inborn errors of the innate immune system. Curr Top Microbiol Immunol. 2006;305:127-60.
3. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med. 2006;3(8):1242-8.
4. Creagh EM. Caspase crosstalk: Integration of apoptotic and innate immune signalling pathways. Trends Immunol. 2014;35(12):631-40.
5. Horai BR et al. Production of ice Deficient in genes for interleukin (IL)-1α, IL-1β, IL-1 α/β, and IL-1 receptor antagonist shows that IL-1β is crucial in turpentine-induced fever development and glucocorticoid secretion. J Exp Med. 1998;187(9):1463-75.
6. Ghayur T et al. Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. Nature. 1997;386(6625):619-23.
7. Bergsbaken T et al. Pyroptosis: Host cell death and inflammation. Nat Rev Microbiol. 2009;7(2):99-109.
8. He WT et al. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res. 2015;25(12):1285-98.
9. Shi J et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526(7575):660-5.
10. Miao EA et al. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat Immunol. 2010;11(12):1136-42.
11. Creagh EM, O'Neill LA. TLRs, NLRs and RLRs: A trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol. 2006;27(8):352-7.
12. Agostini L et al. NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity. 2004;20(3):319-25.
13. Shi J et al. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature. 2014;514(7521):187-92.
14. Rühl S, Broz P. Caspase-11 activates a canonical NLRP3 inflammasome by promoting K+ efflux. Eur J Immunol. 2015;45(10):2927-36.
15. Man SM, Kanneganti TD. Regulation of inflammasome activation. Immunol Rev. 2015;265(1):6-21.
16. Allen IC et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009;30(4):556-65.
17. Rajan JV et al. The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. J Virol. 2011;85(9):4167-72.
18. Rathinam VA et al. TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. Cell. 2012;150(3):606-19.
19. Gross O et al. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. Nature. 2009;459(7245):433-6.
20. Saïd-Sadier N et al. *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. PLoS One. 2010;5(4):e10008.
21. Ritter M et al. Schistosoma mansoni triggers Dectin-2, which activates the Nlrp3 inflammasome and alters adaptive immune responses. Proc Natl Acad Sci U S A. 2010;107(47):20459-64.
22. Dai X et al. Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. J Allergy Clin Immunol. 2011; 127(3):806-14.
23. Halle A et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-β. Nat Immunol. 2008;9(8):857-65.
24. Eisenbarth SC et al. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature. 2008;453(7198):1122-6.
25. Dostert C et al. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science. 2008;320(5876):674-7.
26. Martinon F et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440(7081):237-41.
27. Muñoz-Planillo R et al. K+ efflux is the common trigger of nlrp3 inflammasome activation by bacterial toxins and particulate matter. Immunity. 2013;38(6):1142-53.
28. He Y et al. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature. 2016;530(7590):354-7.
29. Shi H et al. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. Nat Immunol. 2016;17(3):250-8.
30. Hornung V et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol. 2008;9(8):847-56.
31. Milhavet F et al. The infevers autoinflammatory mutation online registry: Update with new genes and functions. Hum Mutat. 2008;29(6):803-8.
32. Hoffman HM et al. Familial cold autoinflammatory syndrome: Phenotype and genotype of an autosomal dominant periodic fever. J Allergy Clin Immunol. 2001;108(4):615-20.
33. Hoffman HM et al. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat Genet. 2001;29(3):301-5.
34. Yu JR, Leslie KS. Cryopyrin-associated periodic syndrome: An update on diagnosis and treatment response. Curr Allergy Asthma Rep. 2011;11(1):12-20.
35. Willingham SB et al. Microbial pathogen-induced necrotic cell death mediated by the inflammasome components CIAS1/cryopyrin/ NLRP3 and ASC. Cell Host Microbe. 2007;2(3):147-59.
36. Dowds TA et al. Regulation of cryopyrin/Pypaf1 signaling by pyrin, the familial Mediterranean fever gene product. Biochem Biophys Res Commun. 2003;302(3):575-80.
37. Stack JH et al. IL-converting enzyme/ caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. J Immunol. 2005;175(4):2630-4.
38. Rosengren S et al. Monocytes from familial cold autoinflammatory syndrome patients are activated by mild hypothermia. J Allergy Clin Immunol. 2007;119(4):991-6.
39. Hoffman HM et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. Lancet. 2004;364(9447):1779-85.
40. Landmann EC, Walker UA. Pharmacological treatment options for cryopyrin-associated periodic syndromes. Expert Rev Clin Pharmacol. 2017;10(8):855-64.
41. Lordén G et al. Lipin-2 regulates NLRP3 inflammasome by affecting P2X7 receptor activation. J Exp Med. 2017;214(2):511-28.
42. Cush JJ. Autoinflammatory syndromes. Dermatol Clin. 2013;31(3):471-80.
43. Sarkisian T et al. Familial

Mediterranean fever in Armenian population. Georgian Med News. 2008;156:105-11.

44. Gershoni-Baruch R et al. Familial Mediterranean fever: Prevalence, penetrance and genetic drift. Eur J Hum Genet. 2001;9(8):634-7.
45. Daniels M et al. Familial Mediterranean fever: High gene frequency among the non-Ashkenazic and Ashkenazic Jewish populations in Israel. Am J Med Genet. 1995;55(3):311-4.
46. Kalyoncu M et al. Are carriers for MEFV mutations "healthy"? Clin Exp Rheumatol. 2006; 24(5 Suppl 42):S120-2.
47. Brenner-Ullman A et al. Possible protection against asthma in heterozygotes for familial Mediterranean fever. Am J Med Genet. 1994;53(2):172-5.
48. Ross JJ. Goats, germs, and fever: Are the pyrin mutations responsible for familial Mediterranean fever protective against Brucellosis? Med Hypotheses. 2007;68(3):499-501.
49. Cattan D. Familial Mediterranean fever: Is low mortality from tuberculosis a specific advantage for MEFV mutations carriers? Mortality from tuberculosis among Muslims, Jewish, French, Italian and Maltese patients in Tunis (Tunisia) in the first half of the 20th century. Clin Exp Rheumatol. 2003; 21(4 Suppl 30):S53-4.
50. Srinivasula SM et al. The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. J Biol Chem. 2002;277(24):21119-22.
51. Yu JW et al. Cryopyrin and pyrin activate caspase-1, but not NF-κB, via ASC oligomerization. Cell Death Differ. 2006;13(2):236-49.
52. Chae JJ et al. Gain-of-function pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. Immunity. 2011;34(5):755-68.
53. Park YH et al. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. Nat Immunol. 2016;17(8):914-21.
54. Xu H et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature. 2014;513(7517):237-41.
55. Miziorko HM. Enzymes of the mevalonate pathway of isoprenoid biosynthesis. Arch Biochem Biophys. 2011;505(2):131-43.
56. Waiczies S et al. Geranylgeranylation but not GTP loading determines rho migratory function in T cells. J Immunol. 2007;179(9):6024-32.
57. Ozen S, Demir S. Monogenic periodic fever syndromes: Treatment options for the pediatric patient. Pediatr Drugs. 2017;19(4):303-11.
58. Ridker PM et al.; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017;377(12):1119-31.
59. Ridker PM et al.; CANTOS Trial Group. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: Exploratory results from a randomised, double-blind, placebo-controlled trial. Lancet. 2017;390(10105):1833-42.
60. Robbins GR et al. Inflammasomes and metabolic disorders: Old genes in modern diseases. Mol Cell. 2014;54(2):297-308.
61. Cordero MD et al. AMP-activated protein kinase regulation of the NLRP3 inflammasome during aging. Trends Endocrinol Metab. 2018;29(1):8-17.
62. Kuo CF et al. Global epidemiology of gout: Prevalence, incidence and risk factors. Nat Rev Rheumatol. 2015;11(11):649-62.
63. Liu-Bryan R et al. Innate immunity conferred by Toll-like receptors 2 and 4 and myeloid differentiation factor 88 expression is pivotal to monosodium urate monohydrate crystal-induced inflammation. Arthritis Rheum. 2005;52(9):2936-46.
64. Landis RC, Haskard DO. Pathogenesis of crystal-induced inflammation. Curr Rheumatol Rep. 2001;3(1):36-41.
65. Dalbeth N et al. Mechanism of action of colchicine in the treatment of gout. Clin Ther. 2014;36(10):1465-79.

Genetically Modified Wheat, Wheat Intolerance, and Food Safety Concerns

Authors: Atul Munish Chander,[1,2] Sanjay Kumar Bhadada,[2] *Devinder Kumar Dhawan[1]

1. Department of Biophysics, Panjab University, Chandigarh, India
2. Department of Endocrinology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

*Correspondence to dhawan@pu.ac.in

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: Mr Chander is thankful to the Indian Council of Medical Research (ICMR), New Delhi, India, for providing fellowship.

Received: 22.05.17

Accepted: 20.02.18

Keywords: Food safety, gluten, metabolomics, RNA interference (RNAi), transgenic wheat.

Citation: EMJ Allergy Immunol. 2018;3[1]:114-122.

Abstract

Wheat intolerance is a common problem for certain individuals. A gluten-free diet is the only option for people with wheat-associated disorders (WAD) to manage their condition. The elimination of immunogenic proteins from the wheat is the most appropriate approach to ameliorate the symptoms of affected individuals, while also meeting their nutritional requirements. RNA interference technology can be exploited to silence the expression of gliadins to produce a wheat variety lacking the immunogenic proteins associated with WAD, but there are challenges before implementation of transgenic varieties in the market will occur. This review is focussed on RNA interference approaches acquired to produce wheat that patients with different WAD can tolerate. The authors also discuss the advantages and disadvantages of current omics approaches that are being used to validate the food safety issues related to the applicability and clinical relevance of genetically modified wheat.

INTRODUCTION

Wheat is the causal factor for a number of diseases, including coeliac disease (CD), wheat-dependent exercise-induced anaphylaxis (WDEIA), wheat allergy, dermatitis herpetiformis, and non-coeliac gluten sensitivity (NCGS). The literature is scarce about the prevalence of most wheat-associated disorders (WAD) except for CD. Recently, the prevalence of wheat allergy in a birth cohort (10 and 11 year olds) was reported as 0.48% in the UK,[1] whereas prevalence was measured between 1.2 and 75.3 per 100,000 people for dermatitis herpetiformis.[2-8] Research conducted in the USA reported the prevalence of NCGS to be 6.0% in a hospital-based study,[9] but another study reported a 0.55% prevalence of NCGS in the general population of the USA.[10] CD is the most studied and prevalent among all wheat-related diseases. CD was first reported to have a prevalence of 1:184 in an Italian school-aged paediatric population.[11] Prevalence of CD varies from 0.006% to 5.600% in different populations across the world.[12-20] The Saharawi

population of Africa accounts for 5.6% of CD cases. CD is considered the most prevalent wheat-related disease across the world.[21] In addition, the prevalence of CD is around 11% in the Type 1 diabetes mellitus population in India.[22] CD is also the cause of other diverse health problems[23,24] because CD is an autoimmune disorder of the small intestine that leads to destruction of intestinal villi as a consequence of inflammation.[25,26] Though there are some genetic and environmental factors associated with disease,[27-31] wheat gluten (wheat protein) remains the antigenic trigger of CD, and its withdrawal from the diet improves the clinical conditions of CD patients. The extent of gluten intake is strongly associated with the severity and prevalence of CD.[20]

Even after decades of research, there is no successful treatment available as an alternative to a gluten-free diet (GFD). Strict lifelong adherence to a GFD always remains a challenge for CD patients and even occasional ingestion of gluten-containing food facilitates reoccurrence of gluten-induced inflammation. Moreover, some CD patients never recover from symptoms, even after remaining on a GFD for a long time, due to intestinal dysbiosis.[32] Although a GFD is beneficial for patients, some clinical reports have shown that its effectiveness is compromised in malnourished patients.[33] Therefore, exclusion of wheat from the diet may lead to secondary problems that may persist, along with an altered intestinal microbiota.[34]

Currently, trials of ongoing therapies in CD patients are still evoking different opinions with regard to eliciting a beneficial response. Thus, a wheat variety lacking the immunogenic components of gluten may prove to be beneficial for patients with this condition. Similarly, RNA interference (RNAi) is being used by the scientific community to produce a wheat variety devoid of immunogenic proteins. Other studies have used metabolomics techniques as markers of biosafety for crops, with an aim to elucidate the effect of fertilisers on the metabolome of plants. Although metabolomics in this field is in its infancy, this review elucidates the use of metabolomics in the development and validation of safety measures with regard to genetically modified (GM) plants. Hence, metabolomic approaches have the potential of elucidating better safety measures from a health point of view.

IMMUNOGENIC GLUTEN PEPTIDES: THE CAUSAL FACTOR FOR COELIAC DISEASE

Gluten is the protein component of wheat that contains α-gliadins, γ-gliadins, and glutenins as immunogenic components, and ω5-gliadins as the allergen component. Human proteolytic enzymes cannot effectively digest gluten proteins,[35] and these proteins have been reported to possess differential immune targets that make them immunotoxic by nature. During proteolytic digestion in the intestine, immunogenic proline and glutamine-rich gluten polypeptides are produced that can stimulate T cells.[36] These peptides are resistant to further hydrolysis due to enrichment of proline residues in the amino acid sequences.[37] The peptides 33-mer and 26-mer, derived from α-gliadin and γ-gliadin, respectively, subsequently trigger immunological responses in the intestines of CD patients.[25,38-40] Peptide 31–49 of α-gliadin was reported to be a potent activator of innate immune processes in the mucosa of CD patients when tested on T cell lines established from the CD intestinal mucosa.[41] Another similar peptide of α-gliadin was tested in a Caco-2 cell line model and was reported to be resistant to digestive enzymes, with the potential to penetrate across a Caco-2 monolayer.[42] The immunogenic potential of some peptides, such as α-9 (57–68) and α-2 (62–75), was confirmed when they were recognised by a T cell line derived from Norwegian CD patients.[43] Gliadin peptide 31–43 promotes an endoplasmic reticulum-stress pathway by inducing Ca^{2+} mobilisation from the endoplasmic reticulum, whereas peptides 31–43 and 57–68 can induce immune dysfunction.[44] In addition, peptides 31–43 and 57–68 can alter immune regulators and induce deamidation of gluten peptides and gliadin–tissue transglutaminase crosslinking in enterocytes.[36,43] As most of the peptides are immunogenic, some of them (p10-mer, QQPQDAVQPF) have protective effects that prevent gliadin-dependent dendritic cell maturation (Table 1).[45]

Table 1: Immunotoxic gluten peptides and their diverse effects.

Immunotoxic peptides	Protein	Study model	Key findings
31–43	α2-gliadin	*In vitro* study on duodenal samples[36]	Protein induces anti-endomysial antibody production.
31–49	Prolamins of α-gliadin	*In vivo* study on CD patients[46]	Patients with CD display variable sensitivity.
56–75	α-gliadin	*In vivo* study on CD patients[47]	Coeliac-specific intestinal morphology and intraepithelial lymphocyte count increased significantly after treatment with both gliadin and the test peptide in all subjects.
57–89, 33-mer epitope	α2-gliadin	CD patients[40]	These peptides induced gut-derived human T cell lines derived from 14 CD patients.
α-9(57–68) and α-2(62–75)	α-gliadin	T cell line derived from CD patients[43]	These are the common α-gliadin epitopes recognised by T cells in Norwegian CD patients.
31–49	α-gliadin	T cell lines established from CD intestinal mucosa[41]	Potent activator of the innate immune activation in CD mucosa.
31–55	α-gliadin	Caco-2 cell line model[42]	The peptide is resistant to digestive enzymes and can penetrate across a Caco-2 monolayer.
31–43 and 57–68	α-gliadin	Caco-2 cell line model[44]	Promote an ER-stress pathway that may be relevant in CD pathogenesis and involved in inflammation.
Decapeptides: QQPQRPQQPF (pRPQ) and its homologue QQPQDAVQPF (pDAV), and from human thyroid peroxidase (hTPO) (LDPLIRGILLARPAKLQV)	Gliadin peptides	Human monocyte-derived DC[45]	These peptides significantly prevent the gliadin-induced maturation of DC.

CD: coeliac disease; DC: dendritic cell; ER: endoplasmic reticulum.

RNA INTERFERENCE AND WHEAT

A conserved biological response to double-stranded RNA, known as RNAi or post-transcriptional gene silencing, mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes. RNAi has been cultivated as a means to manipulate gene expression experimentally and to probe gene function on a whole-genome scale.[48] RNAi technology has been used to produce GM plants, and to provide benefits to CD patients. Gil-Humanes et al.[49] designed hairpin constructs that are expressed in the endosperm of bread wheat and have the potential to downregulate gliadin proteins in the transgenic lines. Three transgenic wheat lines did not elicit T cell responses during *in vitro* treatment with T cell clones derived from intestinal lesions of CD patients. Another study by the same group found that the downregulation of γ-gliadins proved beneficial for enhancing the quality of the dough.[50] The subsequent transgenic clones resulted in stronger dough quality that had tolerance to over-mixing, from an industry perspective. In a recent study,[51] all of the gliadin fraction of wheat was successfully downregulated using RNAi so that the new wheat line exhibited stability

and tolerance to over-mixing, thereby showing better bread-making qualities. Continuing their work on previous wheat lines, they evaluated the physical properties and contents of gliadins, thus further predicting the amount of safe bread intake possible for CD patients. Hence, Gil-Humanes et al.[52] claimed their wheat line possessed similar baking and sensory properties to normal flour but lacked 97% of the gliadin content. The wheat they developed also had better nutritional properties because of higher content of lysine, an essential amino acid. Although, no clinical trial was performed in this study, a safe consumption of 67 g of their bread per day by the CD patients was claimed as per descriptions of per day maximum safe limits of gluten intake by Catassi et al.[53]

The ω5-gliadins are the major sensitising allergens in WDEIA, a disorder in which a patient experiences an allergic response during exercise. To contain such a response, Altenbach et al.[54] generated transgenic lines of wheat that were knocked down for ω5-gliadins. Furthermore, in a set of additional experiments, it was revealed that the protein content of flour was determined by the fertiliser regime in both transgenic samples as well as normal wheat samples. Subsequently, ω5-gliadin was also indicated to have a negative effect on flour quality; thereby, suggesting that transgenic wheat lines produce better flour quality than wild-type wheat.

Altenbach et al.[55] further observed that the allergenic response of ω5-gliadins knock-down wheat lines was reduced in patients, as determined by serum immunoglobulin E reactivity in a clinical trial. Two transgenic wheat lines were assessed for their allergenic potential, in which ω5-gliadin genes were silenced by RNAi. Sera from 7 of 11 WDEIA patients showed greatly reduced levels of immunoglobulin E reactivity to ω5-gliadins for both transgenic lines. However, the sera also showed low levels of reactivity to other gluten proteins, but sera from three patients showed the greatest reactivity to proteins other than ω5-gliadins, which included either high molecular weight glutenin subunits (HMW-GS), α-gliadins, or non-gluten proteins. The complexity of immunological responses among these patients suggests that flour from the transgenic lines would not be suitable for individuals diagnosed with WDEIA. To the best of the authors' knowledge, transgenic wheat varieties knocked down for α-gliadins have not yet entered clinical trials for CD in humans.[56] However, the present trial of ω5-gliadins revealed that its administration in the WDEIA population could reduce the incidence of this food allergy.[55] The current clinical trial has also raised questions on the applicability of transgenic lines, thereby pointing towards the complexity of WAD; i.e., WDEIA, CD, and wheat allergy, and the complex status of overall wheat proteins.

As the wide range of complex immunogenic proteins of wheat is still a challenge in developing immune-tolerant wheat lines, wheat proteins as a whole should be targeted while trying to give an alternative to the GFD. This study also raised questions on the use of such silenced GM plants even after a number of quality checks. The effect of gene knockdown on other proteins and metabolites that remain untraceable by current molecular techniques is another challenge regarding GM plants. Furthermore, what makes other proteins immunogenic for the WDEIA population is still not evident, and whether it is because of a changed proportion of a targeted protein component, a consequence of the gene silencing on the plant's metabolic control, or an imbalanced proportion of proteins is a subject of further investigations. Gil-Humanes et al.[57] reported that silencing induced a strong reduction in all the gliadins but caused a compensatory effect on the synthesis of non-gluten proteins by upregulation.

Furthermore, the effect of gliadin knockdown on nutritional values of wheat remained contradictory; however, Barro et al.[58] attempted to solve this by validating that silencing WAD-associated proteins did not affect the total protein and starch content of wheat. The authors designed a combination of seven plasmids containing RNAi fragments to mask all major components of WAD; i.e., α, γ, ω-gliadins, and low molecular weight glutenin subunits. Out of these combinations, two provided a >90% reduction in gluten content in comparison with the wild-type when measured by anti-gliadin 33-mer monoclonal antibody. However, total protein and starch contents remained unaffected in all types of combinations. Gel electrophoresis, reversed-phase high-performance liquid chromatography, and liquid chromatography–

mass spectrometry were used to measure the extent of silencing. Though promising results were also observed in experiments by Altenbach et al.,[54] questions will remain unanswered until preclinical and clinical trials likewise yield convincing results. Table 2 represents information about the different RNAi approaches used to produce safe wheat lines, and their characteristic features.

FERTILISERS AS PREDOMINATING FACTORS FOR ACCUMULATION OF RISK PROTEINS IN WHEAT

Currently, both the prevalence and incidence of food intolerances are increasing,[59-62] but the reason for widespread food intolerance is unknown. Studies have reported the adverse effects of sulfur fertilisers on gene regulation of wheat plants, thereby increasing the content of risk proteins (immunogenic proteins i.e., gliadins).[63,64] These adverse effects due to genomic level changes need validations using metabolomics and other omics approaches integrated with clinical practice or trials in suitable models. Furthermore, the dysregulations at the genetic level that modulate the metabolic profiles of plants is a food safety concern.[65,66]

Altenbach et al.[63] revealed that environmental factors, including fertilisers, affected the composition of specific flour proteins and their regulation. While conducting experiments, *Triticum aestivum* was grown with and without post-anthesis fertilisation (PAF), followed by quantitative two-dimensional gel electrophoresis of the flour. Subsequently, proteomic profiling clarified that the proportions of 54 unique proteins were altered in the treatment group; PAF treatment resulted in an increased proportion of most ω-gliadins, HMW-GS, serpins, and some α-gliadins.

Table 2: RNA interference approaches to mask the expression of immunogenic proteins and their clinical relevance.

Wheat variety	Silencing technique	Targeted peptides	Preclinical studies	Effect on quality of wheat
'Gazul', 'Podenco', and 'Arpain'	RNAi-mediated silencing	γ-gliadins	No	Downregulation of targeted proteins resulted in improved quality and strength of gluten.[50]
Triticum aestivum cv 'Bobwhite 208' (BW208) and *T. aestivum* cv 'Bobwhite 2003' (BW2003)	RNAi-mediated silencing	α, γ, and ω-gliadin	No	All targeted fractions of gluten were downregulated and produced wheat lines with better tolerance to over-mixing, showing bread making qualities.[51]
T. aestivum cv. Butte 86	RNAi-mediated silencing	ω-5 gliadins	No	Knocked-down wheat lines show improvement in quality and dough mixing properties. Response to fertilisers was also observed.[54]
T. aestivum	RNAi-mediated silencing	ω-5 gliadins	Trial on WDEIA patients	Complex immunological responses in patients were observed in WDEIA patients when administered with transgenic wheat lines, thus unsafe for WDEIA patients.[55]
T. aestivum cv. Bobwhite 208 (BW208)	RNAi-mediated silencing	ω-gliadins and γ-gliadins, and three of these also silenced α-gliadins	*In vitro* antibody test	Gluten content of six wheat lines was observed as highly reduced when antigliadin 33-mer monoclonal antibody. LC-MS/MS confirmed that wheat lines with three plasmid combinations was totally devoid of immunogenic peptides.[58]

LC: liquid chromatography; MS: mass spectrometry; RNAi: RNA interference; WDEIA: wheat-dependent exercise-induced anaphylaxis.

Hurkman et al.,[64] while conducting studies to observe the differential effects of temperature and fertiliser treatment on the development and yield of grain, reported that both the treatments elicited the accumulation of gluten proteins in wheat flour. Under high temperature conditions, gluten protein accumulation was not observed by PAF treatment, but the majority of HMW-GS, ω-gliadins, and some α-gliadins were found to be elevated, thereby affirming that environmental stimuli do influence the accumulation of risk proteins of CD. The studies presented provide evidence that the man-made means for increasing productivity of crops can be a prominent cause of food intolerances because of a lack of food safety measures. The aforementioned studies highlight the adverse effects of sulfur in terms of food safety and that they need to be validated by targeting studies to evaluate the effect of sulfur-induced metabolome change and its effect on food safety in relation to human health. Although there is lack of literature in this regard, there is some evidence in support of the side effects of sulfur fertiliser in GM wheat, as described in the next section.

CROSS TALK ON RNA INTERFERENCE, FOOD SAFETY, AND METABOLOMICS IN WHEAT

It is worthwhile to elucidate the unwarranted side effects of knocked-down wheat lines of α-gliadins on plant metabolites since α-gliadins contain sulfur-rich amino acids, including cysteine and methionine. Furthermore, in wheat lines knocked down for α-gliadin genes, sulfur amino acids used in the synthesis of α-gliadin might remain accumulated in the plant.[67,68] The accumulation of sulfur is reported to induce its incorporation into other plant metabolites that may become a food safety concern, but this was not observed in metabolomic studies in transgenic versus wild lines.[69,70] Therefore, with the support of metabolomics, the recent studies advocated that transgenic varieties are safe, because no change in the metabolomic signature in transgenic verses wild wheat lines was observed.

RNAi-produced wheat lines lack antigenic components but result in diverse side effects on the plants. Initially, evaluation was performed by proteomic analysis of different parts of the plant (grains, leaves, straw, and husk).[39,40] Comparison of proteomic profiles of transgenic lines with that of normal lines gives an overview of the disturbed gene function or a description of knocked-down genes.[63,64] RNAi-induced silencing of 75 α-gliadin genes that completely eliminated all gliadin proteins, as confirmed by proteome analysis,[71] raises the safety issues[70,72] related to such varieties; these should be considered a priority, especially those that are caused by a disturbance in a single plant metabolite or whole metabolome,[73] with and without treatment with fertilisers. Recently, metabolomics has helped in better understanding the effects of any external stimuli (fertiliser) or genetic modifications (gene knockdown) on plant metabolism and the associated issues of biosafety.

Zörb et al.[69] performed gas chromatography mass spectrometry-based metabolite profiling of flour of wheat line silenced for 75 α-gliadin genes and reported that, in comparison to wild-type plants, no appreciable difference in 109 metabolites was seen when plants were grown without sulfur. No unintended side effects of RNAi-induced gene silencing were observed. Conversely, the effect of fertilisation or single nutrient (sulfur) availability in disturbing metabolomic status was much higher than RNAi-induced silencing. The concentration of metabolites was also found to increase with increasing sulfur supply. Zörb et al.[69] also revealed that variable amounts of sulfur supply influenced the yield aspects of grain metabolome in both the wild-type and transgenic wheat line. Plants grown with higher sulfur content showed elevated grain metabolite concentration in comparison to the plants grown without sulfur treatment. Moreover, principal component analysis showed that the levels of β-amino isobutyric acid were affected the most by variation in sulfur supply in wild-type wheat. Furthermore, when α-gliadins were knocked down, alanine, glycine, serine, homoserine, and tyrosine were found to be associated with sulfur-induced differences in the grains. These studies confirm the effects of sulfur fertilisers in GM wheat but, noting the concerns discussed above it is pertinent to elucidate the effects of sulfur alone on gene regulation and also the associated

metabolomics in order to make the food safety concerns relevant.

Collectively, it can be concluded that sulfur was not only potent in inducing genetic dysregulations in wheat,[63] but it might have produced metabolic changes that were reflected in metabolomics studies.[69]

CONCLUSION

The results of various experiments related to proteomics and metabolomics are in accordance with each other and reveal that fertilisers affect the proteome and metabolome of wheat flour; thereby, raising issues related to food safety. Although RNAi-induced gene silencing does not affect the metabolomic signature of wheat flour, fertilisers do have an effect. Sulfur fertilisers remain the determining factor for disturbance in plant metabolism, followed by dysregulation in the proteome. Moreover, proteomic studies demonstrate that sulfur fertilisers induce the accumulation of gliadins in wheat, which makes the flour more immunogenic. Thus, the techniques adopted are useful for validating that fertilisers induce metabolic disturbances in plants. However, clinical trials of wheat lines knocked down for ω-gliadin showed reduction in disease-specific markers in WDEIA patients, but reactivity to other wheat protein components could not be ruled out. This raises controversy on the gene expression status of other gluten proteins that may be dysregulated as a consequence of interference exerted by RNAi. Therefore, though omics techniques are useful to validate the global metabolic control or gene expression status, initial *in vitro* or *in vivo* preclinical studies are needed to guide better future clinical output. Future research in this field should be attributed to validation of food safety issues through improved platforms of studying the transcriptome, proteome, metabolome, and immunome.

References

1. Venter C et al. Very low prevalence of IgE mediated wheat allergy and high levels of cross-sensitisation between grass and wheat in a UK birth cohort. Clin Transl Allergy. 2016;6(1):22.
2. Mobacken H et al. Incidence and prevalence of dermatitis herpetiformis in western Sweden. Acta Derm Venereol. 1984;64(5): 400-4.
3. Salmi TT et al. Prevalence and incidence of dermatitis herpetiformis: A 40-year prospective study from Finland. Br J Dermatol. 2011;165(2):354-9.
4. Christensen OB et al. Natural history of dermatitis herpetiformis in southern Sweden. Derma-tologica. 1986;173(6):271-7.
5. Burrows D. The prevalence of dermatitis herpetiformis. Br J Dermatol. 1972;86(4):437.
6. Moi H. Incidence and prevalence of dermatitis herpetiformis in a country in central Sweden, with comments on the course of the disease and IgA deposits as diagnostic criterion. Acta Derm Venereol. 1984;64(2):144-50.
7. Smith JB et al. The incidence and prevalence of dermatitis herpetiformis in Utah. Arch Derma-tol. 1992;128(12):1608-10.
8. West J et al. Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: Population-based study. Am J Gastroenterol. 2014;109(5):757-68.
9. Sapone A et al. Spectrum of gluten-related disorders: Consensus on new nomenclature and classification. BMC Med. 2012;10:13.
10. DiGiacomo DV et al. Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: Results from the Continuous National Health and Nutrition Examination Survey 2009-2010. Scand J Gastroenterol. 2013;48(8):921-5.
11. Catassi C et al. The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects. Acta Paediatr Suppl. 1996;412:29-35.
12. Mardini HE et al. Racial differences in the prevalence of celiac disease in the US population: National Health and Nutrition Examination Survey (NHANES) 2009-2012. Dig Dis Sci. 2015;60(6):1738-42.
13. Cilleruelo ML et al. Prevalence and natural history of celiac disease in a cohort of at-risk children. J Pediatr Gastroenterol Nutr. 2016;62(5): 739-45.
14. Mustalahti K et al.; Coeliac EU Cluster; Project Epidemiology. The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. Ann Med. 2010;42(8):587-95.
15. Laass MW et al. The prevalence of celiac disease in children and adolescents in Germany. Dtsch Arztebl Int. 2015;112(33-34):553-60.
16. Enroth S et al. Prevalence and sensitization of atopic allergy and coeliac disease in the Northern Sweden Population Health Study. Int J Circumpolar Health. 2013;72.
17. Burger JP et al. Rising incidence of celiac disease in the Netherlands; an analysis of temporal trends from 1995 to 2010. Scand J Gastroenterol. 2014; 49(8):933-41.
18. White LE et al. Childhood coeliac disease diagnoses in Scotland 2009-2010: The SPSU project. Arch Dis Child. 2013;98(1):52-6.
19. Singh P et al. Prevalence of Celiac disease in Asia: A systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;31(6):1095-101.
20. Ramakrishna BS et al. Prevalence of adult celiac disease in india: Regional variations and associations. Am J Gastroenterol. 2016;111(1):115-23.
21. Teresi S et al. Celiac disease seropositivity in Saharawi children:

A follow-up and family study. J Pediatr Gastroenterol Nutr. 2010;50(5):506-9.

22. Bhadada SK et al. Prevalence and clinical profile of celiac disease in Type 1 diabetes mellitus in north India. J Gastroenterol Hepatol. 2011;26(2):378-81.
23. Bhadada SK et al. Changing scenario in aetiological profile of short stature in India-growing importance of celiac disease: A study from tertiary care centre. Indian J Pediatr. 2011;78(1): 41-4.
24. Bhadada SK et al. Does every short stature child need screening for celiac disease? J Gastroen-terol Hepatol. 2008;23(8 Pt 2):e353-6.
25. Schuppan D et al. Celiac disease: From pathogenesis to novel therapies. Gastroenterology. 2009;137(6):1912-33.
26. Schulzke JD et al. Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. Pediatr Res. 1998;43(4 Pt 1): 435-41.
27. Gutierrez-Achury J et al. Fine mapping in the MHC region accounts for 18% additional genetic risk for celiac disease. Nat Genet. 2015;47(6):577-8.
28. Canova C et al. Association of maternal education, early infections, and antibiotic use with celiac disease: A population-based birth cohort study in northeastern Italy. Am J Epidemiol. 2014;180(1):76-85.
29. Chander AM et al. Genome sequencing of Serinicoccus chungangensis strain CD08_5 isolated from duodenal mucosa of a celiac disease patient. Genome Announc. 2016;4(2).
30. Chander AM et al. Genome sequence of Kocuria palustris strain CD07_3 isolated from the duo-denal mucosa of a celiac disease patient. Genome Announc. 2016;4(2).
31. Chander AM et al. Genome insight and comparative pathogenomic analysis of Nesterenkonia jeotgali strain CD08_7 isolated from duodenal mucosa of celiac disease patient. Front Microbiol. 2017;8:129.
32. Wacklin P et al. Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. Am J Gastroenterol. 2014;109(12):1933-41.
33. Radlović N et al. Effect of gluten-free diet on the growth and nutritional status of children with coeliac disease. Srp Arh Celok Lek. 2009;137(11-12):632-7.
34. Sanz Y. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult humans. Gut Microbes. 2010;1(3):135-7.
35. Wieser H. Chemistry of gluten proteins. Food Microbiol. 2007;24(2): 115-9.
36. Sollid LM, Khosla C. Future therapeutic options for celiac disease. Nat Clin Pract Gastroenterol Hepatol. 2005;2(3):140-7.
37. Siegel M et al. Rational design of combination enzyme therapy for celiac sprue. Chem Biol. 2006;13(6):649-58.
38. Koning F et al. Pathomechanisms in celiac disease. Best Pract Res Clin Gastroenterol. 2005;19(3):373-87.
39. Jabri B, Sollid LM. Mechanisms of disease: Immunopathogenesis of celiac disease. Nat Clin Pract Gastroenterol Hepatol. 2006;3(9): 516-25.
40. Shan L et al. Structural basis for gluten intolerance in celiac sprue. Science. 2002;297(5590):2275-9.
41. Mamone G et al. Immunogenic peptides can be detected in whole gluten by transamidating highly susceptible glutamine residues: Implication in the search for gluten-free cereals. J Agric Food Chem. 2013;61(3):747-54.
42. Iacomino G et al. Structural analysis and Caco-2 cell permeability of the celiac-toxic A-gliadin peptide 31-55. J Agric Food Chem. 2013;61(5):1088-96.
43. Arentz-Hansen H et al. The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med. 2000;191(4):603-12.
44. Caputo I et al. Gliadin peptides induce tissue transglutaminase activation and ER-stress through Ca2+ mobilization in Caco-2 cells. PLoS One. 2012;7(9):e45209.
45. Giordani L et al. Two wheat decapeptides prevent gliadin-dependent maturation of human dendritic cells. Exp Cell Res. 2014;321(2):248-54.
46. Sturgess R et al. Wheat peptide challenge in coeliac disease. Lancet. 1994; 343(8900):758-61.
47. Fraser JS et al. Coeliac disease: In vivo toxicity of the putative immunodominant epitope. Gut. 2003;52(12):1698-702.
48. Hannon GJ. RNA interference. Nature. 2002;418(6894):244.
49. Gil-Humanes J et al. Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. Proc Natl Acad Sci U S A. 2010;107(39):17023-8.
50. Gil-Humanes J et al. The introgression of RNAi silencing of γ-gliadins into commercial lines of bread wheat changes the mixing and technological properties of the dough. PLoS One. 2012;7(9):e45937.
51. Gil-Humanes J et al. The shutdown of celiac disease-related gliadin epitopes in bread wheat by RNAi provides flours with increased stability and better tolerance to over-mixing. PLoS One. 2014;9(3):e91931.
52. Gil-Humanes J et al. Reduced-gliadin wheat bread: An alternative to the gluten-free diet for consumers suffering gluten-related pathologies. PLoS One. 2014;9(3):e90898.
53. Catassi C et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr. 2007;85(1):160-6.
54. Altenbach SB et al. Silencing of omega-5 gliadins in transgenic wheat eliminates a major source of environmental variability and improves dough mixing properties of flour. BMC Plant Biol. 2014;14:393.
55. Altenbach SB et al. Assessment of the allergenic potential of transgenic wheat (triticum aes-tivum) with reduced levels of ω5-gliadins, the major sensitizing allergen in wheat-dependent exercise-induced anaphylaxis. J Agric Food Chem. 2015;63(42):9323-32.
56. Gobbetti M. Will Europe toast GM wheat for gluten sufferers? Nat Biotechnol. 2016;34(4):369. Erratum in: Nat Biotechnol. 2016;34:1072.
57. Gil-Humanes J et al. Suppression of gliadins results in altered protein body morphology in wheat. J Exp Bot. 2011;62(12):4203-13.
58. Barro F et al. Targeting of prolamins by RNAi in bread wheat: Effectiveness of seven silencing-fragment combinations for obtaining lines devoid of coeliac disease epitopes from highly im-munogenic gliadins. Plant Biotechnol J. 2016;14(3):986-96.
59. Sasaki M et al. Prevalence of clinic-defined food allergy in early adolescence: The SchoolNuts study. J Allergy Clin Immunol. 2018;141(1):391-8.
60. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention and management. J Allergy Clin Immunol. 2018;141(1):41-58.
61. Polk BI, Dinakar C. Patient-centered outcomes in food allergy. Curr Allergy Asthma Rep. 2017;17(6):39.
62. Backman H et al. Increased prevalence of allergic asthma from 1996 to 2006 and further to 2016–results from three population surveys. Clin Exp Allergy. 2017;47(11):1426-35.
63. Altenbach SB et al. Differential effects of a post-anthesis fertilizer regimen on the wheat flour proteome determined by quantitative 2-DE. Proteome Sci. 2011;9:46.
64. Hurkman WJ et al. Comparative

proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in the developing endosperm and flour from Triticum aes-tivum L. cv. Butte 86. Proteome Sci. 2013;11(1):8.

65. Dixon RA et al. Applications of metabolomics in agriculture. J Agric Food Chem. 2006;54(24):8984-94.
66. Oms-Oliu G et al. Metabolomics for assessing safety and quality of plant-derived food. Food Res Int. 2013; 54(1):1172-83.
67. Granvogl M et al. Influence of sulfur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. J Agric Food Chem. 2007;55(10):4271-7.
68. Rennenberg H et al. Sulfur metabolism in plants: Are trees different? Plant Biol (Stuttg). 2007;9(5):620-37.
69. Zörb C et al. Silencing of the sulfur rich α-gliadin storage protein family in wheat grains (Triticum aestivum L.) causes no unintended side-effects on other metabolites. Front Plant Sci. 2013;4:369.
70. Conner AJ et al. The release of genetically modified crops into the environment. Part II. Over-view of ecological risk assessment. Plant J. 2003;33(1):19-46.
71. Becker D et al. Protein composition and techno-functional properties of transgenic wheat with reduced α-gliadin content obtained by RNA interference. Journal of Applied Botany and Food Quality. 2012;85(1):23.
72. Johnson KL et al. How does scientific risk assessment of GM crops fit within the wider risk analysis? Trends Plant Sci. 2007;12(1):1-5.
73. Moses V et al. GM crops and food: Biotechnology in agriculture and the food chain. Editorial. GM Crops Food. 2012;3(1):1-2.

Challenges in Allergy Diagnostics and Solutions Worth Considering

Authors: *Snjezana Kos,[1] Robert-Jan Sanders[2]

1. Central Laboratory of the Hospital (Maasstad Lab), Department of Clinical Chemistry, Maasstad Hospital, Rotterdam, Netherlands
2. General Practice Laboratory Foundation (Star-SHL), Department of Laboratory Diagnostics, Rotterdam, Netherlands

*Correspondence to Koss@maasstadziekenhuis.nl

Disclosure: The authors have declared no conflicts of interest.

Received: 16.03.18

Accepted: 11.05.18

Keywords: Allergy, molecular components (MC), patient management, pretest probability, specific activity (SA).

Citation: EMJ Allergy Immunol. 2018;3[1]:123-127.

Abstract

The introduction of molecular components has led to exponential growth in the field of allergy diagnosis over the last two decades and allergy testing is now more complex and comprehensive. Most specialists who do not deal with the management of allergy patients on a daily basis may find it difficult to stay up-to-date with current developments in the field, which, in practice, may lead to unnecessary or nontargeted testing. The primary objective of this review is to briefly summarise the major differences in past immunoglobulin E testing compared to modern methods. The secondary objective is to give an overview of approaches that are, in the authors' opinions, worth considering as concepts because they address two fundamental issues in allergy management: how to relate results of immunoglobulin E testing to severity of symptoms, and how to increase the pretest probability of an allergy and facilitate management of an allergic patient.

INTRODUCTION

Diagnostic Challenges in Allergy Patients

In routine allergy diagnostics, immunoglobulin (Ig)E antibody tests are used to detect and monitor the reaction of the immune system to the allergen. According to recent literature, 6.0% of children and 3.7% of adults experience IgE-mediated allergic symptoms following the ingestion of food.[1]

Allergy testing is now more complex and comprehensive due to the introduction of molecular components (MC) over the last two decades.[2] This development in methodology is difficult for most specialists to follow, especially for those who do not deal with allergy on a daily basis. In practice, unnecessary testing or nontargeted testing is often observed. Though it may seem like a less straightforward approach, the correct combination of properties of MC and its use in the right context is important for the success of MC-mediated therapy.[3-5]

Correct use of MC is a complex problem that was highlighted by the European Academy of Allergy and Clinical Immunology (EAACI) and, as such, a taskforce was formed that initiated a new framework for the interpretation of IgE sensitisation tests.[6] In brief, multiple testing

without allergy-focussed clinical history leads to a likelihood of allergy not higher than the background rate.[6] This implies that the pretest probability of allergic disease should preferably be judged prior to commencing allergy diagnostics. Large prospective studies aim to contribute to the solution of this problem by mapping patient-related factors in relation to allergy manifestations, such as the Mechanisms of the Development of Allergy (MeDALL) project, which is still ongoing.[7-9] The approaches that help to increase the pretest probability of allergy are stimulated in addition to the patient-centred approach.[6]

Misused or misinterpreted diagnostics can lead to unnecessary dieting or may postpone referral to an allergy specialist. After the referral process, additional testing may be performed, such as skin prick tests, oral provocation testing, or even more complex tests, such as the Immuno Solid-phase Allergen Chip® (ISAC) (VBC Genomics, Wien, Austria and Phadia, Uppsala, Sweden), which carries out semi-quantitative tests on the chip. Sometimes treatment such as immunotherapy may be offered. For skin prick tests and immunotherapy, the composition of test products used is largely unknown; composition refers to percentages of different MC that the tests are made up of. This makes the comparison between allergy diagnostics (extracts and skin prick tests) and therapy (immunotherapy) difficult, and the difficulty translating diagnostics into therapeutics may be a contributing factor towards creating inconsistent results among studies in the literature.[10-14]

In the recent EAACI guidelines on immunotherapy, a key recommendation is that a 3-year course of subcutaneous or sublingual immunotherapy is recommended for children and adolescents with moderate-to-severe allergic rhinitis triggered by grass or birch pollen allergy to prevent asthma for up to 2 years post-immunotherapy. There is low-quality evidence for the preventive potential of this treatment; however, further high-quality clinical trials are needed.[15]

The primary objective of this review is to summarise the differences in current IgE testing compared to testing in the past. The secondary objective is to give an overview of approaches that are, in the authors' opinions, worth considering as concepts because they address two fundamental issues in allergy management: how to relate results of IgE testing to severity of symptoms, and how to increase the pretest probability of allergy and facilitate management of the allergic patient.

COMPARISON OF MAJOR DIFFERENCES IN SEROLOGICAL IMMUNOGLOBULIN E TESTING: PAST AND PRESENT

Molecular Component Testing in Addition to Extract Testing

Allergy testing is traditionally performed by measuring the presence of specific (s)IgE antibodies to allergen extracts. In the past this was the only serological possibility, but for the last 10 years MC have been introduced and can be identified in clinical laboratories on a regular basis. Egg allergy is a key example of the MC concept: an egg extract can be obtained. This extract is composed of varying percentages of MC, which the patient reacts to with varying severity. The names of the different MC are derived from Latin names of plant or animal sources, which makes having structured nomenclature difficult to remember if not used on a daily basis.

In general, an allergen-independent cut-off for sIgE is used to indicate sensitisation. A sIgE result >0.35 kU_A/L, and in some cases >0.10 kU_A/L, indicates sensitisation to a certain allergenic source, independent of whether sensitisation to extracts or to MC are tested. However, if the reaction to allergen extracts is significant, it still may not indicate its origin, i.e., MC contributing to a positive result.

Functional Stratification of Molecular Components

The MC are functionally stratified into groups, such as lipid-transfer proteins (LTP), storage proteins, and minor and cross-reactive allergens such as PR-10 and profilin. The functional sorting of MC allows more targeted testing for the severity of these major allergens.[16,17] The increased knowledge gained from functional group testing can aid experienced clinicians to better manage their patients.

Different Techniques Used to Detect Specific Immunoglobulin E

sIgE for MC can be determined by different techniques, with the largest portfolio offered by Phadia™ Laboratory Systems (Thermo Fisher Scientific, Waltham, Massachusetts, USA). sIgE can be measured in solution in a quantitative manner (ImmunoCAP Components) or on the chip in a semi-quantitative way (ImmunoCAP ISAC), both of which correlate well with each other.[18,19] Among others, there are two key differences between these two techniques. Firstly, the sensitivity to sIgG interference during the measurement with ImmunoCAP, when approximately a million times more allergen is coated on a cap. This type of measurement is more sensitive and less prone to interference by sIgG naturally generated as a result of activation of the immune system in patients who are allergic to some sources. The reason is that the effect of competition of sIgG, particularly the sIgG4 subtype, with sIgE for the allergen binding site is more pronounced when the amount of allergen available for binding is limited. The difference in the amount of allergen present determines the extent to which the result of sIgE may be influenced. The results of sIgE measured on the ImmunoCAP and ISAC may, therefore, potentially diverge due to sIgG (especially the sIgG4) concentration. Secondly, on ISAC, no routine possibility to determine total (t)IgE exists. The only allergy test for which the World Health Organization (WHO) standard exists is for tIgE (WHO 75/502).[20]

In general, independent of technique, some MC are obtained by complex purification and others through recombinant techniques. The same holds for the allergen extracts used. This can also be a source of differences in sensitivity among different reagent providers, resulting in heterogeneous results among studies.

Correlation of the Patient's Clinical History and Biochemical Testing

In an ideal situation, sIgE testing would correlate with a patient's clinical history and additional testing would not be necessary. However, there are a significant number of patients whose biochemical testing does not correlate with their clinical history, and the test outcomes are not a reliable predictor for severe reactions. Patients may still report anaphylactic shock with the same sIgE values as those who fully tolerate the same food allergen.

POSSIBLE SOLUTIONS

Exploring the Use of Specific Activity of Molecular Components as an Approach to Classify Patients into Severe and Non-Severe Groups

Specific activity of extracts

Progress has been made in biochemical testing and the use of sIgE MC, which allows more specific testing than older methodologies, but the fundamental problem of poor correlation between test results and clinical presentation remains. Studies have shown that the ratio of food extract sIgE to tIgE, the so-called specific activity (SA), is a useful parameter in predicting clinical outcome when compared to the absolute value of sIgE from the extracts.[1,21] The concept behind SA is that it relates sIgE to the total pool of IgE. Theoretically, the sum of all IgE is reflected by tIgE. The IgE receptor does not appear to have a predisposition for any specific type of sIgE; these sIgE instead compete for the same binding sites. The SA of MC might be the missing piece that specialists are looking for to provide a specific test that relates to disease severity.

Specific activity of molecular components

In a preliminary study with a small sample size, IgE antibodies to molecular food components, whether measured on ISAC or ImmunoCAP, were shown to be frequently abnormal and did not reflect disease severity sufficiently. This was reiterated when the cut-off values were increased 10-fold and similar abnormal results were produced. There was sufficient correlation between ISAC and ImmunoCAP. The discrepancy between ImmunoCAP and ISAC could not be explained by IgG4 interference. There was, however, improved correlation between disease severity and clinical outcome with the use of SA MC as a clinical predictor compared to sIgE measurement.[22]

Substantiation of use of specific activity of molecular components

The discrepancy between sIgE and disease severity in allergies is still not fully understood.[23] There are no tests available that can predict severe allergic disease. It was previously shown that SA might be a humoral immune response parameter important in allergy testing using allergen extracts.[21,24] Therefore, SA might be the missing piece to close this fundamental gap in allergy diagnostics.

IgE concentrations (tIgE and sIgE) are age-dependent; therefore, age-dependent reference values for tIgE are used. Since both tIgE and sIgE show this dependency, the use of SA (sIgE or tIgE) overcomes the problem of age dependency. SA may have greatest clinical importance in patients with low tIgE and in monosensitised patients.[1,21,24,25]

Matricardi et al.[25] showed that when a patient is 5 years old, variations in tIgE reflect variations in overall sIgE concentrations. The same study indicated that in childhood, sIgE (to grass pollen) starts as a weak mono or oligomolecular response and evolves rapidly into a polymolecular response giving rise to (severe) clinical symptoms. This implies that allergen-specific immunotherapy should be started as early as possible in the sensitisation process in order to avoid further expansion of sensitisations and escalation of clinical symptoms.[26] In this context, SA of MC might help to stratify patients who may benefit from early intervention from those who may not.

Using the Allergy Algorithm or Another Approach to Increase Pretest Probability of Allergic Disease

The allergy algorithm was developed in Maasstad Hospital, Rotterdam, Netherlands, as a tool to address the challenges faced by clinicians and general practitioners in allergy diagnostics.[27] In the development of this algorithm, the authors have included the most common allergens occurring in the Netherlands, using a rule-based approach. The algorithm is guided by the patients' symptoms, in line with classifications suggested by the EAACI taskforce, but are less extensive.[6] The results are accompanied by interpretation texts that can address some combinations of seven issues: severity of sensitisation based on reflex testing of MC known to be related to severe reactions like storage proteins or LTP upon obtaining positive reactions to extracts; influence of thermal processing of food; possible cross-reactivity; referral to allergy specialist advice; quantity of allergens tested in relation to symptoms and pretest probability of allergy; advice on immunotherapy based on recent guidelines; and reflex testing for immunotherapy MC upon request.

The aims of the tool are to provide a patient-specific diagnostic process and assist physicians with interpretation of the results. Although frequently underestimated, the improper interpretation of allergy results and inadequate treatment can lead to the development of asthma and a possible lifelong need for corticosteroids, which may in turn lead to the development of adrenal insufficiency.[28]

CONCLUSION

Allergy testing has changed dramatically and, through the introduction of MC, is now more complex and comprehensive. The diagnostic value of the MC sIgE is questionable because the cut-off indicates only sensitisation and is the same for all components, extracts, and ages. Furthermore, the information remains incomplete as patients may still report anaphylactic shock on the same sIgE values as those who fully tolerate some allergens, including food.

A key approach in the management of patients with allergy is testing that can help to increase pretest probability of allergy. In the right context, the tIgE is useful because it enables specialists to use SA of MC; moreover, it is the only standardised allergy test. Although good studies are lacking, preliminary studies show that the use of SA can be helpful in the management of patients and effective triage. Finally, considering the challenges in allergy diagnostics, the possible solutions presented in this manuscript may provide worthwhile consideration.

References

1. Gupta RS et al. Predicting outcomes of oral food challenges by using the allergen-specific IgE-total IgE ratio. J Allergy Clin Immunol Pract. 2014;2(3):300-5.
2. Villalta D et al. Recommendations for the use of molecular diagnostics in the diagnosis of allergic diseases. Eur Ann Allergy Clin Immunol. 2018;50(2):51-8.
3. Riccio AM et al. Molecular diagnosis and precision medicine in allergy management. Clin Chem Lab Med. 2016;54(11):1705-14.
4. Lin J, Alcocer M. Overview of the commonly used methods for food allergens. Methods Mol Biol. 2017;1592:1-9.
5. Gupta M et al. Diagnosis of food allergy. Immunol Allergy Clin North Am. 2018;38(1):39-52.
6. Roberts G et al. A new framework for the interpretation of IgE sensitization tests. Allergy. 2016;71(11):1540-51.
7. Anto JM et al. Mechanisms of the development of allergy (MeDALL): Introducing novel concepts in allergy phenotypes. J Allergy Clin Immunol. 2017;139(2):388-99.
8. Westman M et al. Windows of opportunity for tolerance induction for allergy by studying the evolution of allergic sensitization in birth cohorts. Semin Immunol. 2017;30: 61-6.
9. Wilson JM et al. Allergen sensitization in a birth cohort at midchildhood: Focus on food component IgE and IgG4 responses. J Allergy Clin Immunol. 2018;141(1):419-23e5.
10. Huss-Marp J et al. Spiking with recombinant allergens to improve allergen extracts: Benefits and limitations for the use in routine diagnostics: Part 19 of the Series Molecular Allergology. Allergo J Int. 2015;24:236-43.
11. Kleine-Tebbe J, Jakob T. Molecular allergy diagnostics using IgE singleplex determinations: Methodological and practical considerations for use in clinical routine: Part 18 of the Series Molecular Allergology. Allergo J Int. 2015;24:185-97.
12. Larenas-Linnemann D et al. Debates in allergy medicine: Allergy skin testing cannot be replaced by molecular diagnosis in the near future. World Allergy Organ J. 2017;10(1):32.
13. Schuyler AJ et al. Underestimation of specific IgE measurements using extract-based assays on undiluted sera revealed through dilution. J Allergy Clin Immunol Pract. 2018;6(3):1070-72.e4.
14. Wojtalewicz N et al. Six years of INSTAND e. V. sIgE proficiency testing: An evaluation of in vitro allergy diagnostics. Allergo J Int. 2017;26(2):43-52.
15. Halken S et al. EAACI guidelines on allergen immunotherapy: Prevention of allergy. Pediatr Allergy Immunol. 2017;28(8):728-45.
16. Canonica GW et al. A WAO - ARIA – GA²LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J. 2013;6(1):17.
17. Heaps A et al. The utility of the ISAC allergen array in the investigation of idiopathic anaphylaxis. Clin Exp Immunol. 2014;177(2):483-90.
18. Jensen-Jarolim E et al. Debates in allergy medicine: Molecular allergy diagnosis with ISAC will replace screenings by skin prick test in the future. World Allergy Organ J. 2017;10(1):33.
19. Hamilton RG. Microarray technology applied to human allergic disease. Microarrays (Basel). 2017;6(1):3.
20. National Institute for Biological Standards and Control. WHO Reference Reagent Serum Immunoglobulin E (IgE) Human NIBSC code: 75/502 Instructions for use. (2011). Available at: http://www.nibsc.org/documents/ifu/75-502.pdf. Last accessed: 17 May 2018.
21. Hamilton RG et al. IgE antibody-specific activity in human allergic disease. Immunol Res. 2010;47(1-3):273-84.
22. Kos S et al. Preliminary study in specific activity of molecular components in allergy: Implications for diagnostics and relationship with disease severity. Clin Chem Lab Med. 2017;55(6):e113-7.
23. Du Toit G et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015;372:803-13.
24. Hamilton RG, Saito H. IgE antibody concentration, specific activity, clonality, and affinity measures from future diagnostic confirmatory tests. J Allergy Clin Immunol. 2008;122(2):305-6.
25. Matricardi PM et al. Longitudinal trends of total and allergen-specific IgE throughout childhood. Allergy. 2009;64(7):1093-8.
26. Matricardi PM. Molecular profile clustering of IgE responses and potential implications for specific immunotherapy. Curr Opin Allergy Clin Immunol. 2013;13(4):438-45.
27. Kos S et al. Allergy algorithm to increase pre-test probability of allergic disease. J Lab Precis Med. 2017;2:64.
28. Broersen LH et al. Adrenal insufficiency in corticosteroids use: Systematic review and meta-analysis. J Clin Endocrinol Metab. 2015;100(6):2171-80.

Revising the Criteria for Occupational Mould-Related Disease: Arguments, Misconceptions, and Facts

Authors: Tamara Tuuminen,[1,2] *Jouni Lohi[3]

1. Medicum, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland
2. Kruunuhaka Medical Center, Kaisanimenkatu, Helsinki, Finland
3. Medicum, Department of Pathology, University of Helsinki, Helsinki, Finland

*Correspondence to Jouni.j.lohi@gmail.com

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors would like to thank Prof Ville Valtonen for the useful discussions and critical reading of the manuscript. They would also like to thank all the patients who shared their medical records and experiences, and Ewen MacDonald for his assistance in proofreading this article.

Received: 23.11.17

Accepted: 06.06.18

Keywords: Allergic alveolitis (AA), asthma, dampness, hypersensitivity, medical ethics, microbiota, mould hypersensitivity, mould-related disease, serial inhalation challenge (SIC), serial peak expiratory flow (PEF).

Citation: EMJ Allergy Immunol. 2018;3[1]:128-135.

Abstract

Occupational exposure to indoor air moulds and the consequent development of dampness and mould hypersensitivity syndrome (DMHS) may cause lung damage; in most cases, this is not allergic asthma mediated by specific immunoglobulin E-class antibodies. Instead, it is often a hypersensitivity pneumonitis or bronchopneumonitis. In Finland, the current diagnostic criteria for occupational DMHS have been adapted from knowledge of immunoglobulin E-mediated asthma; however, the safety of the methods used in occupational medicine have been insufficiently addressed in the literature. Accordingly, the aim of this paper is to raise awareness about the safety of current methods: specific inhalation challenge, workplace peak expiratory flow monitoring, and histamine provocation tests, by illustrating four cases. The medical records of these four cases with documented occupational DMHS were reviewed. The presented evidence suggests that the methods applied to study the occupational nature of lung damage are not suitable and the current ethics are questionable. The authors claim that, in particular, serial inhalation challenge with extracts from moulds, workplace serial peak expiratory flow leading to continuous exposure to mycotoxins, and histamine provocation tests may irreversibly damage the health of DMHS patients. Therefore, there is a prompt need to revise current practice guidelines to assess occupational DMHS. The guidelines should not be based on old dogmas, nor should they be influenced by insurance considerations. Instead, they should be based solely on medical evidence and, crucially, they should be safe for the patient and, therefore, should be implemented with caution.

INTRODUCTION

Mould-related disease, or dampness and mould hypersensitivity syndrome (DMHS), has been extensively described;[1-9] it is a complex multiorgan disorder with activation or impairment of the immune system,[10,11] systemic inflammation,[12,13] recurrent infections, or reactivation of latent infections. Only some cases develop into immunoglobulin (Ig)E-mediated allergy and asthma,[14,15] and DMHS may also be associated with invasive fungal infections.[16] In reality, DMHS is a mycotoxicosis,[17,18] a systemic chronic inflammation,[12,13] and an oxidative stress reaction.[17] The differences in response patterns between patients have been reviewed through the prism of evolutionary coadaptation of moulds and humans over millennia of coexistence.[19]

DMHS is very common in Finland, although exact data on the number of incidences are missing. The diagnostic coding R68.81 implemented in Finland in 2015 does not refer specifically to DMHS, but includes all environmental hypersensitivities. DMHS is therefore considered as a trait or a functional disorder, not a disease, and does not guarantee the patient access to any social security benefits. In Finland, the incidence of DMHS in adults and children is increasing alarmingly and is highlighted in media publications and on social networks. However, officially recognised occupational cases, mainly occupational asthma (OA), are steadily decreasing. Only 5–6% of DMHS patients receive compensation for OA (Irmeli Lindström, unpublished data, 2016) and DMHS is accepted as an occupational disorder only if it results in the development of asthma; all other forms of DMHS[12] are not considered.[20,21] This health policy is determined by the Finnish Institute of Occupation Health (FIOH), which is partly financed by insurance companies.[22]

From 1995 to 2009, the FIOH studied the causality of OA and mould infestation in the workplace by applying the specific inhalation challenge (SIC); this was mandatory for all subjects undergoing investigations for occupational lung conditions.[21] This exposure was performed without ethical approval. During the SIC, an extract of *Aspergillus fumigatus* or *Cladosporium cladosporioides* (ALK-Abelló, Copenhagen, Denmark) was inhaled by a sensitised person in a specific chamber. Importantly, these preparations contained impurities[23] and had never been intended for inhalation but only to study IgE-mediated immunity. Fungal preparations were also administered to individuals without specific IgE-class antibodies (Cases 1 and 2). During this period, the SIC test was performed on several hundreds of people, some of whom became unconscious after the exposure, and many experienced acute health deterioration (Cases 1 and 2), requiring hospitalisation. After the SIC exposure, some of these individuals were diagnosed with allergic alveolitis (AA) (Cases 1 and 2). After many complaints, the SIC was replaced by workplace serial peak expiratory flow (PEF) monitoring, which became mandatory.[21] To the best of the authors' knowledge, the safety of these tests has not been addressed in the scientific literature. This case series reviews the medical records of four patients with an explicit occupational exposure to dampness microbiota documented by state-of-the-art environmental investigations.

CASE 1

The Specific Inhalation Challenge Test Evoked an Acute Neutrophilic and Lymphocyte Influx into the Pulmonary Alveoli

A 50-year-old non-smoker experienced dizziness and a feverish feeling at his workplace; he had previously had a massive exposure with an unconscious episode due to indoor air moulds while standing below a ventilation output in October 2000. The replacement air in the remediated building was taken from under the floor. Extensive growth of *Streptomyces* bacteria, along with other damp-related species, had been cultured prior to remediation. In 2001, the man was placed on sick leave and thereafter did not return to his workplace. He was referred to the FIOH, where he tested IgE-negative for all available mould antigens and IgG-positive for some fungal antigens, but not for *A. fumigatus*. In March 2002, the first SIC was performed with *A. fumigatus* and was repeated after 1.5 weeks. The patient felt feverish and fatigued after the *A. fumigatus* exposure but did not react to the *C. cladosporioides* and *Acremonium* exposures; altogether he was exposed to SIC four times over 12 days. A few days after the first

exposure to *A. fumigatus*, he underwent his first bronchoalveolar lavage (BAL) investigation, which was repeated in July 2002. During the bronchoscopy, the mucosa was found to be fragile and covered with bloodstains. Finally, in 2002, he was given a diagnosis of occupational AA and received a disability pension for several years. After SIC exposure, he experienced vertigo, felt feverish although his body temperature remained at only 36°C, had shortness of breath, and his walking ability declined dramatically. In February 2013, he was admitted to hospital due to the presence of right pleural exudate that was treated with pleural decortication. The patient continues to experience vertigo and sick building syndrome, and a mouldy environment exacerbates his symptoms (e.g., pain). In summary, the patient in this case was exposed to dampness microbiota at his workplace; he had four sequential SIC tests and immediate BAL investigation revealed acute inflammation, leading to a diagnosis of occupational AA.

CASE 2

The Specific Inhalation Challenge Test Exacerbated Pulmonary Effusion in Dampness and Mould Hypersensitivity Syndrome

A 49-year-old non-smoker worked in an office with dampness in 1990 and 4 years later started to experience recurrent sinusitis. Starting from 2000, she experienced unexplained bruising and, in 2001, moved to another building because moisture damage microbiota (e.g., *Chaetomium* and *Aspergillus*) and asbestos had been found. In this new office, she started to experience a non-productive cough, fatigue, dyspnoea, palm tingling, and fever. In 2001, she had leukopenia and thrombocytopenia and, due to high fever, she was admitted to hospital; however, pneumonia was not diagnosed. Thereafter, she often missed work due to illness, which improved her condition. In 2002, she was referred to the FIOH, where skin prick tests for moulds were negative, as were IgE-class antibodies to 16 common damp microbiota moulds. High-resolution computed tomography (HRCT) revealed minor fibrosis, and a histamine provocation test in 2002 confirmed asthma. Subsequently, she was given a 3-month sick leave period, was prescribed asthma medication, and was then able to cycle 20–30 km per day. She returned to work in a third building and soon afterwards was again referred to the FIOH where spirometry showed evidence of obstruction. She experienced pain in her chest when sneezing and in the evenings she was hypothermic. She was again referred to the FIOH where she was exposed to SIC tests with *A. fumigatus* and *C. cladosporioides* extracts under powerful corticosteroid medication with *A. fumigatus* antigen and *C. cladosporioide* antigen in 2003. Thereafter, she had a burning and seizure-like sensation in her chest, tingling of her left arm, mouth numbness, and a heavy feeling beneath the scapulae. The SIC results were interpreted as an intrinsic but poorly balanced asthma. She became sensitised to environmental moulds and could not tolerate damp weather. She developed multiple allergies, including to bananas, strawberries, and apples. She returned to work at the end of 2002, but was soon placed on sick leave again. In 2003 (1 year after SIC), she underwent BAL and ultimately a diagnosis of occupational AA was made. In 2004, she was examined again at the FIOH, where her condition worsened, and she needed oxygen inhalation. In 2006, a BAL examination was performed again that confirmed her previous diagnosis. In 2009, she underwent biopsy of her lungs that caused massive oedema of her neck. In 2014, the BAL examination again confirmed AA but insurance companies have refused to compensate her medical expenses since 2014. In summary, the patient described in Case 2 was exposed to dampness microbiota in multiple workplaces, where serial PEF monitoring was performed two times with inconclusive results. She was exposed once to SIC and BAL investigations that revealed chronic inflammation compatible with AA.

CASE 3

Mandatory Serial Peak Expiratory Flow Monitoring for Legal Evidence Caused a Health Deterioration

A healthy 50-year-old non-smoker started to feel unwell shortly after she moved to a new office in January 2009. She had severe flu-like symptoms with cough, rhinitis, eye infections, palpitations

in the extremities, loss of voice, headache, fatigue, and fever. During her time off work in the summer, her symptoms eased, but when she returned to work in August she started to experience dyspnoea. In addition, she began to experience nausea, vomiting, and chest pain. Species, including *Actinomycetes*, *Acremonium*, and *Penicillium,* were detected at her workplace. In December 2009, she was placed on sick leave due to new-onset asthma and she was referred to the FIOH for professional evaluation. The FIOH insisted on serial PEF monitoring, although she felt unwell in the office. The return to work aggravated her illness during workplace PEF monitoring and she was subsequently admitted to hospital. The attending physician doubted her ability to continue PEF monitoring, but nonetheless its completion was deemed mandatory in order to gather evidence of an occupational illness. In March 2010, regular medication for asthma was started. In June 2010, she received a diagnosis of OA and a pension for her professional disability for only 2 years; the reasoning for this decision was that other diseases, such as multiple chemical sensitivity (MCS), were her main ailments. At present, she has chronic fatigue syndrome, hyperhaemoglobinaemia (haemoglobin: 170 g/L; reference for females: 117–155 g/L), secondary (compensatory to chronic toxicosis) polycythaemia, and hypokalaemia (plasma potassium: 3.1–3.2 mmol/L; reference: 3.3–4.9 mmol/L) despite potassium substitution. In 2017, a disturbance in her autonomic nervous system balance was documented (Figure 1). Due to her MCS, she found it difficult to leave her home and rarely had visitors. At the time of this communication, she is not a recipient of any social security benefits. In summary, the patient in Case 3 was exposed to dampness microbiota at her workplace; she undertook workplace serial PEF twice, despite her health deterioration, and finally received a diagnosis of OA.

CASE 4

Controlled Significant Decline of Peak Expiratory Flow During a Bronchospasm is of No Legal Value

A 58-year-old non-smoker started to experience a non-productive cough at her workplace when studying petri dishes with bacterial growth. She started to experience flu-like episodes in the workplace after the weekends. She was previously healthy, taking no medication. In June 2014, after a holiday, she received two antibiotic courses for sinusitis that did not relieve her symptoms. She lost her voice and her cough became so intense that she experienced pain in her chest and ribs and felt extremely fatigued. During a 2-week period of sick leave, her voice almost returned to normal but her cough continued and worsened after her return to work. Finally, a fungal growth was found in the proximity of her office, and it was no longer disputed that her disease was associated with working in the office. In August 2014, she underwent six maxillary punctures, but the cultures were negative. The workplace serial PEF in August was unsuccessful due to abundant secretions of mucus from her nose, shortness of breath, and pain in her ribs and chest. From mid-August to December, she was placed on sick leave due to severe cough and laboratory remediation.

At the FIOH, in January 2015, incipient asthma was suspected because of the variation in the daily PEF, the insignificant response to bronchodilators, and slight hyper-reactivity, but these findings did not meet the clinical criteria for asthma. She returned to work in January 2015 when remediation of the laboratory had been completed; she quickly started to experience a loss of voice and her cough exacerbated. On her last working day, when she experienced a bronchospasm, her PEF measurements dropped to 280–280–300 L/min (normal value: 450–500 L/min; recorded by a nurse) and 4 hours later, after 2 hours of outdoor walking, her PEF had slightly recovered (360–370–360 L/min; recorded by a physician). A diagnosis of asthma was made in August 2015 at the Helsinki University Hospital, Helsinki, Finland. Lung HRCT in 2016 revealed multiple lymphatic nodules in the interstitia that were still present 6 months later. Since her serial PEF had been unsuccessful, it was deemed that she did not have OA and thus did not qualify for benefits. *Cladosporium*, *Penicillium*, and *Aspergillus versicolor* were cultured in her workplace. In summary, the patient presented in Case 4 was exposed to dampness microbiota; workplace serial PEF were not performed but a significant drop in PEF on her last working day was of no legal value with regard to government benefits.

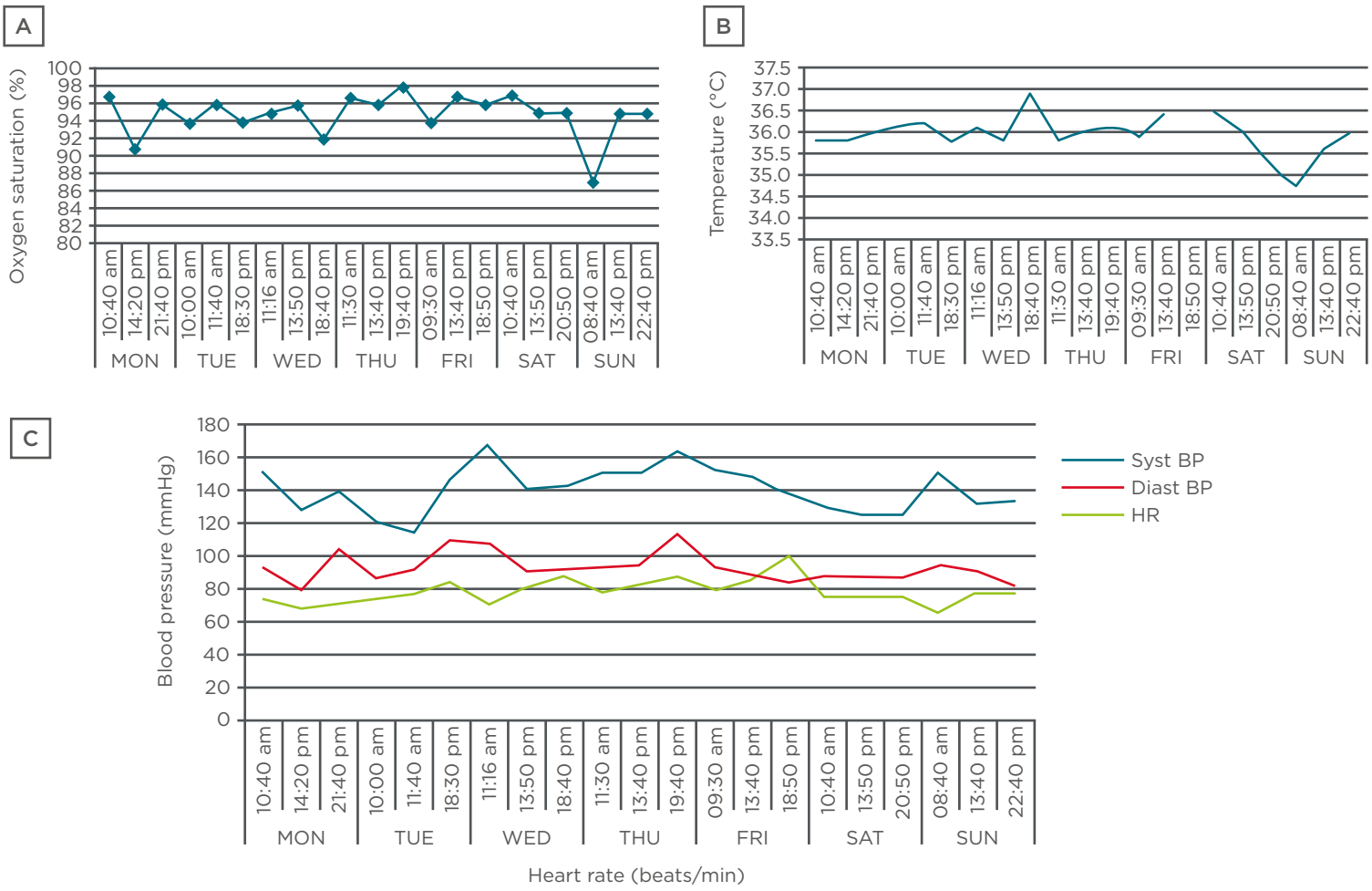


Figure 1: Physiometry parameters indicating dysregulation of the autonomic nervous system.

A) Monitoring of oxygen saturation (Jumper Medical Equipment Co., Shenzhen, China); B) Body temperature (Beurer, Ulm, Germany); C) Monitoring of systolic and diastolic blood pressure, and heart rate. P-glucose was also monitored (Bayer, Leverkusen, Germany) but not recorded in this figure. During the episodes of hypoglycaemia or hypothermia, the patient felt so unwell that she was bedridden.

Diast BP: diastolic blood pressure; HR: heart rate; syst BP: systolic blood pressure.

DISCUSSION

These cases illustrate that the diagnostic methods and criteria[24] applied to an IgE-mediated immune response are being erroneously applied to a disease that, in most cases, is not IgE-mediated (Cases 1 and 2).[15] Medical and legal abuse of patients exposed to indoor air dampness microbiota in their workplaces continues to take place in Finland with the unspoken approval of all the appropriate monitoring authorities. The Declaration of Helsinki,[25] signed in 1964 and widely regarded as the cornerstone document on human research ethics, is being violated.

The authors have shown that patients developed sick building syndrome (Cases 2, 3, and 4), meaning that while being away from the workplace their condition improved, but worsened upon return. It has also been illustrated that DMHS is mostly not an IgE-mediated allergy; none of the cases reported here were IgE-positive to the most common fungal antigens. DMHS patients may develop a loss of tolerance, becoming sensitive to allergens they could tolerate before (Case 2), and exposure to an inhaled fungi antigen caused acute inflammation (Case 1), while a BAL test performed 1 year after SIC revealed chronic inflammation (Case 2). Both Cases 1 and 2 illustrate the deleterious

health effects of SIC. These cases show that DMHS may also associate with MCS (Case 3). Most importantly, continued exposure to mycotoxins endangers patients' health (Cases 2, 3, and 4) and workplace serial PEF may be inconclusive (Case 2), may be of no help for the patient's legal rights (Case 3), and may aggravate symptoms (Case 3).

On the basis of the presented data, the authors conclude the following:

Deduction 1

Exposure to indoor air moulds may cause lung damage; in most cases, this is not an allergic asthma mediated by specific IgE-class antibodies, but is AA or hypersensitivity pneumonitis (HP).[26] DMHS has a myriad of clinical presentations.[1-9] Lung effusion due to the inhalation of spores and mycotoxins often is not only asthma; instead, in the majority of cases, it is AA or HP.[26] It is the opinion of the authors that the criteria and the protocol devised for OA[27,28] should not be applied to DMHS. The recommendations that an individual who has lost their tolerance should continue to inhale toxic air endangers their health, disregards the consequential symptoms and conflicts with the universally accepted healthcare principle of *primum non nocere* (first, to do no harm). Gathering legal evidence should never take precedence over medical ethics.

Mycotoxins cause so-called ion channel disease by forming novel ion channels that disrupt the membrane potential of the mitochondria because of the influx of Na^+ and efflux of K^+ from the cell.[18] Mycotoxins are broad-spectrum toxins with cytotoxic and immunomodulatory effects.[5-7,18] Chronic exposure to moulds may induce an inflammatory response that can be measured by cytokine and chemokine production from peripheral blood mononuclear cells.[29] Long-term exposure to indoor air dampness microbiota is the foundation for the development of MCS (Case 3).[9] One may argue that the reported consequences of mycotoxin exposure refer to the oral administration route; however, mycotoxins are also absorbed via the inhalation route, after which they can gain access to the blood circulation without being detoxified in the enterohepatic circulation, or alternatively they can penetrate directly into the brain via the nervus olfactorius.[30]

Since inhaled particles such as spores of pathogenic indoor moulds are only 0.005–5.000 µm in diameter,[31] it is easy to comprehend that these xenobiotics penetrate deep into the lungs, creating inflammation *in situ*, not only airway hyper-reactivity and inflammation. When a post-mortem examination was performed on an individual who had inhaled large quantities of mould xenobiotics through a bagpipe, a severe HP was revealed.[26] Thus, inhalation of mould components may lead not only to the inflammation of the large airways (asthma), but to an overwhelming inflammation of the parenchyma[26] and small airways.[32] When both are present, this condition may be called bronchopneumonitis (BP). Moreover, the strict definition of asthma has been questioned.[32] There are several reasons to suspect that mould exposure is not primarily allergic asthma but in fact causes HP or BP: a) patients report a poor response to bronchodilators because the inflammation is mainly in the small airways or in the parenchyma; b) poor response to corticosteroids because of the involvement of the T helper 17 inflammatory cell arm;[33] c) during auscultation, wheeze is not predominant in mould-exposed individuals and, instead, shortness of breath and even chest pain at rest are usually reported; d) spirometry curves are often compatible with a restriction defect rather than with an obstruction pattern; e) HRCT may reveal lymphatic nodes in the interstitial parenchyma (Case 4) or incipient fibrosis (Case 2); and f) an influx of lymphocytes, with the typical ratios of their subsets, is compatible with AA (Case 2).[34] Immediately after the exposure to impure mould extracts, a pathology compatible with acute inflammation (neutrophilic influx) was documented (Case 1).

Deduction 2

Clinical criteria and the protocol for evaluating mould-related lung disease in DMHS should be revised. Exposure to wet mouldy grains is not the only reason an individual can develop HP or BP. Thus, it is not only a farmer's disease and is not synonymous with organic dust toxic syndrome (ODTS).[35] In ODTS, the exposure is massive and caused mainly by spores, whereas the exposure to damp microbiota is associated with mycotoxins and volatile organic compounds. Indoor mycotoxins may be different from outdoor mycotoxins;[18] indoor mycotoxins

have been demonstrated to inhibit the growth and function of antigen-presenting cells and lymphocytes.[11,12] Therefore, AA, HP, or BP due to indoor mycotoxin-producing moulds may develop, even though there are low lymphocytic cell counts. Thus, the criteria adopted for diagnosing occupational AA associated with DMHS should be different from those of farmer's disease or ODTS. AA (or HP or BP depending on the agreed terminology) should be examined appropriately in every patient exposed to dampness microbiota.[36]

Deduction 3

The SIC test is by no means the most accurate test[27,28] to study causality in OA, especially in DMHS. The test is invasive and may cause irreversible health damage (Cases 1 and 2). As performed in Finland, the SIC test has been responsible for many serious and under-reported health problems in DMHS patients. The large number of SIC tests performed in Europe[28] is, in fact, a shameful history, not an achievement of advanced occupational medicine.

Deduction 4

Workplace serial PEF monitoring to prove causality in DMHS should be discontinued. Workplace serial PEF was originally suggested as a way of assessing OA with positive specific IgE-class antibodies.[24] IgG and IgE-class antibodies to dampness moulds have been extensively studied in Finland. It was found that specific IgE elevation to 11 species of moulds was observed in <5% of exposed children attending problematic schools (approximately n=500; age: 7–13 years).[15] The majority of IgE-positive children were atopic. Moreover, the PEF test has a sensitivity of only 75% (specificity: 95%),[24] which is insufficient for screening purposes. Rather, the possibilities of using cytokine and chemokine measures of blood in the diagnosis of asthma caused by mould exposure should be considered.[29] PEF measurement per se is not harmful, but serial workplace PEF measurements will cause continued harmful exposure of a person to indoor air mycotoxins (Case 3) and therefore should be banned.

Deduction 5

Histamine provocation tests in the evaluation of hyper-reactivity of bronchi in DMHS patients should be abandoned. So far, there is no evidence about the safety of this intervention. Many patients with DMHS in whom MCS has developed[9] exhibit a disruption in the permeability of their blood brain barrier (BBB). Iatrogenic exposure to histamine that penetrates the BBB will aggravate inflammation in the brain. In DMHS patients, neuroinflammation recorded as a structural brain injury with increased permeability of the BBB has been documented.[37]

CONCLUSION

Finally, it is undisputable that DMHS is not primarily an invasive fungal disease. Therefore, immunity guidelines developed for invasive infection are not applicable to this clinical entity.[38,39] The authors argue that DMHS is primarily a mycotoxicosis. Evaluation of the SIC test[40-43] shows that it lacks safety considerations and a careful assessment by independent clinicians of medical ethics, and may cause possible long-term adverse effects and even iatrogenic damage. The future directions for diagnosing and treating HP with an incidence of 0.3–0.9 per 100,000, irrespective of its cause, were highlighted by Vasakova et al.[44]

Based on the presented arguments, the authors challenge current practices related to the interpretation of occupational DMHS. The causality should be proven with a safer technique; for example, assaying the biomarkers of the inflammation cascade and oxidative stress.[44] These biomarkers should have a short half-life but be stable enough to permit analysis. The possibilities are within our reach; we need goodwill and an open mind to improve our practices.

References

1. Curtis L et al. Adverse health effects of indoor molds. J Nutr Environ Med 2004;14(3):261-74.
2. Gray MR et al. Mixed mold mycotoxicosis: Immunological changes in humans following exposure in water-damaged buildings. Arch Environ Health 2003;58(7):410-20.
3. Genuis SJ. Clinical medicine and the budding science of indoor mold exposure. Eur J Intern Med 2007;18(7):516-23.
4. Hope JA. Review of the mechanism of injury and treatment approaches for illness resulting from exposure to water-damaged buildings, mold, and mycotoxins. ScientificWorldJournal. 2013.
5. Thrasher JD. Fungi, bacteria, nano-particulates, mycotoxins and human health in water damaged indoor environments. J Comm Pub Health Nurs. 2016;2:115.
6. Pestka JJ et al. *Stachybotrys chartarum*, trichothecene mycotoxins, and damp building–related illness: New insights into a public health enigma. Tox Sci. 2008;104(1):4-26.
7. Rasimus-Sahari S et al. The peptide toxin amylosin of *Bacillus amyloliquefaciens* from moisture-damaged buildings is immunotoxic, induces potassium efflux from mammalian cells, and has antimicrobial activity. Appl Environ Microbiol. 2015;81:2939-49.
8. Tuuminen T, Rinne KS. Severe sequelae to mold-related illness as demonstrated in two Finnish cohorts. Front Immun. 2017;8:382.
9. Valtonen V. Clinical diagnosis of the dampness and mold hypersensitivity syndrome: Review of the literature and suggested diagnostic criteria. Front Immunol. 2017;8:951.
10. Kankkunen P et al. (1,3)-β-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J Immunol. 2010;184(1):6335-42.
11. Stanzani M et al. *Aspergillus fumigatus* suppresses the human cellular immune response via gliotoxin-mediated apoptosis of monocytes. Blood. 2005;105(6):2258-65.
12. Edmondson DA et al. Immune Response among patients exposed to molds. Int J Mol Sci. 2009;10(12):5471-84.
13. Korkalainen M et al. Synergistic proinflammatory interactions of microbial toxins and structural components characteristic to moisture-damaged buildings. Indoor Air. 2017;27(1):13-23.
14. Tischer CG et al.; ENRIECO consortium. Meta-analysis of mould and dampness exposure on asthma and allergy in eight European birth cohorts: An ENRIECO initiative. Allergy. 2011;66:1570-9.
15. Taskinen T. Moisture and mould problem in school children. Academic dissertation. Kuopio KTL, A9/2001.
16. Marcos CM et al. Anti-immune strategies of pathogenic fungi. Front Cell Infect Microbiol. 2016;6:142.
17. Chang HT et al. Mechanisms underlying effect of the mycotoxin cytochalasin B on induction of cytotoxicity, modulation of cell cycle, Ca^{2+} homeostasis and ROS production in human breast cells. Toxicol. 2016;370:1-19.
18. Mikkola R et al. Toxic indole alkaloids avrainvillamide and stephacidin B produced by biocide tolerant indoor mold *Aspergillus westerdijkiae*. Toxicon. 2015;99:58-67.
19. Daschner A. An evolutionary-based framework for analyzing mold and dampness-associated symptoms in DMHS. 2017;7:672.
20. Käypä hoito. A patient with symptoms of moisture and lesions: Current Care Guideline. Available at: http://www.kaypahoito.fi/web/kh/suositukset/suositus?id=hoi50111. Last accessed: 18 September 2017. (In Finnish).
21. Karvala K. "Asthma in damp indoor environments," Mertanen V (ed.), (2012), Tampere: Suomen Yliopistopaino Oy - Juvenes Print, pp.1-172.
22. Selinheimo S et al. Comparing cognitive-behavioural psychotherapy and psychoeducation for non-specific symptoms associated with indoor air: A randomised control trial protocol. BMJ Open. 2016;6(6).
23. Esch RE. Manufacturing and standardizing fungal allergen products. Allergy Clin Immunol. 2004;113(2):210-5.
24. Fishwick D et al.; British Thoracic Society Standards of Care Subcommittee Guidelines on Occupational Asthma. Standards of care for occupational asthma. Thorax. 2008;63(3):240-50.
25. World Medical Association. Declaration of Helsinki. Available at: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/. Last accessed: 18 September 2017.
26. King J et al. Bagpipe lung: A new type of interstitial lung disease? Thorax. 2016;72(4).
27. Vandenplas O et al. Diagnosing occupational asthma. Clin Exp Allergy. 2017;47(1):6-18.
28. Suojalehto H, Cullinan P. Specific inhalation challenge tests for occupational asthma in Europe: A survey. Eur Respir Rev. 2014;23(132):266-70.
29. Rosenblum Lichtenstein JH et al. Environmental mold and mycotoxin exposures elicit specific cytokine and chemokine responses. PLoS One. 2015;10(5):1-22.
30. Islam Z et al. Neurotoxicity and inflammation in the nasal airways of mice exposed to the macrocyclic trichothecene mycotoxin roridin a: Kinetics and potentiation by bacterial lipopolysaccharide coexposure. Toxicol Sci. 2007;98(2):526-41.
31. Pringle A. Asthma and the diversity of fungal spores in air. PLoS Pathog. 2013;9(6):e1003371.
32. Löwhagen OJ. Diagnosis of asthma - New theories. Asthma. 2015;52:538-44.
33. Zhang Z et al. β-Glucan exacerbates allergic asthma independent of fungal sensitization and promotes steroid-resistant TH2/TH17 responses. J Allergy Clin Immunol. 2017;139(1):54-65.
34. Wolff H et al. Patients referred to an indoor air health clinic: Exposure to water-damaged buildings causes an increase of lymphocytes in bronchoalveolar lavage and a decrease of CD19 leucocytes in peripheral blood. Scand J Clin Lab Invest. 2009;69:537-44.
35. Spagnolo P et al. Hypersensitivity pneumonitis: A comprehensive review. J investing Allergol Clin Immunol. 2015;25:237-50.
36. Alenius H et al. Recommendations on how to deal with moisture damage. Finnish Medical Journal. 2007;62. (In Finnish).
37. Shoemaker RC et al. Structural brain abnormalities in patients with inflammatory illness acquired following exposure to water-damaged buildings: A volumetric MRI study using NeuroQuant®. Neurotoxicol Teratol. 2014;45:18-26.
38. Wheeler ML et al. Immunity to commensal fungi: Detente and disease. Annu Rev Pathol. 2017;12:359-85.
39. Williams PB et al.; Environmental Allergens Workgroup. Innate and adaptive immune response to fungal products and allergens. J Allergy Clin Immunol Pract. 2016;4(3):386-95.
40. Masuo M et al. Factors associated with positive inhalation provocation test results in subjects suspected of having chronic bird-related hypersensitivity pneumonitis. Respir Investig. 2016;54:454-61.
41. Hu C et al. Specific inhalation challenge: The relationship between response, clinical variables and lung function. Occup Environ Med. 2017;74(8):586-91.
42. Muñoz X et al. Diagnostic yield of specific inhalation challenge in hypersensitivity pneumonitis. Eur Respir J. 2014;44(6):1658-65.
43. Morell F et al. Hypersensitivity pneumonitis: Challenges in diagnosis and management, avoiding surgical lung biopsy. Semin Respir Crit Care Med; 2016;37(3):395-405.
44. Vasakova M et al. Hypersensitivity pneumonitis: Perspectives in diagnosis and management. Am J Respir Crit Care Med. 2017;196:680-9.

Events

29TH AUGUST–2ND SEPTEMBER 2018

The 2018 Joint South Africa Paediatrics Association (SAPA) and Allergy Society of South Africa (ALLSA) Conference (ALLPAEDS 2018)

Cape Town, South Africa

This exciting joint congress organised by the SAPA and ALLSA boasts a fantastic scientific programme that pays particular... Read More

4TH–8TH SEPTEMBER 2018

29th Annual Conference of the Australasian Society of Clinical Immunology and Allergy (ASCIA)

Canberra, Australia

Want to learn about allergy and immunology in the sun this autumn? Then Canberra is the place for you, as the Australian capital hosts the 29th annual meeting of the ASCIA... Read More

12TH–16TH SEPTEMBER 2018

The 2018 Canadian Society of Allergy and Clinical Immunology (CSACI) Annual Scientific Meeting

Halifax, Canada

An event with a magnificent history, the CSACI annual meeting, Canada's foremost allergy and immunology gathering, returns this September for the 73rd time. With abstracts... Read More

27TH–29TH SEPTEMBER 2018

13th German Allergy Congress 2018 (DGAKI 2018)

Dresden, Germany

Germany is a nation steeped in allergy and immunology history, providing a number of groundbreaking advances over recent years that changed the face of the field. The motto of the DGAKI is "For a world without... Read More

30TH SEPTEMBER–2ND OCTOBER 2018

The British Society for Allergy and Clinical Immunology (BSACI) Annual Meeting 2018

Telford, UK

A mere stone's throw from the historic town of Shrewsbury, UK, the annual meeting of BSACI will this year be held at the Telford International Centre, Telford. Joint sessions are... Read More

18TH–20TH OCTOBER 2018

Food Allergy and Anaphylaxis Meeting 2018 (FAAM 2018)

Copenhagen, Denmark

The City of Spires and Danish capital, Copenhagen is the place to be for food allergy and anaphylaxis experts this year as the EAACI FAAM Congress moves to the city. This event comes highly recommended by the... Read More

15TH–19TH NOVEMBER 2018

American College of Allergy, Asthma and Immunology 2018 Annual Scientific Meeting (ACAAI 2018)

Seattle, Washington, USA

Not to be confused with its European counterpart, the annual ACAAI congress will this year be held on the west coast of the USA, in Seattle, Washington. The theme of... Read More

1ST–5TH JUNE 2019

European Academy of Allergy and Clinical Immunology (EAACI) Congress 2019

Lisbon, Portugal

Moving from the heart of Germany to the beaches of Portugal, the 2019 EAACI Congress will be held in beautiful Lisbon. The focus of the 2019 congress will be... Read More

Coming soon.

EMJ provides influential articles, presentations of scientific research and clinical practice, and in-depth reviews of international medical congresses.

Subscribe to *EMJ Rheumatology* for free.

EUROPEANMEDICAL-JOURNAL.COM /SUBSCRIBE

Buyer's Guide

- ABBOTT
- ABIONIC
- AIMMUNE THERAPEUTICS
- ALK
- ALLERGOPHARMA GMBH & CO., KG
- ALLERGY THERAPEUTICS PLC
- ANALLERGO
- ASTRAZENECA PLC
- BAUSCH + LOMB
- BIOCHECK GMBH
- BÜHLMANN LABORATORIES AG
- CIRCASSIA LTD.
- CITEQ BIOLOGICS
- CSL BEHRING
- DBV TECHNOLOGIES
- DIATER
- DR. FALK PHARMA GMBH
- DR. FOOKE-ACHTERRATH LABORATORIEN GMBH
- DST - DIAGNOSTISCHE SYSTEME & TECHNOLOGIEN GMBH
- EUROIMMUN AG
- EXBIO PRAHA, A.S.
- FAES FARMA
- GLAXOSMITHKLINE
- HAL ALLERGY
- HANGZHOU ZHEDA DIXUN BIOLOGICAL GENE ENGINEERING CO., LTD.
- HELMUT HUND GMBH
- HITACHI CHEMICAL DIAGNOSTICS INC.
- HYCOR BIOMEDICAL
- INDOOR BIOTECHNOLOGIES LTD.
- IMMUNOTEK S.L.
- INVITALIS GMBH
- LABORATORIOS LETI
- LOFARMA
- LOW DERMA™
- MACRO ARRAY DIAGNOSTICS
- MENARINI GROUP
- MYLAN
- NASALEZE INTERNATIONAL LTD.
- NOVARTIS PHARMA AG
- NUSS/ANAPHYLAXIE NETZWERK E.V.
- NUTRICIA
- PHARMING GROUP N.V.
- PROBELTE PHARMA S.L.V.
- R-BIOPHARM AG
- RED MAPLE TRIALS INC.
- ROXALL GROUP
- ROYAL PHILIPS
- S. KARGER AG
- SANOFI GENZYME AND REGENERON
- SHIRE INTERNATIONAL GMBH
- SMARTPRACTICE
- STALLERGENES GREER
- THERMO FISHER SCIENTIFIC
- URIACH

VIEW CONGRESS REVIEW ←