EMJ EUROPEAN MEDICAL JOURNAL

# ALLERGY & IMMUNOLOGY

ISSN 2398-9130 Vol 4.1 • July 2019 • europeanmedical-journal.com

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*"The EAACI congress continues to go from strength to strength, with this year's event being larger than ever before. "*

Spencer Gore, CEO

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VIEW IN FULL ←

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Front cover and contents photograph: Lisbon, Portgual, home of the EAACI 2019. © saiko3p / 123rf.com

# EMJ Allergy.



# Welcome

This year, I was lucky enough to travel with the EMJ team to Lisbon, Portugal, for yet another fantastic European Academy of Allergy and Clinical Immunology (EAACI) congress. Celebrating all the past year's incredible research in the field of allergy and immunology, this congress is always a real joy to attend. Now, 6 weeks later, I am proud to present the latest edition of *EMJ Allergy & Immunology*, our open access eJournal dedicated to sharing the key happenings from this world-leading congress.

As a historic seafaring nation, Portugal has a strong pedigree of discovery and exploration, so it was most fitting that this nation hosted such a ground-breaking event. The EAACI congress continues to go from strength to strength, with this year's event being larger than ever before. As explained in the opening ceremony, one of EAACI's focusses this year was on precision medicine, including our rapidly evolving understanding of the microbiome. This theme is reflected in this edition's Congress Review section, where we reflect the top headlines from the event, including breakthroughs in medical Apps and mHealth, oral immunotherapy for food allergies, and *Staphylococcus aureus* as a risk factor of food allergy in children with atopic dermatitis.

Likewise, the journal's Abstract Reviews section has a handpicked selection of fascinating studies, with each summary penned by the congress presenter themselves. Once again, the theme of precision medicine is clearly prominent here, including an abstract review summarising the importance of clear patient–physician communication to ensure adherence to inhaled medication.

Finally, *EMJ Allergy & Immunology 4.1* provides a host of peer reviewed articles for your enjoyment. Topics here include the role of natural killer cells in the immune response, the elusive topic of allergic asthma, new insights into the function of IL-10, and much more.

Thank you, as always, to the many contributors that make this journal possible. The authors, peer reviewers, Editorial Board members, and of course the wonderful EMJ staff have all worked exceptionally hard to bring you this beautiful journal, and of that I am very proud. I wish you all the best for the upcoming year and hope you enjoy this publication.

Spencer Gore

Chief Executive Officer, European Medical Group

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# Foreword

Dear Readers,

It is with great excitement that I introduce to you this year's edition of *EMJ Allergy and Immunology*.

The Annual Meeting of the European Academy of Allergy and Clinical Immunology Congress (EAACI) was held in Lisbon, Portugal, in June this year. The theme was 'Mapping the new world of allergy', words that have never been so accurate in describing such an event! This year was the prime opportunity to present an update of where our knowledge currently stands and which direction the new concepts, in either basic or clinical immunology and allergy, will guide us in a near future.

The congress had, as always, a wonderful atmosphere with a multitude of interesting presentations to attend. Furthermore, there were opportunities to get involved in some exciting exchanges and discussions, including some tremendous pros and cons sessions, and a number of interesting poster presentations. In this journal you will find an excellent review of the event, giving you the opportunity to get in touch with all the major topics in the field of allergy. By including the key details from abstract presentations and discussion sessions, this review will allow you to revisit your favourite highlights or catch up on what you missed from the largest allergy congress in the world.

In addition, you will find some great interviews with some leading medical experts who explain their views of what the future of allergy and immunology holds for us, keeping in mind that we are in constant challenge with the new emerging discoveries, year after year.

The quality of the papers in this year's journal are excellent, but my particular favourite has to be the paper titled 'Well Known and Unknown Asthma Phenotype: Allergic Asthma'. More than ever we are realising the importance of personalised treatments and their management. This is all mediated with the fact that our knowledge of physiopathology, including asthma phenotypes, commands more specific treatment. The era of 'one disease, one drug' is gone!  A large number of well-proven specific treatments are now available, but these treatments should always be properly targeted to the right patient.

I would like to thank you all for your enthusiasm to this latest version of *EMJ Allergy and Immunology*. This edition will definitely generate some interesting exchanges with our colleagues, achieving one of the main objectives of the journal as a whole.

Kind regards,

**Prof Dr Jacques Bouchard**

Université Laval, Québec, Canada, and CIUSSS Capitale-Nationale, Québec, Canada

# Congress Review

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


Location: Lisbon, Portugal

Date: 1st - 5th June 2019

Citation: EMJ Allergy Immunol. 2019;4[1]:10-20. Congress Review.


Gothic architecture and cobbled backstreets created the breath-taking backdrop for this year's European Academy of Allergy and Clinical Immunology (EAACI) Congress. Portugal's bustling capital city of Lisbon hosted >7,000 delegates for the 5-day event that has become a staple in the budding allergist and immunologist's calendar. The EMJ team were spoilt for choice in Lisbon this year, with a cornucopia of ground-breaking research, thought-provoking sessions, >60 symposia, and a wealth of abstract and poster presentations on offer.

Prof Luis Delgado, Vice President of Education and Specialty for EAACI, discussed Lisbon and EAACI's rich histories in the opening ceremony, highlighting the many developments within the fields of allergy and immunology, such as enhancements in the mapping of allergy structure and biological treatments. This year's event was no different in its ambitions to advance knowledge in every corner of the field. There was a plethora of abstract and poster presentations and we have compiled a range of abstracts that were of particular interest. Summaries of these select abstracts can be found inside *EMJ Allergy and Immunology 4.1*'s congress review, penned by the authors themselves to give you a first-hand insight into the abstract presentations.

Prof Leif Bjermer's thought-provoking session looked at the barrier of respiratory diseases, particularly asthma, for aspiring athletes. Prof Bjermer outlined how the very nature of athleticism puts the lungs under additional pressure, leaving athletes more susceptible to respiratory diseases. He warned against immune remodelling and suppression, activities that can leave athletes in a vulnerable position. The session ended on a positive note, encouraging enjoyment and a focus on the positive aspects of exercise while avoiding stress.

This edition also brings you a summary of a EAACI Position Paper that studies the prevalence of allergology specialty and sub-specialty in Europe and nearby countries,

as well as the level and availability of care for allergic disease in these countries. The write-up also discusses a SWOT analysis carried out in the surveys created by the position paper authors to gain insight into the perceived strengths, weaknesses, opportunities, and threats throughout these countries surrounding the speciality of allergology. The paper calls for further training opportunities and freedom of movement for allergologists to ensure the profession attracts young talent and better facilitates a high level of allergy care.

*...the very nature of athleticism puts the lungs under additional pressure, leaving athletes more susceptible to respiratory diseases.*

The congress was packed full of fascinating sessions, targeting poignant and topical issues over the 5-day period, while enabling the sharing of knowledge to create a better future for the care and treatment of allergy. We already have our sights set on the next annual congress for EAACI, which will be held in the historical city of London, UK. We hope to see you all there for what is sure to be another fantastic event opening a gateway to the vital sharing of knowledge within allergy and immunology. In the meantime, please sit back and enjoy our highlights from the incomparable congress that was EAACI 2019.


EAACI 2019 REVIEWED →

# Assessment of Oral Immunotherapy for Paediatric Allergy

PAEDIATRIC food allergy affects 8% of children in Westernised countries and is characterised by medical and social implications for the child that are of utmost importance to the healthcare community. Much focus is given to the prevention of severe reactions in this vulnerable demographic through education and limiting of high-risk exposure scenarios, and deservedly so. However, now more than ever there has been an increased focus towards developing therapeutic means to alleviate or eliminate paediatric allergy.

Prof Montserrat Alvaro Lozano from the Sant Joan de Déu Children's Hospital in Barcelona, Spain, provided her expert insight into the use of oral immunotherapy for the treatment of allergic children during one of EAACI's pros and cons sessions. This involves the desensitisation of the child's allergy through periodic exposure of initially small amounts of the specific allergen in the child's food. Through gradual increases of dosage and clearance of allergic symptoms, an optimal end goal can feasibly be achieved in which an adequate nutritional state is attained, and the child's social life is markedly improved.

Prof Lozano was open in her admiration and optimism concerning this therapeutic approach, but dutifully highlighted to the audience that there is still much work to be done towards making immunotherapy the standard of care. Due to the high-risk and intensive nature of the treatment, not to mention the accuracy with which the treatment must be administered, standard practice is for it to be performed in tertiary centres with experienced medical personnel and appropriate resuscitation facilities available. This is to prepare for adverse events, which are still frequent and can be severe.

Because of these practical and logistical considerations, Prof Lozano was of the opinion that we are currently not at a stage to widely offer immunotherapy for allergic children in the clinic. Speculating on the future of paediatric allergy treatment, she commented on the exciting prospect of developing immunotherapy further to improve efficacy and safety, and also using it to identify biomarkers in patients. Through identifying these biomarkers for a good or bad response, treatment can be tailored to the individual and hopefully lead to noticeable improvements across the paediatric allergy landscape.

*"Apps are instantly helpful – which is what someone with a severe allergy needs."*

# mAPPing the New World of Allergy

IN THE CURRENT digital world, mobile phones assist us in numerous aspects of our daily life, and are even helping us to monitor our health, so it is no surprise that applications can now be used to assist precision medicine approaches. All fields of medicine will benefit from the use of these technologies, including allergy and clinical immunology. A new avenue for controlling allergic rhinitis and allergy by mHealth technology was presented at the EAACI Congress 1st-5th June 2019.

Not only will patients benefit directly from the installation of these technologies into healthcare, but so will research, epidemiology studies, and general standard of care. Patients will gain independence and control of their disease, in addition to improved communication with their doctors. The attending doctor will be able to keep a photographic history of the disease, analyse longitudinal data to retrieve suggested diagnosis and treatment, and adjust treatment to ensure it remains optimal.

Multiple mobile applications for allergic disease have already been developed and are being used by patients every day. Mobile Airways Sentinel Network uses a visual analogue scale for nose, eye, and asthma symptoms to link allergy to work impairment. Patients with pollen allergies can use pollen diaries to compare the different seasons and aerobiological particles to identify the possible allergen or forecast worsening of pollen allergy symptoms. Applications can even advise those with food allergies in the selection of allergy-free foods.

"Apps are instantly helpful – which is what someone with a severe allergy needs. Sadly, we have seen that people can't always rely on restaurants and cafes to keep them safe, so an App puts the control back into the hands of the patient," says Leah Ryz, 38, Hove, UK, who uses a mobile application to scan allergen-free foods.

# Advice for Aspiring Elite Athletes

BECOMING a gold medal Olympian or scoring the winning goal in the World Cup final are the kinds of dreams held by millions of young people. Yet many of those who strive to make this ambition of being an elite athlete a reality will be stopped in their tracks due to the development of respiratory problems such as asthma. In a poignant session at this year's EAACI Congress, Prof Leif Bjermer, Respiratory Medicine and Allergology, Lund University, Lund, Sweden, offered advice on how to minimise the chances of these issues occurring, using the latest research findings in this area.

Prof Bjermer began with a sombre message for a significant number who hold aspirations of becoming an elite sportsperson; your natural genetic make-up can make it very difficult to succeed at such a high level. In particular, if your maximum level of oxygen consumption ($VO_2$) is not high enough, your body is probably unlikely to be able to cope with the demands an elite sportsperson must endure. He highlighted a case report of an aspiring cross-country skier who was initially very successful at junior level. Sadly, she began to repeatedly develop respiratory infections. The source of these problems was her limited $VO_2$ maximum, at 54 mL/kg/min: not high enough to compete at an elite level, which meant her respiratory system struggled to cope with the intensity of training. "I see this quite often where there's someone who can never become an elite athlete with that maximum optimal uptake. They are depressed, they don't understand why they can't succeed, but the problem is they just do not have the ability," commented Prof Bjermer.

The risk of developing asthma is particularly strong in those undertaking endurance sport, in which high levels of oxidative stress are placed on the airways. Prof Bjermer highlighted studies of marathon runners in which evidence of damage and stress to the airways have been gathered, the latter indicated by a higher degree of lipid peroxidation and vitamin E turnover. Therefore, minimising oxidative stress is critical in people

*"...I think as leaders who are dealing with young athletes in their growing career, it's really important to protect them from being over-stressed."*

for sports such as marathon running. Studies in mice models have shown the potential for antioxidative drugs to decrease muscular fatigue during strenuous exercise and increasing vitamin E levels appears to reduce neutrophilic inflammation and mucus production. Whether such approaches can be applied in a clinical setting remains to be seen.

Endurance sport can also make people especially vulnerable to respiratory infection, with lower neutrophil activity leading to reduced immune capacity. There is a period following intense exercise in which people are immunosuppressed. It is crucial for aspiring athletes to therefore take steps to avoid infection during this period. Prof Bjermer outlined how elite sports teams often implement strict measures to prevent scenarios where infections can be caught, such as staying in a separate hotel, not talking to journalists, and not shaking hands with anyone. Clinical trials have also demonstrated the efficacy of some medications in reducing the chances of getting a respiratory infection, including taking probiotic supplements. He added: "My advice is to prevent respiratory infection and immune remodelling and most importantly of all, if you feel you are underweight or have a cold, don't stress yourself, don't compete: the best training activity you can do is to stay in bed."

Prof Bjermer later went on to emphasise the importance of maintaining a healthy nose. This should be an ongoing priority for aspiring young athletes, according to Prof Bjermer, because nose problems are more frequent compared to the general population in athletes in any form of sport and setting, indoor or outdoor. "We know that if you improve nasal patency and nasal ventilation, it will help you to get better matching of the ventilation perfusion between the lower airways, thereby improving performance," he stated.

Finally, Prof Bjermer put the whole issue into perspective during his closing remarks, with advice for young athletes, coaches, and medical professionals alike: "My final advice is to enjoy what you are doing. Doing endurance exercise is a health hazard: you face the risk of developing asthma. But when you balance all the positive effects of exercise it's worth it. But you need to face it, and I think as leaders who are dealing with young athletes in their growing career, it's really important to protect them from being over-stressed."

# World Environment Day

WORLD Environment Day, a United Nations initiative supported by EAACI, this year focussed on the topic of air pollution and its impact on global health, climate change, and asthma. A EAACI press release dated 5th June 2019 gave more detail on connections between air pollution and allergic diseases, as well as the ways in which the EAACI is working to reduce the incidence of allergic diseases caused by air pollution.

Ever since the industrial revolution, allergic and respiratory diseases have been on the rise, with multi-faceted causes and complex pathogeneses. The increase in emissions of greenhouse gases has led to more $CO_2$ in the atmosphere and higher temperatures across the globe. "Depending where you live, higher temperatures may result in more plant growth. A shift in allergen exposure from pollen is to be expected, the direction depends on where you live," explained Dr Jeroen Buters, Technical University of Munich, Munich, Germany, and past Chair of the EAACI Working Group on Aerobiology and Pollution.

However, according to EAACI, $CO_2$ is not the only gas to make pollen more allergenic. Birch and ryegrass pollen that had an increased exposure to traffic pollutants like nitrogen oxides, ozone, and diesel exhaust particles was found to have a higher volume of allergens than pollen in urban parks. In addition, rates of asthma are increased by exposure to traffic fumes and prenatal exposure can impact the development of the respiratory system in children.

"Exposure to particulates from diesel vehicle emissions is linked to asthma and allergies. Although everyone is susceptible to diesel pollution, children, the elderly, and individuals with pre-existing respiratory conditions are the most vulnerable. As vehicles equipped with advanced diesel emissions controls will enter the market, it will be important to ensure that emission levels are maintained throughout the life of the vehicle by periodic testing," said Isabella Annesi-Maesano, Institut National de la Santé et de la Recherche Médicale (INSERM).

EAACI's commitment to reducing these challenges is set out in the 2018 White Paper on Research, Innovation, and Quality Care.[1] Therein, EAACI suggested "Exposome-focused projects are needed to examine the complex interplay of environment and genetics to determine the most cost-effective interventions for reducing the risk of allergic disease," adding "Once key exposures and potential interventions are identified, a comprehensive approach among clinicians, patients, healthcare organisations, insurance providers, government agencies, and urban planners must be undertaken to establish cost-effective primary and secondary prevention strategies to reduce these risks and promote wellness."

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# eHealth: Striding for Digital Healthcare


*eHealth has numerous benefits: at-home monitoring, reliable data collected through standardised recording, and a cut down on unnecessary GP appointments, to name just a few.*

IN THE DIGITAL age, there is a smartphone app for just about everything; it is time for healthcare to catch up and embrace the digital shift. eHealth is enabling this change: taking strides into the app world to offer patients the possibility to record their data on their smartphone, tablet, or wearable device. eHealth has numerous benefits, including at-home monitoring, reliable data collection through standardised recording, and a cut down on unnecessary GP appointments, to name just a few.

The EAACI opening ceremony session on eHealth, Mobile Health Technologies in Allergy Care, explores the digital universe of eHealth, and the many possibilities it presents. eHealth refers to digital tools relating to healthcare and offers software solutions that can be ran on a mobile device and can often connect with wearables. There are a range of stakeholders, most notably patients and doctors, many of whom can see the benefit of eHealth; the session reviewed a questionnaire surveying 1,200 physicians across 9 specialties that showed that these doctors could see the potential that digital solutions offer for their patients.

The session also discussed the World Health Organization (WHO), which has published guidelines around digital health solutions, aiming to assist countries who need to create eHealth policies. But it is not as simple as just creating a policy; as options expand and evolutions occur, the requirements for a good policy become more complex. It is important to remember that, as with the entire digital world, eHealth changes every day, feeding into the complexities that come with policy creation.

One challenge to consider is the high prevalence of people downloading apps and then not using them. These healthcare apps would have ~to cause minimal disturbance to the patients' day-to-day life and be immediately beneficial to keep usage rates high. The world of eHealth offers great potential for patients and doctors alike, but it is critical that barriers such as this are faced to ensure that this digital avenue can be explored to its highest potential.

# Bacterial Infection Linked to Food Allergy in Children with Atopic Dermatitis

DURING the Presidential Symposium on Immunomodulation in Food Allergy, held at this year's congress, findings from an international collaboration were presented in which *Staphylococcus aureus* bacteria colonisation was shown to be closely correlated with likelihood and persistence of food allergy in children with atopic dermatitis (i.e., eczema). Prof George du Toit from King's College London, London, UK, was on-hand to talk about the findings.

*S. aureus* infection has previously been associated with severe eczema, a dermatological condition which itself is a risk factor for food sensitisation or allergy. The research group reasoned that there may exist a direct link between *S. aureus* status and food allergy through the regulatory influence of eczema severity. Participants from the Learning Early About Peanut Allergy (LEAP) study who had eczema were assessed for severity of their condition and had skin/nasal swabs taken and analysed.

Across the cohort, bacterial colonisation was significantly associated with eczema severity, except at age points 12 and 60 months where a deterioration in severity was associated. However, skin colonisation correlated with high levels of hen's egg white and peanut sIgE at any time point compared to those without colonisation, a finding that was independent of eczema severity. A positive correlation was also observed with persistence of the food allergy to age 5 or 6 years, a point by which the majority of allergic children have become desensitised. This effect was also independent of eczema severity.

Dr Olympia Tsilochristou, lead author, commented: "We yet do not know the exact mechanisms that lead from atopic dermatitis to food allergy; however, our results suggest that *Staphylococcus aureus* could be an important factor contributing to this outcome." Another of the contributors suggested that further longitudinal studies involving advanced techniques and interventional strategies aimed at eliminating *S. aureus* in early stages of life might hold potential for determining the environmental factors that aid progression of eczema and food allergy.

*"We yet do not know the exact mechanisms that lead from atopic dermatitis to food allergy; however, our results suggest that Staphylococcus aureus could be an important factor contributing to this outcome."*

# The Roadmap for the Allergology Specialty and Allergy Care in Europe and Adjacent Countries

## Summary of the EAACI Position Paper

**Kirstie Turner**
Editorial Administrator

## INTRODUCTION

There is a high prevalence of allergic disease throughout Europe: 17.0–29.0% prevalence of allergic rhinitis,[1] 1.3–11.0% asthma,[2] and 26.5% atopic eczema,[3] among others. This high level of allergic disease places a burden of care that must be met by highly trained specialists. Fyhrquist et al.[4] carried out a study regarding the availability of care services and specialist training availability. The findings are discussed in their paper: 'The roadmap for the allergology specialty and allergy care in Europe and adjacent countries. An EAACI position paper'. This article will summarise the methodology, findings, and analysis of this European Academy of Allergy and Clinical Immunology (EACCI) position paper.

## METHODOLOGY

The study was conducted using a survey created by the EAACI National Allergy Society Committee and the Union Européenne des Médecins Spécialistes (UEMS) Section and Board (S&B) of Allergology. The survey was compiled using questionnaires developed in previous research: the 2016 NASC registry data collection and the UEMS S&B and speciality committee 2016 survey for UEMS delegates. The survey questioned participants on the level of care services and training availability for the specialities and sub-specialities included within allergic care. Additionally, a strengths, weaknesses, opportunities, and threats (SWOT) analysis of the allergic disease care options was completed. This was sent to 51 NASC members and 30 countries linked with UEMA S&B, most of which were European.

## RESULTS AND DISCUSSION

### Speciality and Subspeciality

An important outcome of the questionnaire was the confirmation that most countries had

recognised the full speciality. However, a small number of countries considered allergology as a subspeciality and some recognised neither the speciality nor subspeciality. The results also showed the number of new registered specialists and subspecialists each year in the surveyed countries: Italy, Spain, and Poland had the most specialists, with 40–42, 40–55, and 30, respectively; Germany was found to have the most new subspecialists each year, with 140 registering, although this number is decreasing. There was a mixed response in terms of the number of specialists and subspecialists being sustained, growing, or falling, although the majority of responding countries declared a sustained or growing number, which is positive news for the discipline. The researchers highlighted the importance of free movement for these specialists and subspecialists.

The number of subspecialists and specialists per 100,000 people varied greatly across Europe, highlighting the lack of standardisation across allergy disease care. For instance, there are 5.96 full speciality allergologists per 100,000 people in Georgia, while the corresponding figure for Luxembourg is 0.17. Additionally, although allergic disease is highly prevalent, the discipline reports a lower number of specialists per 100,000 people than many other specialities. For instance, dermatology has 5.84 per 100,000, compared to allergologists and subspecialists for allergology, which saw results of 1.81 and 1.84, respectively: an estimated 1 subspecialist available for every 53–54,000 people. However, it should be noted that this figure, for many countries, is simply a theoretical estimate as many specialists do not work as allergologists following completion of their study.

## Training for Speciality and Subspeciality

The paper also goes on to highlight the variation of training in the speciality. Training duration for specialists and subspecialists ranges from 1.5–7.25 years, with a mean duration of full speciality and subspecialty measuring at 4.55 and 5.08 years, respectively. A point for concern was that many training schemes were not meeting the current requirements for the speciality of Allergology and Clinical Immunology (as amended in 1997–2003), which should be at least 2 years in a common trunk and a minimum of 3 years in the speciality. It was recommended that these requirements should be updated to reflect current training standards. Furthermore, the authors suggested efforts should be made to ensure countries followed harmonised training, while preserving the ability of individual countries to organise specialities nationally. While the requirements were often not being met, there were grounds for positivity when considering training for a subspeciality in Allergology. EAACI's recommendation was that the minimum training requirement here was 18 months, a duration that most countries were exceeding.

## Strengths, Weaknesses, Opportunities, and Threats Analysis

The SWOT analysis completed within the survey provided an in-depth exploration of allergic disease care. Participant responses often contradicted one another; diversity between the surveyed countries can be attributed to different local circumstances and policy, resulting in differing priorities and opinions. For example, a speciality could be present in one country, and therefore seen as a strength, or could be lacking in another and therefore seen as a weakness, or even a threat.

The low attractiveness of the role or specialisation was seen as a threat by some countries. Some also highlighted the danger of weak prioritisation of the discipline among authorities. A lack of funding and reimbursement opportunities were also a key concern, along with a need for standardisation and more prevalent availability of immunotherapy products.

On the other hand, several opportunities were identified, including the discipline's continual growth, improved awareness of allergic diseases, better methods of prevention, focus on improvements of patient care, and investment in young doctors to specialise. The researchers considered answers given by ≥3 country representatives to be the most important topics. An overview of the SWOT analysis can be seen in Table 1.

# CONCLUSION

The researchers concluded that the specialty of allergology is recognised in most European countries, as well as in adjacent countries. There

are, however, considerable variations across Europe regarding the care services available. Diversity within care systems includes the type of caregivers, specialist numbers, the training of specialists, and availability of training. Therefore, they recommended that standardisation of training should be implemented for allergologists and subspecialists. The authors recognised that having a mix of precise data and estimations was a limitation of the survey.

It was further recommended that there should be investment to train and specialise young doctors, as well as to create further opportunities for a full speciality in countries. The authors also outlined that free movement of allergologists should be better facilitated. The paper offers an important evaluation of the care availability for allergy diseases and well-informed recommendations to improve the availability and standard of care.

**Table 1: Strengths, Weaknesses, Opportunities, and Threats analysis of allergic disease care.[4]**

| Strengths | | Weaknesses | | Opportunities | | Threats | |
|---|---|---|---|---|---|---|---|
| A high level of training. | 8 countries | Lack of full speciality. | 6 countries | Further research and translational medicine for improvement of care. | 6 countries | Declining number of specialists. Fragmentation of full and subspecialities. | 10 countries |
| Networking, including interdisciplinary. | 6 countries | Poor training and discrepancies between full and subspeciality training. | 5 countries | Growth of the discipline due to allergy epidemic. | 6 countries | Discipline being low priority resulting in lack of funding. | 6 countries |
| Presence of full speciality. | 10 countries | Unappealing discipline leading to lack of specialists. | 13 countries | Increased awareness and prevention of allergy. | 6 countries | Reduction in availability of immunotherapy products. A lack of standardisation. | 6 countries |
| Research activity. | 6 countries | Weak collaboration; competition with other disciplines. | 3 countries | Investment in young specialists. | 4 countries | Threat of losing full speciality. | 5 countries |

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# Incorporation of the Microbiome into Precision Medicine

**Layla Southcombe**
Editorial Administrator

Precision medicine has already had a big impact on the diagnosis and treatment of diseases across a multitude of therapeutic fields. Oncology is one of the main disciplines benefitting from the installation of a precision medicine infrastructure. The field of allergy and immunology is one of many fields that would benefit from taking a precision medicine approach to patient care. Recently, the microbiome, dynamic communities of microbes that colonise the body, has been introduced into precision medicine and has already illuminated aspects of the relationship between the microbiome and numerous diseases.[1]

*"With such a diverse influence over disease, it is no surprise that many hours are being invested into researching the microbiome and including the data in precision medicine approaches."*

Independent studies, including the Human Microbiome Project, have proved that disruptions in the normal balance of the microbiome results in dysbiosis.[2] The resilience, yet plasticity, of the microbiome is a very important factor; it is much more mutable than human cells.[3] An imbalance in the microbiome has been associated with numerous diseases, for example chronic diseases, autoimmune disorders, inflammatory bowel disease, neurodevelopmental disorders, and more.[2] With such a diverse influence over disease, it is no surprise that many hours are being invested into researching the microbiome and including the data in precision medicine approaches.

Evidence is mounting that disruptions in the microbiome in early life contributes to the establishment of food allergies.[3] Progressing our knowledge on this interaction may allow us to predict or even prevent food allergies from a young age, avoiding unnecessary reactions and hospital stays, and possibly even deaths. Before this is possible, in-depth knowledge about the relationship between specific colonies in the microbiome and specific allergies need to be identified.

The three major subtypes of inflammatory bowel disease (ulcerative colitis, Crohn's disease, and indeterminate colitis) not only differ in their presentation but all require different treatments for optimal dampening of symptoms.[4] There is an importance for the precise diagnosis of the individual subtypes and the microbiome can be used to achieve this. The gut microbiome signatures differ between all three and have even been associated with surgical outcomes in Crohn's disease, in which increases in *Faecalibacterium prausnitzii* in the ileal mucosa are associated with decreased disease recurrence

at 6 months post-surgery.[4] Familiarity with the composition of the gut microbiome of each specific disease will allow physicians to monitor the progression or improvement of each of the conditions, allowing informed decisions about treatment regimens to be made.

*"Familiarity with the composition of the gut microbiome of each specific disease will allow physicians to monitor the progression or improvement of each of the conditions, allowing informed decisions about treatment regimens to be made."*

The gut microbiome is not the only localised biome that needs to be characterised; lung and skin microbiome also play an important role in the pathogenesis of allergic disease by modulating immune responses in their respective organs. In the lung, by adjusting the balance between Th2 and Th17 patterns, the microbiome plays a role in driving allergic asthma endotype polarisation.[5] Bacteria that reside on the skin modulate inflammation, with dysbiosis influencing chronic inflammatory diseases such as atopic dermatitis and psoriasis.[5] By quantitating the changes that lead to allergic reactions and inflammation in these organs, the diagnosis and treatment of these diseases can be optimised leading to better overall patient care and quality of life.

Inclusion of microbiome studies into the infrastructure of precision medicine approaches to the allergy and immunology field will benefit and improve all aspects of patient care. This was a hot topic at the EAACI congress held in Lisbon, Portugal, this year and will continue to be for many years. Characterising localised microbiomes has already helped the prediction of treatment outcome; the progression of the knowledge of the relationship between the microbiome and diseases will remain of interest in all fields of medicine, including allergy and immunology.


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# Congress Interview


## Mariana Couto

EAACI Working Group on Allergy, Asthma & Sports


The European Medical Journal caught up with Mariana Couto about her role in the EAACI Working Group on Allergy, Asthma & Sports, as well as interdisciplinary collaborations with other working groups and the major challenges faced by doctors in this field.

### Q1 Could you tell us about how you came to be part of the EAACI Working Group on Allergy, Asthma & Sports?

I have been interested in this field of knowledge since very early in my career. I had a fellowship at the Norwegian School of Sports Sciences, under the supervision of Prof Kai-Håkon Carlsen, and in a blink of an eye I became so enthusiastic that I decided to do my PhD in this area. My thesis was entitled: 'Asthma and sports: Mechanisms and effects of airway damage in elite athletes.'

*"...it is very important these interdisciplinary collaborations occur so that information can reach a lot of physicians to ensure the best possible and homogenised care of these patients."*

Almost at the same time, the EAACI was having elections and I applied to the Junior Members (JM) Working Group (WG). Given my interest in this field, I became the JM representative of the WG on Allergy, Asthma & Sports. I served two terms as a JM, then I applied to become a senior member of the Board and was elected Secretary during one term, and then Chair during this last term.

### Q2 What is the Working Group currently focussing on?

Besides spreading the news related to allergy, asthma, and sports through active participation in Congresses with symposiums and workshops devoted to this area, the WG has two major projects running: one focussing on effects of exposure to chlorine by-products in swimming pools, and the other related to the role of exercise in rehabilitation of patients with asthma; this one is in collaboration with the EAACI Asthma Section and the European Respiratory Society (ERS).

## Q3 There is a EAACI-ERS Joint Task Force focussing on the recognition and diagnosis of exercise-related asthma, respiratory, and allergic disorders in sports. How important are such interdisciplinary collaborations?

Patients with sports-related respiratory symptoms search for the assistance of different doctors, not only allergists but also pulmonologists, general practitioners, sports medicine physicians, and more. So, it is very important these interdisciplinary collaborations occur so that information can reach a lot of physicians to ensure the best possible and homogenised care of these patients.

## Q4 The use of therapeutic use exemptions (TUE) has come under suspicion from the public in recent years as a result of high-profile cases. Would you say greater public understanding of the link between intensive endurance training and possible airway injury is necessary to combat this? Alternatively, should increased scrutiny be placed on TUE?

There are many studies demonstrating that inhaled beta 2 agonists, the main bronchodilators used for asthma and airway hyperresponsiveness, do not enhance sports performance, and therefore a lot of voices advocate their free use in sports without the need for a TUE. However, I do not personally agree because we have shown in a study[1] that there is a need for TUE to justify the use of such medication in order to reduce the number of athletes taking these drugs. Furthermore, although inhaled beta 2 agonists do not enhance their sports performance, these athletes were taking medication for which there was no clinical indication for their need, and this could result in potential side effects (which may occur with any drug). So, I believe the need for TUE improves athlete care, but this is of course a personal opinion and not an institutional statement, because this reality may vary between countries.

## Q5 With approximately 7–8% of Olympic athletes affected by asthma or airway hyperresponsiveness, it is clear that they are not necessarily a barrier to participating in exercise. However, what considerations need to be borne in mind when managing these conditions?

Athletes' care needs further attention and more studies are needed to further investigate how the asthma phenotype of elite athletes differs from that of classical asthma in the general population. Being able to define such distinct phenotypes would give further knowledge and understanding of the underlying mechanisms of asthma in elite athletes and would improve diagnosis and treatment. Different therapeutic modalities could then be specifically applied for the targeted phenotypes, rather than for asthmatic athletes in general, which is the current management approach.

*"...more studies are needed to further investigate how the asthma phenotype of elite athletes differs from that of classical asthma in the general population."*

## Q6 Moving away from elite-level athletes, how can we encourage all those with allergies and asthma to participate in exercise?

Compared to inactive young people, physically active children and youth have higher levels of cardiorespiratory endurance. Regarding asthma, evidence has shown that physical training improves cardiopulmonary fitness and may even improve the quality of life of both children and their caregivers. It has been suggested that moderate intensity physical training may decrease both total and allergen-specific IgE levels and reduce the need for asthma medication. So, physical activity should be recommended as a supplementary therapy to medication in asthmatic individuals.

But it must be noted that physical exertion is a powerful trigger of bronchoconstriction and symptoms in patients with asthma. Symptoms of asthma during exercise may result in avoidance of physical activity leading to detrimental

consequences to physical and social wellbeing of patients with asthma. So, it is very important the patient has adequate asthma control and regular appointments with their allergist to ensure the medication is efficacious in preventing symptoms.

*"...asthmatic patients are able to perform any sport as long as their asthma is managed and under control."*

## Q7 On this theme, are there any sports that are particularly suitable or unsuitable for those with allergy and asthma?

It has been previously shown that intense swimming activity causes a lung growth greater than normal in children and adolescents and teaches airway control. Also, the hot and humid conditions of swimming training have been pointed out to be less asthmogenic. So, swimming is one of the best sports for asthmatics, but exposure to chlorine by-products has raised some concerns in the last years, and that is why the WG is now collecting evidence to evaluate this situation.

Golf, yoga, and gentle biking are also less likely to trigger asthma flare-ups. Other sports that are favourable for asthmatics include baseball, football, gymnastics, and shorter track and field events.

Some sports can be challenging for patients with asthma. These include endurance sports like long-distance running or cycling or sports that demand a lot of energy without a lot of rest time. Cold-weather sports like cross-country skiing or ice hockey can also be difficult. It is very important to note that although these sports may not be the best choice, the asthmatic patients are able to perform any sport as long as their asthma is managed and under control.

## Q8 With allergy and asthma diagnoses on the rise in many countries, does this present you with any challenges in your work?

From year to year we have larger numbers of children and youth that need to be monitored in our allergy departments, and that demands extra effort from the doctors obviously.

## Q9 With ultra-endurance sports growing in popularity, do people need to be aware of the link between intensive endurance training and possible airway injury?

Yes, it is very important that people are aware of this link and that they recognise the possible symptoms associated so that they can promptly search for a doctor who, if needed, can perform exams. It is very common that people who perform sports at extreme conditions interpret their respiratory symptoms as 'normal' in the context of heavy training and delay seeking medical help, which impairs their treatment and may lead to pathological conditions as a result of this acute injury.

## Q10 What are some of the issues you expect the WG to have to tackle in the future?

I am leaving the WG this year, I have been elected to the Asthma Section, but I hope the new Board and its Chair keep up the enthusiasm for informing the medical community and society about the news in this field and continue contributing with research that may increase knowledge about diagnosis and treatment of these conditions.


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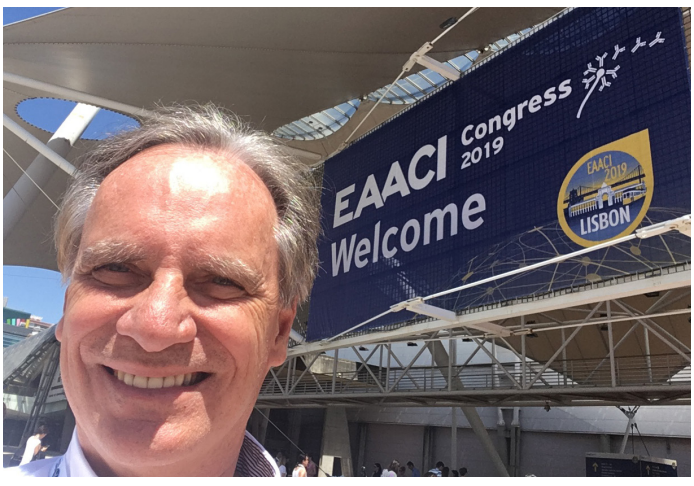
# Meeting the *EMJ Allergy & Immunology* Editorial Board

One of the key benefits of attending congresses across the continent is the opportunity it gives us to talk directly with our Editorial Board. Whilst topics of discussion can pertain to the journal itself, importantly we get to hear first-hand impressions of the congress and learn from the experts themselves what topics they are looking forward to in the future of their respective field. At EAACI this year, we had the pleasure of meeting three of our esteemed contributors: Dr Sarah Karabus, Prof Jacques Bouchard, and Prof Nikolaos Papadopoulos.

Dr Karabus enthusiastically talked about some of the sessions she had attended at this year's event, including an interesting talk on the link between atopic dermatitis and the microbiome. The microbiome, as she explained, is such an evolving field of discussion and one that holds unmet therapeutic potential across a multitude of diseases. The sessions she had seen had made her reflect on the differences in allergic severity seen across her and her siblings, differences she accounts to varying potential influences of animal proximity they experienced growing up.



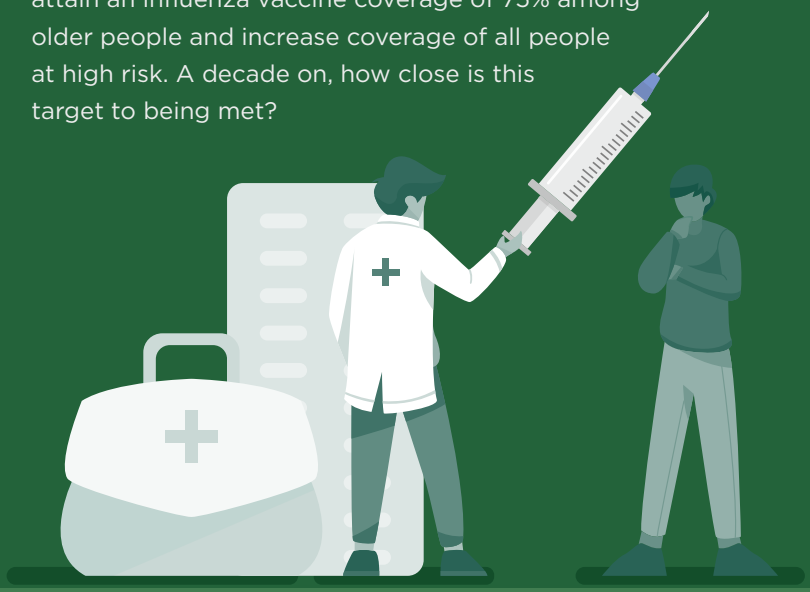

Prof Bouchard highlighted the congress' central theme of precision medicine, and how the potential to tailor specific therapies to specific patients represents a new way of thinking for allergists. Regarding EAACI, Prof Bouchard singled out the pro and con sessions as particularly valuable, as they provide a platform for unbiased and constructive discussion over a range of relevant topics. One of these topics, Prof Bouchard explained, is the role of skin in allergy manifestation, and how treatment or prevention of atopic dermatitis can complement efforts taken to prevent or desensitise children to their allergies.

Finally, Prof Papadopoulos reflected on the EAACI congress as a whole. As past President of EAACI, he commented on how the congress has grown over the years and continually covered almost all relevant topics in the allergy field: both a good and bad feature due to the difficulties it presents in trying to prioritise your schedule accordingly! He chimed that despite EAACI's size, it has a unique flair and community-feel that makes the congress stand out from others and cements it as a truly important event that any allergist or immunologist would not want to miss out on. These were inspiring words and provided a fitting context for our attendance of the congress.

# VACCINATION

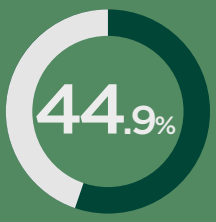
## COVERAGE IN PRIORITY GROUPS

In the northern hemisphere, annual influenza epidemics affect approximately 5–15% of the population.[1] Up to 44,000 people in the World Health Organization (WHO) European region are estimated to die annually of respiratory diseases associated with seasonal influenza.[2] In 2009, the European Council issued a recommendation that urged Member States to attain an influenza vaccine coverage of 75% among older people and increase coverage of all people at high risk. A decade on, how close is this target to being met?



### Median vaccination coverage


For older age groups†




For those with chronic medical conditions‡


Number of EU/EEA Member States that responded

† Nineteen

‡ Twelve

§ Seven

### Recommendations of EU/EEA Member States for seasonal vaccination of priority groups (out of 30 that responded)[3]


**30**

For older age groups


**23**

For all healthcare workers


**28**

For pregnant women in their 2nd/3rd trimester and/or with a chronic illness


**19**

For all pregnant women

### Priority groups for vaccination include



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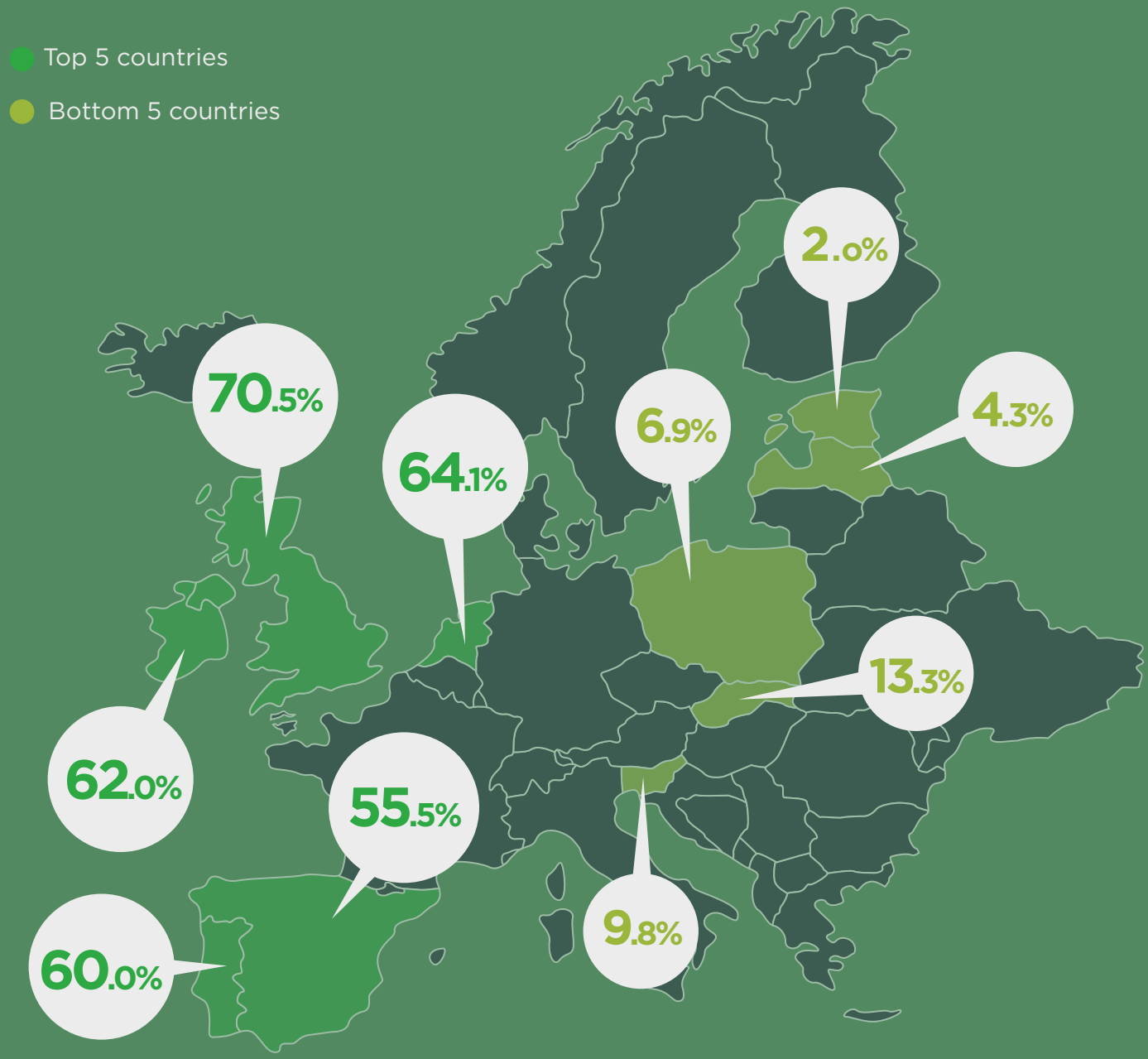
Seasonal influenza vaccination coverage rates for elderly people 2016-17 season based on the administrative method (reported immunisation data)[3]




WOMEN

CHILDREN AGED 6–59 MONTHS

INDIVIDUALS WITH CHRONIC DISEASES

RESIDENTS OF LONG-TERM CARE FACILITIES

HEALTHCARE WORKERS

# Expanding Options in House Dust Mite Allergy Immunotherapy: Optimising Individual Patient Outcomes

This symposium took place on 4th June 2019, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Lisbon, Portugal


**Chairpeople:** Moisés Calderón,[1] Thomas Casale[2]

**Speakers:** Petra Zieglmayer,[3,4,5] Philippe Gevaert,[6,7] Pascal Demoly[8]

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4. Allergy Center Vienna West, Vienna, Austria
5. Thermo Fisher Diagnostics Austria, Vienna, Austria
6. ENT Clinical Department, University Hospital Ghent, Ghent, Belgium
7. Upper Airways Research Laboratory, University of Ghent, Ghent, Belgium
8. Division of Allergy, Department of Pulmonology, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, France



**Disclosure:** Prof Zieglmayer has received lecture fees from ALK Abelló, Allergopharma, Allergy Therapeutics, HAL Allergy, MEDA, Merck, Novartis, Stallergenes Greer, and Thermo Fisher Scientific; industry-sponsored grants from Allergopharma, Allergy Therapeutics, Biomay, Calistoga, GSK, HAL, MSD, Ono, Oxagen, RespiVert, Stallergenes Greer, and VentiRx; consultancy fees from Bencard, Meda, Merck, Sigmapharm, and Stallergenes Greer; and is Medical Director at Thermo Fisher Scientific, Scientific Director of Vienna Challenge Chamber, and Lecturer at the Medical University Vienna. Prof Gevaert served as an advisor or speaker and received grant/research support from 3NT, Ablynx, ALK, Argenx, Bekaert Textiles, Genentech, GSK, HAL Allergy, Medtronic, Novartis, Regeneron, Roche, Sanofi-Genzyme, Stallergenes Greer, Teva, and Thermo Fisher Scientific. Prof Demoly has received fees/grants from Stallergenes Greer, ALK, IQVIA, Thermo Fisher Scientific, and ASIT Biotech, and serves as Chair of the French National Professional Council of Allergology. Prof Casale has received fees from Stallergenes Greer and been an investigator on studies funded by Stallergenes Greer. Prof Calderón reports personal fees from ALK, Stallergenes Greer, Hal Allergy, Allergopharma, Merck, and ASIT-biotech, outside the submitted work.

**Acknowledgements:** Writing assistance was provided by Dr Julia Granerod, London, UK.

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

**Citation:** EMJ Allergy Immunol. 2019;4[1]:32-39.

## Meeting Summary

Prof Calderón opened the symposium by noting its overall aim: to improve outcomes in patients with allergic diseases. Currently, patients can wait an average of 8.5 years to see an allergy specialist and this places a significant burden on individuals. Traditional therapeutic management of patients with allergies is suboptimal, and while appropriate use of allergen immunotherapy (AIT) maximises its impact, treatment guidelines are complex.

Prof Zieglmayer explained that house dust mite (HDM) allergy is a global problem. Allergic rhinitis (AR) drives asthma, with the highest risk in children. Symptoms associated with AR and allergic rhino-conjunctivitis can be different in children compared with adults and adolescents. A holistic approach is needed that treats not only the classic symptoms of AR, but also the accompanying physical and mental impairment. It is critical that clinicians gain a greater understanding of the unique burden of AR in order to better treat their patients.

Prof Gevaert emphasised that precision medicine is key to optimising patient outcomes and that advances have been made in this area, particularly with artificial intelligence. For children, the ultimate aim is to achieve an early diagnosis and use precision medicine for early prevention and treatment with AIT. For adults, better help is required with uncontrolled allergic disease. Precision medicine will make use of all available data to help select patients who are most likely to benefit from AIT.

Prof Demoly summarised data from a Phase III randomised, double-blind, placebo-controlled clinical trial that demonstrated the efficacy and safety of a 300IR HDM sublingual immunotherapy (SLIT) tablet in adults and adolescents with HDM-associated AR. Concluding, Prof Casale reiterated that AIT is a crucial tool in the therapeutic armamentarium against HDM allergy and should be used for early prevention and early treatment, without delay.

## Introduction

### Professor Moisés Calderón

Prof Calderón emphasised that the aim of the symposium was to improve outcomes for patients with HDM allergy, noting that:

a) Waiting for effective treatment places a significant burden on individuals. Patients with allergic diseases can wait an average of 8.5 years to see an allergy specialist, with the wait for AIT being even longer.[1,2]
b) Traditional therapeutic management of patients with allergies is suboptimal. Symptomatic drugs do not slow or halt progression of allergic disease, and many patients continue to display allergy symptoms despite optimal pharmacotherapy.[3-9]
c) Appropriate use of AIT maximises its impact on allergic diseases, but treatment guidelines are complex, and none propose care pathways. Recently, care pathways for AIT have been proposed by the Allergic Rhinitis and its Impact on Asthma (ARIA) group.[10]

## Burden of House Dust Mite-Allergic Respiratory Diseases

### Professor Petra Zieglmayer

Mite allergy is of global relevance.[11-20] The proportion of the population affected by mite allergy ranges from 4% in China and Korea to almost 50% in Australia and New Zealand. Approximately 25% of the population in Europe and North America is affected by respiratory allergies. In Europe, this is expected to increase to >50% by 2025. The problem lies in that almost half of all allergy patients in the European Union (EU) are misdiagnosed, resulting in delayed diagnosis and treatment.[21-23]

AR is known to drive the development of asthma.[24] This is more relevant in the paediatric population, as a German study found that AR in children up to the age of 5 years was a predictor for the development of wheezing at school age.[25] An Australian study reported a 7-fold increased risk of developing asthma in children aged 7–12 years displaying AR compared with the healthy population. This risk decreased with increasing age but was still 2-fold greater in adults displaying

AR compared with those who have never displayed AR.[26] This has important implications for the individual.[27]

A total of 70 of 100 million Europeans with AR display asthma.[23] Patients with AR may feel impaired in daily social activities; rhinorrhoea and sneezing may be perceived as socially unacceptable. Nasal obstruction can lead to reduced quality of sleep. Systemic allergic symptoms can lead to tiredness and a general sense of feeling unwell. This, along with the side effects of medication, can result in malaise that can lead to reduced performance at school and work, loss of productivity, and an economic burden to society.[23] Thus, AR and allergic asthma result in significant individual and societal costs.

HDM allergy is not easy to diagnose as patients present with unspecific symptoms such as loss of smell, general tiredness, and recurring sinusitis. In a European survey, patients with severe HDM-induced respiratory allergies reported peaks in their symptoms in spring, autumn, and (to a lesser extent) mid-winter.[28] The same survey reported a 2-year period from the first symptoms until consultation with a specialist. Furthermore, patients consult general practitioners (GP) and specialists several times a year and often consult several healthcare professionals simultaneously.[28] It is evident the extent to which patients are affected. A total of 30% of GP were aware of ARIA guidelines but only 10% implemented them.[29] Thus, it is not surprising that patients who primarily consult their GP for mite allergy are not adequately treated. To further complicate matters, mite allergic patients are not only sensitised to mites but also present with other allergies (e.g., grass pollen and cat dander) and report comorbidities such as headache, conjunctivitis, sinusitis, and otitis.[28]

Sleep disorders, rhinorrhoea, and nasal obstruction were reported to be the most bothersome symptoms in both children and adults with HDM allergy; however, the order of importance was different.[30] Irrespective of age, however, more symptoms resulted in greater impairment. A study of adults and children with AR showed that the severity of rhinitis has more of an effect on quality of life (QoL), school or work productivity, and daily activities than the duration of rhinitis.[31] A further online, questionnaire-based study reported nasal congestion as the most bothersome symptom.[32] Patients with the most severe symptoms had an impairment in productivity with some unable to work. AR has also been shown to increase the risk of driving accidents, with a quarter falling asleep easily, especially when behind the wheel.[33] Sneezing increases the risk of an accident as one sneeze renders the driver blind for 100 metres. In fact, the degree of impairment is comparable to that seen at a blood alcohol level of 0.05%, the legal limit in many countries.[34] This is not only related to side effects of histamines, as driving ability is even more impaired with untreated AR.

Symptomatic treatment, including antihistamines, nasal steroid sprays, eye drops, and oral steroids, is used by most patients.[5] Immunotherapy may be prescribed to only 2% of patients. Certain medication for AR is reimbursed by the social system of different countries; however, preparations that have been on the market for over 20 years generally need to be paid for by individuals. Costs related to loss of productivity and sick leave also need to be considered. A French study looking at medical resource utilisation and related costs for perennial AR, with or without concomitant allergic asthma, showed a clear increase in costs with severity of AR and poor control of asthma.[35] Concomitant asthma increased medical resource costs at least 2-fold. Household adjustments, such as replacement furniture, humidifiers, or air cleaners, and disposal of soft furnishings, are expensive with little perceived benefit.[5]

Prof Zieglmayer concluded that mite allergy is of global relevance, and that AR drives asthma. The symptoms associated with AR and allergic rhino-conjunctivitis can be different in children compared with adults and adolescents. She emphasised that a holistic approach is needed that treats the classic symptoms of AR in addition to the accompanying physical and mental impairment. It is critical that clinicians gain a greater understanding of the unique burden of AR in order to better treat their patients.

## Precision Medicine to Optimise Patient Outcomes

### Professor Philippe Gevaert

Prof Gevaert mentioned that 20 years ago, he and his colleagues had dreamt that patient samples (blood and nasal secretions) containing a biological marker could be used to personalise treatment with precision medicine. This dream, based on predicted outcome, has altered, and it is now understood that one marker might not be sufficient to achieve this goal.

The case of a 6-year-old boy with a constant cold, blocked nose with open-mouth breathing, rhinorrhoea, and nightly cough all year round was presented. Removal of the adenoids was indicated as a possible treatment option for this patient; however, this child was eventually diagnosed with allergy. Diagnosis in a child is very difficult due to their immature immune systems and the presence of infections and other environmental factors that may lead to rhinitis symptoms. AR patients are classified into two groups: 'sneezers and runners' and 'blockers'. The 'sneezers and runners' often have itching, sneezing, and conjunctivitis. Their symptoms are worse during the day and improve at night. Diagnosis may take some time, but generally after two seasons they are diagnosed with hay fever. 'Blockers', on the other hand, often have severe nasal blockage with little sneezing and no itching. Symptoms are constant but may be worse at night. Diagnosis usually takes a long time. The standard of care is to conduct a skin prick test (SPT) in these patients. Of 2,320 Belgian patients who underwent a SPT, 40% had sensitisation, of which 80% were symptomatic. Almost 30% of the Belgian population had AR symptoms and sensitisation. In those aged 20–40 years, approximately 45% had AR symptoms and sensitisation, implying an increase in allergy with age.[36]

Nowadays, further analyses can be conducted, including measuring specific genes and allergen components. The evolution of sensitisation in children and adolescents to HDM was investigated and was found to begin with Der p 2, Der p 1, and Der p 23.[37] Other allergen components (e.g., Der p 5, Der p 7) were important with increasing age. These data can potentially be used to facilitate better diagnosis and predict better outcomes. Recent data show that mite-specific IgE testing carried out on nasal secretions by means of allergen microarray might soon become an option in the diagnostic workup of AR.[38]

The severity and frequency of symptoms determines therapy as per the ARIA recommendations for the management of AR. With regards to primary and secondary prevention, data are lacking and recommendations are vague. Breastfeeding is recommended regardless of the atopic background of the infant. No general recommendation can be made regarding early-life exposure to pets and HDM avoidance, and dietary manipulations are not recommended. Environmental tobacco smoke should be avoided in pregnant women and children, and the primary prevention of occupational allergy is recommended. Secondary prevention of asthma remains a matter of debate. Early indoor aeroallergen exposure does not appear to affect development of allergic sensitisation or AR in high-risk children.[39] Once a patient is allergic, prevention entails eliminating HDM from the home. According to ARIA guidelines, there is some evidence that encasing bedding in impermeable covers, washing bedding on a hot cycle (55–60 °C), and replacing carpets with hard flooring has some effect on allergen levels but the clinical benefit is less apparent. There is weak or no evidence, however, for acaricides and/or tannic acid; minimising the number of objects that accumulate dust; using vacuum cleaners with integral high-efficiency particulate air filter and double-thickness bags; and removing, hot washing, or freezing soft toys. A systematic review found no effect of chemical or physical methods to reduce exposure to HDM antigens in the homes of people with mite-sensitive asthma.[40]

Prof Gevaert returned to the case of the 6-year-old boy. The child had been prescribed numerous courses of antibiotics and vaccination with Broncho-Vaxom; however, a SPT revealed AR and HDM allergy. Antihistamine and nasal steroids were given following diagnosis. The atopic march, which occurs when the individual develops multiple atopic (allergic) conditions with increasing age, typically starts early with food allergies and eczema, finally ending in asthma and/or AR.[41] At present, AIT is only given once a clinical diagnosis of AR has been made.

Early treatment would be recommended in this young child to prevent disease progression. Prof Gevaert emphasised the need to consider preventative AIT and combine all patient data in order to use precision medicine to select a high-risk child for early AIT.

Prof Gevaert also presented the case of a 46-year-old man with a blocked nose, rhinorrhoea, post-nasal drip, disturbed sleep, snoring, nightly cough, and all-day tiredness. His symptoms lasted all year round. He had a nasal endoscopy and septal deviation was evident. A septoplasty alone however would not cure this patient as further tests showed he also had HDM allergy, emphasising the importance of multidisciplinary efforts to ensure a complete diagnosis. This man took antihistamines and nasal corticoids with no effect. He also had a history of long-term overuse of decongestants. After seeing his GP, he was prescribed a shot of depot systemic corticoids. His HDM allergy remained uncontrolled with medication. Severe chronic upper airways disease defines those patients whose symptoms are inadequately controlled despite adequate (i.e., effective, safe, and acceptable) pharmacological treatment based on guidelines.[8] These patients have impaired QoL, social functioning, sleep, and school or work performance. With optimal treatment, >20% of patients with rhinitis are totally uncontrolled. This could be explained by disease-related (e.g., exogenous, endogenous, or genetic), diagnosis-related (e.g., incorrect diagnosis), patient-related (e.g., poor adherence), or treatment-related (e.g., inadequate treatment) factors.[42]

A Belgian study investigated the control of persistent rhinitis in a real-life community pharmacy setting. Uncontrolled symptoms were reported in 60% of presenters despite medication. Dissatisfaction with the way their rhinitis symptoms were controlled at present was reported by 40%. The nasal spray technique was subsequently evaluated in 1,276 patients. The results indicated a suboptimal spray technique in >80%, with patients not always shaking the medication, tilting the head forward, or spraying away from the septum. Adherence was also a problem, with 54.8% under-adherent (i.e., <80.0% adherence). Decongestant overuse has been reported in 50% of people with persistent rhinitis.[43] Surprisingly, only 3% of presenters use systemic glucocorticosteroids to control AR. However, use of depot-steroid injections has been shown to increase the risk of osteoporosis and diabetes.[44] Thus, AR should not be treated with systemic corticosteroids long term.

Precision medicine is increasingly recognised as the way forward for improving patient outcomes. A consensus on the position and gradual implementation of the principles of precision medicine within existing adult treatment algorithms for AR and chronic rhinosinusitis has been published.[45] Prediction of success of the initiated treatment and patient participation in the treatment plan can be implemented at the time of diagnosis. Strategies to prevent progression of disease, in addition to prediction of therapy success and patient participation in the long-term therapeutic strategy, are included in the second-level approach. Personalised care should be positioned at the tertiary level. Prof Gevaert returned to the case of the 46-year-old man and suggested that this patient would be a candidate for AIT.

The technological revolution means that nowadays all patient data are digitalised. When computers begin to read patient letters and connect data on a large scale (big data), artificial intelligence techniques may facilitate better diagnosis and precision medicine. The goal is to achieve an early diagnosis in children and use it for early prevention and early treatment with AIT. In adults, better help is needed for uncontrolled allergic disease. Prof Gevaert emphasised that precision medicine will help select patients who are most likely to benefit from AIT.

## The 300IR 'Solution' and Future Trends

### Professor Pascal Demoly

HDM SLIT drops have been shown to alleviate the burden of HDM allergies in children and adults with HDM-associated AR and/or asthma.[46] The drop format offers dose flexibility which is important for tailored individual treatment and the development of tablets with the same composition would offer simplicity.

New drugs are developed in accordance with regulatory guidelines.[47] Clinical significance

and clinical relevance should be demonstrated. In AR, the primary trial outcome must reflect both symptom severity and intake of rescue medication (total combined score [TCS]). Secondary outcomes, defined *a priori*, contribute supplementary information on the effect size and safety. The estimation of the effect size must be precise enough to be able to reasonably eliminate the possibility that the effect may be too small to have benefit (positive benefit/risk balance). Prof Demoly emphasised that clinical relevance is not synonymous with statistical significance. Clinical relevance can be quantified with different metrics including effect size, relative clinical impact, numbers of patients needed to treat, and minimal important difference.[48] However, one can also focus on symptoms which are known to be bothersome (e.g., severe blocked nose), factors of which impact is more relevant (e.g., QoL), or specific groups of patients who may be more responsive (e.g., more symptomatic patients).

Prof Demoly reported the results of a confirmatory Phase III, randomised, double blind, placebo-controlled clinical trial which aimed to evaluate the efficacy and safety of the 300IR HDM SLIT tablet when administered for 12 months to adults and adolescents with HDM-associated AR.[49-52] The trial was conducted at 231 centres in Canada, USA, EU, Russian Federation, and Israel. The study included male and female outpatients aged 12–65 years with HDM-associated AR (with or without concomitant asthma) for at least 1 year, sensitised to Der p and/or Der f, and with an average TCS of ≥5 over the baseline evaluation period. A 4-week run-in period was included prior to randomisation with the 300IR tablet (n=802) or placebo (n=805) to allow for the selection of patients with more severe symptoms. The primary evaluation period was the same duration as the run-in period and comprised the last 4 weeks of the treatment period. The primary endpoint of the trial was the average TCS calculated as the average daily TCS during the 4-week evaluation period. The TCS is the sum of two patient daily scores: rhinitis total symptom score and rescue medication score. Additional key outcomes used to assess clinical benefit included efficacy (individual rhinitis symptom scores, QoL, days with AR symptoms under control) and safety.

The average TCS over time showed symptom improvement and reduction in rescue medication use. The relative least squares mean difference (95% confidence interval) versus placebo was -16.9%.[49] Improvements versus placebo in the secondary endpoints rhinitis total symptom score and rescue medication score were also significantly reduced in the 300IR group versus placebo. AIT with 300IR HDM sublingual tablet among adults and adolescents with HDM AR significantly improved nasal symptoms compared to patients receiving placebo despit a higher consumption of rescue medication in the latter group. This treatment was particularly efficient at relieving blocked nose which is a troublesome symptom with a significant socioeconomic burden.[50] QoL scores, as measured by the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ), were taken at the end of the treatment period. Significant improvements in QoL score were seen; this was observed across the seven domains of the RQLQ including sleep and daytime activities.[51] Adults and adolescents with HDM-associated allergic rhinitis benefited from treatment with 300IR HDM SLIT tablet by having more days with their AR symptoms under control compared to patients on placebo.[52] The SLIT tablet was generally well-tolerated, with no reports of severe anaphylactic reaction and no deaths. The most common adverse events were application-site reactions. The safety profile was similar in adults and adolescents, and consistent with previous studies.

Prof Demoly concluded that different solutions are available for different patients. HDM AIT SLIT drops offer dose flexibility for children and adults with AR and/or asthma. The 300IR HDM SLIT tablet is effective and safe in adolescents and adults displaying persistent AR due to HDM.

## Final Remarks

### Professor Thomas Casale

Prof Casale confirmed that a significant amount of morbidity is associated with HDM AR. He emphasised that a clinician's job is to improve patient outcomes by gaining a greater understanding of the unique burden of AR and providing better treatment. Early prevention and early treatment with AIT are crucial, especially in children, and AIT should therefore be used now without delay.

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# A Novel Approach to the Reduction of Cat Allergen Fel d1 Through Inclusion of an Egg Product Ingredient Containing Anti-Fel d1 IgY Antibodies in the Feline Diet

These poster presentations took place on 1st-5th June 2019, as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Lisbon, Portugal


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**Disclosure:** Dr Satyaraj is an employee of Nestlé Purina Petcare, St. Louis, Missouri, USA. Dr Wedner receives research support from Nestle-Purina, Takeda, CSL Behring, Biocrist, and Griffols; is on the advisory boards of Takeda, CSL Behring, and Pharming; and receives speaking fees from Sanofi-Genzyme, Regeneron, GSK, Genentech, and AstraZeneca.

**Acknowledgements:** Medical writing assistance was provided by Satyendra Shenoy, Describe Scientific Writing & Communications, Germany.

**Support:** This article was funded by the Purina Institute and reviews data presented at the European Academy of Allergy and Clinical Immunology (EAACI) Congress held on 1st–5th June in Lisbon, Portugal. It may include opinions of speakers that do not necessarily reflect those of the Nestle Purina Petcare Company.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:40-46.


## Introduction

Domestic cats, *Felis catus*, are one of the major sources of indoor allergens responsible for various allergies, including respiratory disorders.[1] There has been a steady rise in the prevalence of sensitisation to cat allergens,[1] which may be manifested as atopic symptoms in a substantial proportion of allergic individuals.[2,3] Cat ownership is fairly common in Western countries; almost a quarter of households in the USA[4] and Europe[5] own a pet cat, and it can be reasonably assumed that some of these households will have individuals who are allergic to cats. Consequently, management of cat allergens poses a substantial burden on these individuals.[6,7]

Cats produce a number of potential allergens. To date, ten allergens, recognisable by their interaction with specific IgE, have been identified in extracts derived from feline hair, saliva, serum, and urine.[8,9] Moreover, eight of these allergens, Fel d1–8, have been registered with the World Health Organization (WHO) and International Union of Immunological Societies (IUIS).[1] Of these, Fel d1 is the most important allergen to which most individuals with cat allergies are sensitised.[10,11] Fel d1 is a 35–39 kDa glycoprotein produced primarily by the salivary and sebaceous glands in cats.[12] While the exact biological role of Fel d1 is yet to be determined, it is proposed to have numerous functions such as skin protection or transport of lipids, especially steroids, hormones, and pheromones.[1] Fel d1 is transferred to the

hair when cats groom and is subsequently shed with hair and dander. Owing to its small size and molecular structure, Fel d1 can be airborne for long periods of time as well as adhere to fabrics and indoor furniture,[13,14] thereby increasing the probability of exposure. On exposure in sensitised individuals, the unbound form of Fel d1, termed active Fel d1 (aFel d1) in this report, binds IgE and leads to mast cell degranulation, thus initiating the allergic response cascade.

In most cases, treatment approaches for cat allergies are palliative and consist of anti-allergy medications (e.g., antihistamines or decongestants) or avoiding exposure through restriction of access, physical removal of the cat, and improvement of air quality using filtration units. Another line of treatment is allergen immunotherapy, which in principle involves exposure to increasing amounts of allergens to desensitise the individual and induce immune tolerance.[15] While this approach has had success for other allergens, there is little data to support allergen immunotherapy for regular use against cat allergies.[16] Moreover, the process is long-term, contraindicated in conditions such as asthma, and cost-restrictive.[17]

The authors' approach to managing cat allergens takes advantage of the antibody-antigen interaction by generating anti-Fel d1 antibodies using the avian IgY system. The avian IgY is equivalent to the mammalian IgG and is naturally produced in domestic birds, such as chickens, in response to antigens. These antibodies are transferred to the egg where they provide passive immunity to the hatchlings. These antigen-specific IgY antibodies subsequently attach to targeted antigens and neutralise or mark them for destruction by cells of the immune system. The use of avian IgY is not a new discovery: it has already been in use for numerous animal and human applications.[18,19] However, the element of novelty in the authors' approach is that instead of administering the IgY antibodies to the human patient, this research focusses on incorporating the anti-Fel d1 IgY antibodies in the cat's diet, through a safe and nutritious egg product, with the intention of neutralising the aFel d1 in cat saliva. While it was not expected that such an approach would alter the amount of Fel d1 secreted, it was hypothesised that it would reduce the amount of aFel d1 transferred to the hair during grooming and dispersed into the cat's environment.

In this article, the speakers review and share some of the data presented in four posters at the European Academy of Allergy and Clinical Immunology (EAACI) Congress held from the 1st–5th June 2019 in Lisbon, Portugal.

## Variability in Fel d1 Levels in Cat Saliva

There are many misconceptions and conflicting reports related to cat allergens,[20] including the propensity of specific cat breeds to secrete lower levels of Fel d1, and hair colour or length and gender being associated with Fel d1 levels.[21-24] Hence, one of the first questions Bastien et al.[25] sought to answer in the study presented in this poster was whether specific factors such as age, weight, and breed had any effect on the levels of Fel d1 secreted in feline saliva.

In this study, the authors recorded data from 64 healthy, neutered cats of either gender, aged 1.2–15.3 years, and varying in breed, weight, hair colour, and pattern. The principal measurement was salivary levels of Fel d1 in samples collected twice a day, pre and post-feeding, every other day for a year. Fel d1 levels were measured using a commercially available Fel d1 ELISA kit (Indoor Biotechnologies, USA).

It was observed that there was no association between salivary Fel d1 levels and factors such as bodyweight, body condition score, and the colour and pattern of the hair. However, it was interesting to note that Fel d1 production varied substantially not only between cats, but within the same cat over the course of a year. In addition, the age of the animal was found to influence salivary Fel d1 levels; older animals (11–15 years) had significantly lower salivary levels of Fel d1 in comparison to younger animals (1–5 years; $p<0.001$). There was an 80-fold difference in Fel d1 levels between the highest and lowest Fel d1-producing cats. Moreover, cats with a lower average Fel d1 had lower variability over the year compared to cats with higher average Fel d1 levels. These results indicate that salivary samples taken at a single point are not ideal for estimation of the cat's average Fel d1 level and biological variability of Fel d1 should be taken into account in future studies. The methodology and results of these studies have recently been published in a peer-reviewed article.[26]

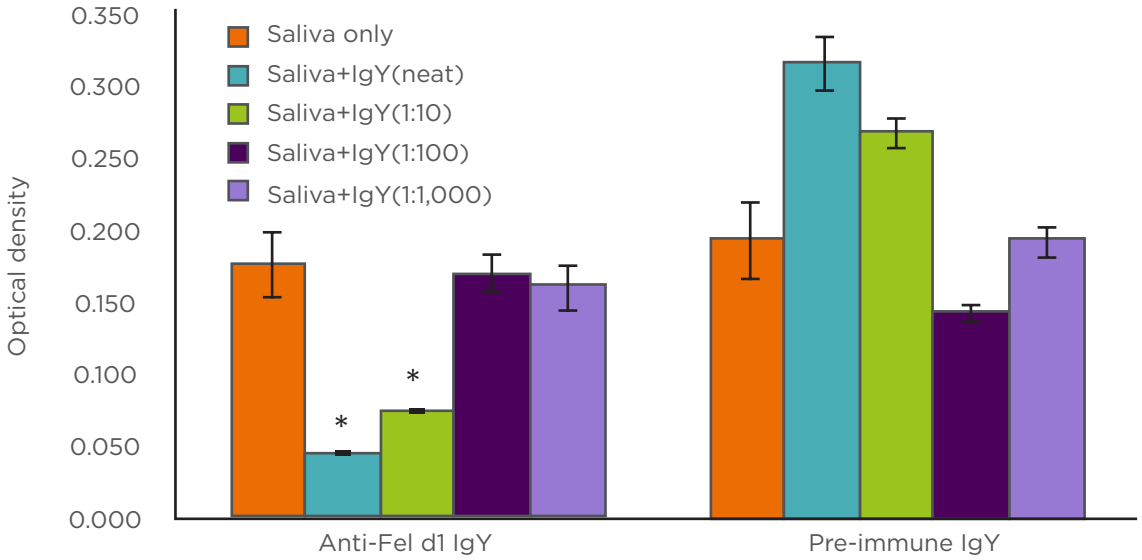


**Figure 1: Chimeric ELISA comparison of IgY antibodies isolated from eggs produced by Fel d1-immunised (anti-Fel d1 IgY) hens compared to IgY from the same hens prior to immunisation (pre-immune IgY).**

*p<0.001

## Fel d1 Blocking Antibodies Against the Major Cat Allergen Fel d1

To test whether anti-Fel d1 antibodies have the potential to block feline Fel d1 and downstream degranulation of mast cells, Satyaraj et al.[27] conducted a series of mechanistic studies using a chimeric ELISA and the beta-hexosaminidase release assay, respectively. The authors first tested the specificity of the antigen-antibody binding using various dilutions of two distinct rabbit-derived anti-Fel d1 antibodies: the Indoor Poly (Indoor Biotechnologies Inc., USA) polyclonal antibody, which recognises multiple Fel d1 epitopes; and Fel d1 Major allergen 1 polypeptide chain 1 antibody (FGI; Fabgennix International Inc., USA), a monoclonal antibody that identifies the peptide sequence covering amino acids 23–40 in chain 1 of Fel d1, a known IgE-binding site.[28] The authors discovered a dose-dependent inhibition of salivary Fel d1 and human IgE complex formation with the Indoor Poly antibody ($p<0.001$) but none with the monoclonal FGI antibody (unpublished results). This indicates that multiple epitopes on Fel d1 need to be blocked to prevent its interaction with IgE and the subsequent mast cell degranulation. It was next investigated if either anti-Fel d1 antibodies could attenuate the Fel d1-mediated activation of IgE and the subsequent allergic cascade, measured through the release of beta-hexosaminidase in humanised rat basophilic leukaemia cells. A specific and dose-dependent reduction in beta-hexosaminidase release was discovered when the polyclonal, but not monoclonal, anti-Fel d1 antibodies were incubated with cat saliva samples.

The authors next sought to examine whether these observations could be replicated using anti-Fel d1 IgY antibodies isolated from egg yolks instead of the polyclonal anti-Fel d1 antibodies. Combining the results of the chimeric ELISA (Figure 1), as well as beta-hexosaminidase assay, a specific and dose-dependent reduction in Fel d1 binding to IgE and downstream mast cell activation was discovered.

The authors next aimed to ascertain if these *in vitro* results could be translated in an animal model. To answer this question, a pilot feeding trial was conducted in 20 cats with moderate-to-high, yet reasonably stable, salivary Fel d1 concentrations. Following a control diet period of 1 week, the cats were split into two groups: one to continue with the control diet, and the other to receive the control diet supplemented with an egg product ingredient containing anti-Fel d1 IgY antibodies, for 4 weeks. Saliva samples

were collected weekly and Fel d1 levels therein were estimated using commercially available direct ELISA (Indoor Biotechnologies, USA). It was discovered that, by Week 3, cats receiving the ingredient containing anti-Fel d1 IgY in their diet had an approximately 24% reduction in their salivary aFel d1 levels in comparison to baseline.

With these data, it can be concluded that Fel d1-specific IgY antibodies bind Fel d1 in cat saliva, thereby blocking the ability of the latter to bind IgE and induce IgE-mediated degranulation. Since monoclonal anti-Fel d1 antibodies could not produce the same effect, it can be concluded that multiple epitopes of Fel d1 must be blocked to prevent its binding to IgE and initiation of the IgE-mediated allergic response cascade. Perhaps the most interesting observation made was the reduction in salivary aFel d1 levels in cats fed a diet with an egg product ingredient containing anti-Fel d1 IgY, which then offers a novel strategy to neutralise aFel d1 in cat saliva without altering production.

## Effects of Fel d1 Blocking Antibodies on Levels of Fel d1 on Cat Hair

After conclusively demonstrating that anti-Fel d1 IgY dose-dependently blocked salivary Fel d1 *in vitro* and reduced salivary levels of aFel d1 in cats whose diet was supplemented with anti-Fel d1 IgY antibodies, Satyaraj et al.[29] next sought to ascertain whether this effect could be replicated on the levels of aFel d1 on hair in cats fed a diet with an egg product ingredient containing the anti-Fel d1 IgY antibodies.

Thus, 105 domestic shorthair cats were enrolled, aged 7 months to 17 years, of either gender, and neutered or intact, in a 2-week baseline period on a control diet followed by separation into two groups. Cats in the control group continued to receive the same diet for a further 10 weeks while cats in the test group were fed the control diet supplemented with an egg product ingredient containing anti-Fel d1 IgY for 10 weeks. Hair samples were collected from the most commonly groomed areas (belly, shoulders, and sides) twice weekly during the baseline period and once per week during the 10-week study phase. Approximately 100 mg of each sample was quantitatively analysed for aFel d1 levels using the previously mentioned ELISA kit.

It was observed that by the end of the study period 97% of the test group cats showed reduction of aFel d1 levels on hair compared to baseline; in 86% of the cats the reduction was ≥30%. Substantial reductions in aFel d1 levels were observed from Week 3 onwards ($p<0.001$), and on average the reduction in aFel d1 levels was 47% by Week 10 (Figure 2A). Thus, it can be concluded that feeding cats a diet with an egg product containing anti-Fel d1 IgY resulted in reduction of aFel d1 levels in the hair and dander and the greatest decreases were observed in cats with initially high levels of Fel d1 (Figure 2B). These results reinforce the previous findings on a larger scale and establish the proof-of-concept of a novel approach for managing cat allergies. The details and results of this study were recently published in a peer-reviewed journal.[12]

## Pilot Evaluation of Cats Fed Anti-Fel d1 Antibodies and Human Allergy

Having demonstrated a blockade of Fel d1 by anti-Fel d1 IgY *in vitro* and *in vivo* accompanied by subsequent reduction in aFel d1 secreted in feline saliva and hair, Wedner et al.[30] intended to investigate if this resulted in beneficial effects in humans with cat allergies. To do this, a pilot study was conducted in which human volunteers were exposed to bedding used by cats fed either a control diet or a test diet with an egg product ingredient containing anti-Fel d1 IgY.

Briefly, blankets on which cats (fed on one of these two diets) had laid and shed hair were used as source of Fel d1 in environmental chambers (FlowerHouse Inc., USA) set up to provide controlled exposure. Eleven adult human subjects with a history of cat sensitivity confirmed by a positive skin prick test to cat allergens and a documented variable response to high and low Fel d1 levels were recruited to participate in this 4-week randomised, double-blinded, crossover study. Subjects underwent a priming exposure during Week 1, in which they spent 3 hours in the environmental chambers containing blankets from cats fed the control diet. Following this,

subjects underwent two more exposures during Week 2 and Week 4 to hair from cats that had been fed either the control diet or the test diet. Subjects randomly assigned to control exposure in Week 2 were exposed to the test condition in Week 4 and vice versa, thus each subject served as their own control. During the exposure period, subjects filled in questionnaires assessing Total Nasal Symptom Score (TNSS) and Total Ocular Symptom Score (TOSS) every 15 minutes. The control and test exposures were compared to the initial priming exposure for statistical evaluation to reduce potentially confounding placebo effects.

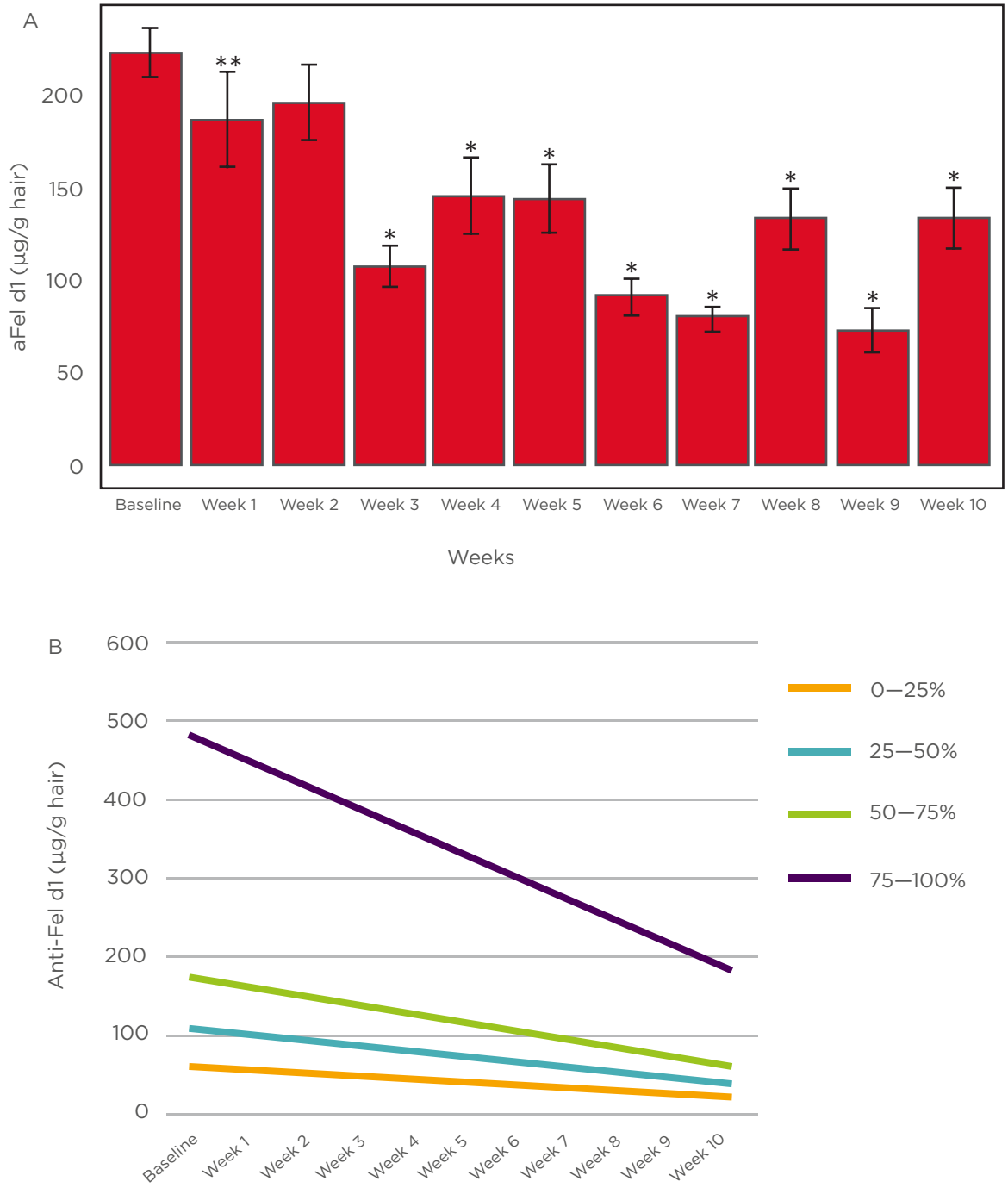


**Figure 2: A) Mean active Fel d1 levels (µg/g hair) across 10 weeks. B) Mean change in Fel d1 levels based on initial concentrations.**

*p<0.001

**p<0.050

It was noted that the levels of aFel d1 were lower in chambers loaded with blankets from cats fed the test diet in comparison to those with blankets from cats fed the control diet. Overall TNSS score and the subscore for nasal congestion were substantially reduced in subjects exposed to hair from cats fed the test diet in comparison to the priming exposure (p=0.0350 and p=0.0055, respectively). The authors also observed improvement in other allergic symptoms such as nasal itching, sneezing, and runny nose; however, the reduction in these scores was not statistically significant. While reduction in overall TOSS scores did not reach statistical significance, subscores for scratchy eyes and itchy eyes were significantly decreased (p=0.0150 and p=0.0072, respectively).

With this study, it was demonstrated that humans with feline allergies had substantial improvement in their TNSS scores and some ocular scores when exposed to bedding from cats fed a diet with an egg product ingredient containing anti-Fel d1 IgY as compared to bedding from control diet-fed cats. While these results are encouraging and hold the promise of reducing incidence of allergies in cat-owners sensitive to cat allergens, specifically Fel d1, this needs to be replicated in a larger human population.

## Summary and Conclusions

While the typical approaches to managing feline allergies have involved either desensitising cat-allergic patients or mitigating the allergic symptoms, this approach was unique in the sense that the authors aimed at neutralising the major cat allergen, Fel d1, after its production but before human exposure, by incorporating an egg product ingredient containing anti-Fel d1 IgY antibodies into the cat's diet. In our series of studies, the mechanism by which anti-Fel d1 IgY bound feline salivary Fel d1 and prevented it from binding human IgE was demonstrated, thereby curtailing the subsequent mast cell-mediated allergic response. In addition, it was shown that cats fed a diet with an egg product ingredient containing anti-Fel d1 IgY have lower aFel d1 levels in their saliva and hair. However, the most clinically relevant of these data showed an improvement in nasal and some ocular symptoms in individuals sensitised to Fel d1 with this approach. This set of results offers proof-of-concept of a novel and cutting-edge approach to management of allergies in individuals sensitised to Fel d1. Further research will be required to demonstrate the usefulness of this approach for managing cat allergens in the home.

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# Human Milk Oligosaccharides: New Ways to Shape the Gut Microbiome in Cow's Milk Protein Allergy

These symposia took place on 2nd and 3rd June 2019 as part of the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Lisbon, Portugal


**Chairpeople:** Anna Nowak-Wegrzyn[1] and Liam O'Mahony[2]

**Speakers:** Liam O'Mahony,[2] Harald Renz,[3] Elizabeth Forbes-Blom,[4] Anna Nowak-Wegrzyn[1]

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**Disclosure:** Prof Nowak-Wegrzyn is a speaker and investigator for Nestlé Health Science. Prof O'Mahony is a speaker for Nestlé Health Science, Nutricia, and Novartis, and has received research support from Alimentary Health and GSK. Prof Renz has been a speaker, adviser, or investigator for Nestlé Health Science, Allergopharma, Novartis, ThermoFisher, Danone, Mead Johnson Nutritional, Bencard, DFG, BMBF, EU, Land Hessen, DAAD, ALK, Stiftung Pathobiochemie, Ernst-Wendt-Stiftung, Mead Johnson Nutritional, Beckman Coulter, and sterna-biologicals (co-founder). Dr Forbes-Blom is an employee of Société des Produits Nestlé S.A.

**Acknowledgements:** Writing assistance was provided by Stephen Jeffreys on behalf of EMJ. Dr Forbes-Blom thanks Norbert Sprenger for his scientific contribution, including preparation of the 'What are Human Milk Oligosaccharides' presentation.

**Support:** The publication of this article was funded by Nestlé Health Science. The views and opinions expressed are those of the presenters. Content was reviewed by Nestlé Health Science and the speakers for medical accuracy.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:48-54.


## Meeting Summary

Prof O'Mahony began by discussing how the human gut is colonised by a wide diversity of microbes. He went on to review the evidence for how they exhibit highly evolved synergistic relationships to provide essential biological functions to the host and how the gut microbiome is influenced by many factors in early life. Prof Renz proceeded to describe the importance of establishing a stable gut microbial community, which closely tracks host growth and immune development. The mechanisms whereby delays or alterations in the establishment of these communities can lead to microbiome immaturity, raise the risk of allergy development including cow's milk protein allergy (CMPA). Dr Forbes-Blom introduced the multiplicity of human milk oligosaccharides (HMO) and explained their position as multifunctional components that shape the developing gut microbiome and influence the developing immune system. Finally, Prof Nowak-

Wegrzyn reviewed the latest trial data on supplementing specialty formulas for the management of CMPA with different HMO and reported the results.

## The Development of the Gut Microbiome and its Role

### Professor Liam O'Mahony

In the last few years, the human gut microbiome has become a very exciting area of research following the delineation that there are between $10^{13}$ and $10^{14}$ gut micro-organisms, with >1,000 species identified so far. This represents half of all cells in the human body, and, with 2–3 million genes, the microbiome substantially dwarfs the host genome which is estimated to have approximately 25,000 genes. The gut microbiome consists of bacteria, fungi, viruses, and bacteriophages. They secrete many primary and secondary metabolites which may be absorbed to influence multiple body sites. A third of all metabolites measured within human urine are bacterial in origin.

The host benefits for such a complex organ are extensive and can be categorised into four broad spheres of influence: i) these non-pathogenic bacteria displace pathogenic species; ii) many bacterial species are responsible for secreting a diverse range of molecules that stimulate the host immune system and drive immune tolerance; iii) gut bacteria impact nutrition and metabolism because they are responsible for generating additional nutrients which humans are incapable of producing from our ingested food as well as influencing our metabolism; iv) gut bacteria have been shown to influence behaviour and mood. These four spheres of influence can be far-reaching, and disruption of microbiome–host interactions have been implicated in diarrhoeal disorders, inflammatory bowel disease, allergies, asthma, autoimmunity, obesity, diabetes, and liver disease, as well as depression, anxiety, and attention deficit hypersensitivity disorder.

Development of an infant's gut microbiome requires 1–2 years and is not bequeathed at birth but instead acquired over the first years of life. Koenig et al.[1] completed an in-depth case study on the development of one infant's gut microbiome over the first 2.5 years of life, showing how increasing abundance and diversity of bacterial species corresponded to shifts in diet and health events. To determine whether it is environmental or genetic influences that shape the gut microbiome, Rothschild et al.[2] studied >1,000 adults with diverse genetic backgrounds and showed genetic kinship was only weakly associated with shaping their gut microbiomes and that it was non-genetic factors such as diet that had a much greater influence. Indeed, diet-bacterial interactions can result in beneficial metabolites; Brussow and Parkinson[3] showed that, following consumption of indigestible plant fibre, bacteria resident in the gut can ferment these fibres to generate short chain fatty acids (SCFA) such as acetate, butyrate, and propionate. Propionate can directly initiate a gut–brain neural circuit which has beneficial effects on host physiology.

Roduit et al.[4] examined 301 children from a birth cohort and measured SCFA levels in their faecal samples by high-performance liquid chromatography at 1 year of age. Associations with early life exposures, especially diet, allergy, and asthma later in their lives, were also examined. Children with the highest levels of faecal butyrate and propionate at the age of 1 year had significantly less atopic sensitisation and were less likely to have asthma by 6 years. They were also less likely to have a reported diagnosis of food allergy or allergic rhinitis.

Prof O'Mahony proposed the analogy of an infant's gastro-intestinal tract being similar to a newly emerged island which undergoes a succession of colonisations as new species arrive and form communities, replete with extinction events when species are wiped out by environmental change or competition. Early life events, such as mode of delivery, breastfeeding, mother's diet and health status, antibiotic and other drug usage in pregnancy and early childhood, the early-life environment such as presence of siblings, and the proximity to pets or farm animals, significantly affect the timing of bacterial colonisation and establishment, which can modify the risk of developing allergies and asthma.[5] Additionally, humans have evolved over millions of years within an environmental and

social context, which has facilitated a reliable transmission and dispersal of gut symbionts. Membership of the human microbiome metacommunity has also been driven by evolutionary factors and these human-adapted symbionts might only be acquired through contact with other humans. Modern lifestyles and changed social interactions may disrupt these metacommunity pathways which could lead to perturbations in human microbiomes. The consequences of this might be a cause for the observed increase in immune-mediated diseases. It is important that infants are given the opportunity to build up a full experience of such symbionts in early life.

## The Role of the Gut Microbiome in Early Immune Development and Allergies

### Professor Harald Renz

Prof Renz opened his talk with a short introduction to the gastrointestinal tract showing the difference between immune tolerance and breakdown of tolerance to ingested antigens (Figure 1).

Dysbiosis in the gut microbiome can be defined by a qualitative and quantitative dysregulation of the composition of the microbiota indicating an impaired microbiome and can be observed preceding disease. A microbial exposome is defined as the measure of all the microbial exposures of an individual in a lifetime and how those exposures relate to health. The window of opportunity at the start of life enables the infant to acquire a library of environmental microbial encounters from the mother during pregnancy and birth, as well as intimate contacts from nasal, throat, milk, and skin encounters. Additionally, indoor pet encounters and microbial encounters outdoors and through ingestion of solid foods all contribute to this exposome. Feehley et al.[7] have hypothesised that alterations in the microbiome interfere with immune system maturation, resulting in IgA production impairment, reduced regulatory T cell (Treg) abundance, and Th2-skewing of baseline immune responses to drive aberrant responses to innocuous (food) antigens.

Exposure to respiratory syncytial virus, rhinovirus, caesarean delivery, antibiotic use prenatally and in infancy, and infant obesity are all modifiable allergy and asthma risk factors contributing to the exposome. It has been shown that the development of the gut microbiome varies depending on the mode of birth delivery. Those born vaginally start with a *Lactobacillus* and *Prevotella*-dominated microbiome, while infants born through caesarean section have a *Staphylococcus, Proprionibacterium, Streptococcus*, and *Corynebacterium*-dominated microbiome. Sevelsted et al.[8] investigated the link between caesarean delivery and the development of immune diseases such as asthma, allergy, inflammatory bowel disease, and Type 1 diabetes mellitus. There were 1.9 million full-term children born by caesarean delivery in the period from 1977–2011, who were analysed for chronic immune diseases as recorded in the Danish national registries. Children delivered by caesarean section had significantly increased risk of asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel disease, immune deficiencies, and leukaemia.

In a large population-based cohort study, Wu et al.[9] reported that *in utero* and early-life events, including maternal urinary tract infections during pregnancy, maternal antibiotic use, caesarean delivery, infant antibiotic use, and having no older siblings at home were all associated with an increased risk of childhood asthma. In addition, there were strong dose-dependent relationships between a number of maternal urinary tract infections during pregnancy, maternal antibiotic use, infant antibiotic use, and older siblings at home with asthma risk. Individuals with extremes of multiple exposures had nearly 8-fold increased odds of developing asthma by the age of 6 years.

The modifiable protective factors in the developing microbiome were reviewed. Breast feeding, exposure to pets, and (in certain countries) the presence of *Helicobacter pylori*, have been shown to have beneficial effects. As the infant develops, the introduction of solid foods impacts the gut microbiome. During breast feeding, the gut microbiota is dominated by *Bifidobacterium, Lactobacillus*, and *Veillonella*. These microbes contribute to immune system development. As solid foods are started, so the taxa are seen to shift in favour of *Bacteroides*

and *Clostridiales* which contribute to the development of SCFA. Roduit et al.,[4] as reported by Prof O'Mahony, has shown how SCFA play a significant role in reducing atopic sensitisation, asthma, and food allergy at 6 years of age. The concept of the microbial exposome, in which high microbial burden confers inflammatory resilience, was discussed. McDade et al.[10] had investigated whether patterns of DNA methylation in inflammatory genes in young adulthood would be predicted by exposure to the exposomes of early life nutritional, microbial, and psychosocial events. The pattern of results showed that repeated microbial exposure in infancy led to a trained immunity with strong resilience to chronic inflammation in adulthood, while low microbial exposure in infancy led to low resilience.

Dr Renz explained the importance of the 'window of opportunity': the critical period in life during which environmental factors exert a lasting effect on the individual and determine an individual's susceptibility to developing allergies and certain lifestyle diseases in adult life. This period spans from intrauterine development through to at least the first 2 years in postnatal life, and is referred to as the 'first 1,000 days'.[11] By proactively managing their exposomes in this period, it is anticipated that children's gut microbiomes will be optimised for future healthier lives.

## What are Human Milk Oligosaccharides?

### Doctor Elizabeth Forbes-Blom

A mother's milk is the best nutrition for her infant and is associated with health benefits such as a lower risk of respiratory and gastrointestinal infections, obesity, diabetes, and possibly allergies.

Human breast milk contains many macro and micro-nutrients as well as HMO. Besides water content, lactose is the major constituent of breast milk with 70 g/L, followed by lipids at 40 g/L (Figure 2).

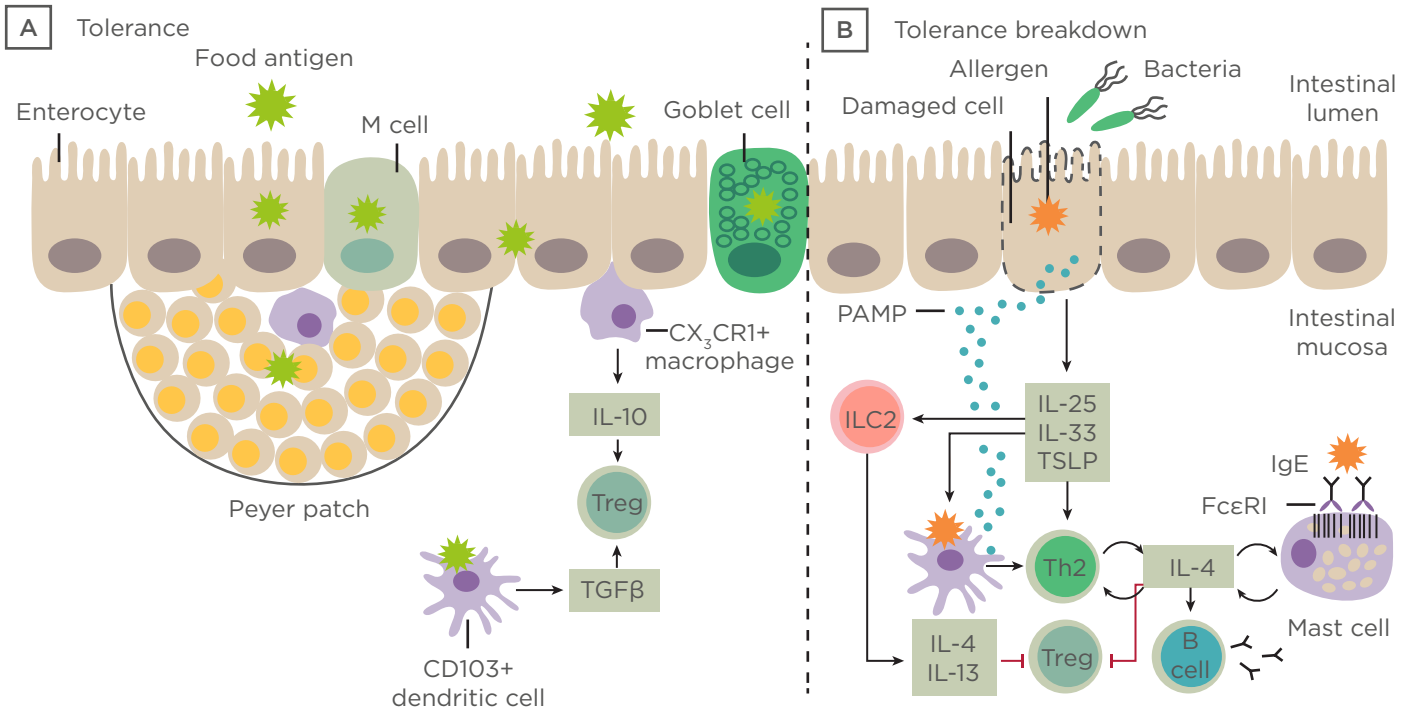


**Figure 1: Immune tolerance and breakdown of tolerance to ingested antigens.**

A) Under normal conditions, food antigens in the gastrointestinal lumen pass into the intestinal mucosa by transiting between enterocytes or by active transport. B) Tolerance breaks down in situations in which danger signals arise.

ILC: innate lymphoid cells; PAMP: pathogen-associated molecular pattern molecules; Th; T helper cell; Treg: regulatory T cell; TSLP: thymic stromal lymphopoietin.

*Adapted from Renz et al., 2018*[6]

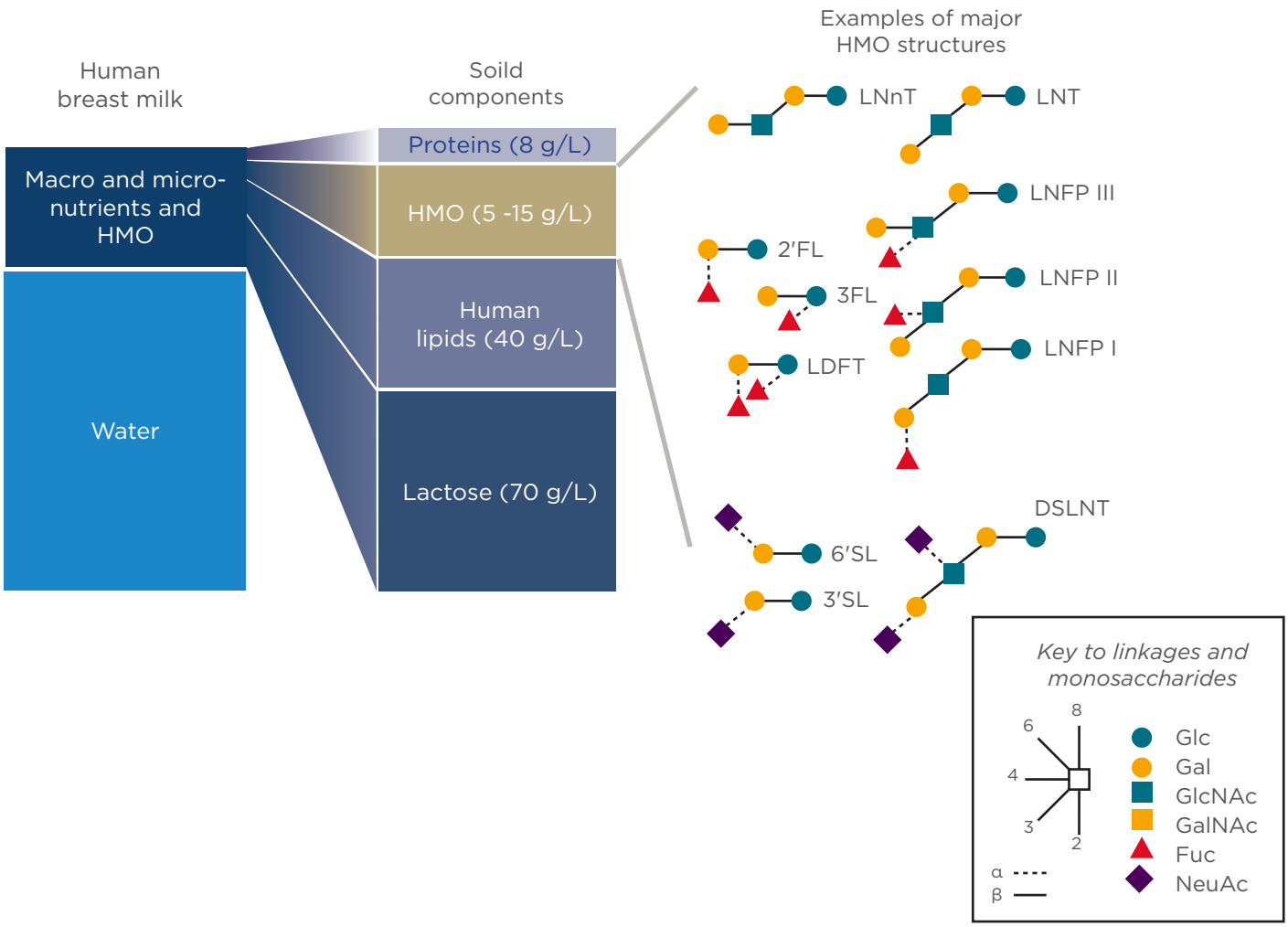


**Figure 2: Gross composition of human milk with examples of major HMO structures.**

2′FL: 2′-fucosyllactose; 3FL: 3fucosyllactose; 3′SL: 3′sialyllactose; 6′SL: 6-sialyllactose; DSLNT: disialyllacto-N-tetraose; Fuc: Fucose; Gal: galactose; GalNAc: N-Acetylgalactosamine; Glc: glucose; GlcNAc: N-acetyl-glucosamine; HMO: human milk oligosaccharides; LDFT: lactodifucotetraose; LNFP: lacto-N-fucopentaose; LNnT: lacto-N-neotetraose; LNT: Lacto-N-tetraose; NeuAc: sialic acid.

Human milk components can be categorised into those with nutritive value for the infant (lactose, proteins, and lipids) and those without nutritive value per se, as they are not digested and exert bioactivities; HMO are part of this second category. One of their main roles identified to date is to support the developing infant gut microbiome. HMO are structurally different from classical prebiotics such as galacto-oligosaccharides and fructo-oligosaccharides. Based on a lactose backbone, HMO are decorated and elongated with the monosaccharides galactose (Gal), N-acetyl-glucosamine (GlcNAc), fucose (Fuc), and sialic acid (NeuAc) with different linkage arrays (Figure 2). They resemble mucosal glycans at the host–microbe interface, and this molecular mimicry supports HMO to play important roles in orchestrating the host–microbial interactions via multiple mechanisms. These mechanisms include preventing pathogen growth and adhesion, reducing inflammatory responses and aiding the mucosal barrier function, as well as promoting an early life microbiome dominated by bifidobacteria. These data taken together demonstrate that HMO promote a gut ecosystem that is unfavourable for invading pathogens, known as colonisation resistance, and influence appropriate education of the developing immune system.

Proposed roles of HMO to protect against infections were explored as part of the secondary

objectives in a randomised, placebo-controlled intervention trial by comparing infants fed a control formula and a formula supplemented with two HMO: 2'fucosyllactose (2'FL) and lacto-N-neotetraose (LNnT).[12] The group of infants fed with the formula supplemented with HMO had a significantly lower risk to experience at least one reported respiratory tract infection and to require antibiotics during the first year of life.

Further investigations conducted by Berger et al.[13,14] compared microbiota compositions at 3 months of age across two randomised formula-fed groups (formula supplemented with HMO and formula without HMO) and a breastfed reference group. The faecal microbiota composition could be categorised into three distinct faecal community types: A, B, or C. In the group that was fed formula supplemented with HMO and the breastfed reference group there was a higher percentage of infants with faecal community type B and a lower percentage of infants with faecal community type C, as compared to the group fed the control formula. Notably, formula fed infants with faecal community type C, typical of the control formula fed infants, had a hazard ratio of 2 (95% confidence interval [CI]: 1.1–3.9; p=0.02) to require antibiotics during the first year of life, as compared to formula-fed infants with faecal community type B that was typical for breastfed infants. Berger and Sprenger[15] further segmented changes in the faecal microbiota composition of infants based on vaginal as compared to caesarean birth within each of the formula groups and the breastfed reference group. These results showed that feeding a formula supplemented with HMO reduced the risk for reported lower respiratory tract infections primarily in the caesarean section-born infants, together with positive changes in microbiota composition such as a strong increase in *Bifidobacterium* abundance.

## Human Milk Oligosaccharides in the Dietary Management of Cow's Milk Protein Allergy

Professor Anna Nowak-Wegrzyn

HMO come in different lengths and many act as nutrients for non-pathogenic bacteria such as *Bifidobacterium infantis*. Shorter chain HMO are almost exclusively consumed by these bacteria and are metabolised to produce SCFA. HMO have also been shown to restrict potential pathogens by providing decoy lectin-binding sites which mimic similar structures found in host epithelia.[16]

HMO are a complex mixture of bioactive components supporting the immune development of breastfed infants. Dendritic cells (DC) play a central role in the regulation of immune responses, being specialised in antigen presentation and driving T cell priming as well as differentiation. Xiao et al.[17] reported on their elucidation of the effect HMO have on the maturation of the immune systems. They showed that a HMO mixture, isolated from pooled human milk, consistently induced semi-maturation of human monocyte-derived DC (moDC). HMO-conditioned human moDC promoted Treg generation from native CD4+ T cells. HMO contain tolerogenic factors that influence human moDC, and thereby modulate the development of the neonatal immune system.

Sprenger et al.[18] studied HMO composition and genetic variation between women. HMO fucosylation is mediated by two fucosyl transferases: FUT2 and FUT3. Non-secretor mothers, who lack the functional FUT2 enzyme, also lack most α1-2-fucosylated oligosaccharides such as 2'FL and lacto-N-fucopentaose (LNFP). Infants fed by non-secretor mothers show a delay in establishing a bifidobacteria-laden microbiome. To compare the HMO composition in breast milk received by infants who develop CMPA with that in infants without CMPA, Seppo et al.[19] classified infants into types of CMPA. They showed that all mothers of infants with delayed-onset CMPA were secretors (active FUT2, milk containing 29FL and LNFP I), while those with an infant with immediate-type (IgE-mediated) CMPA did not have active FUT2. Regardless of CMPA type, after correction for multiple comparisons, the level of LNFP III remained significantly lower in mothers with an infant with CMPA (29 mM versus 57 mM; 95% CI: 11–43; adjusted, p=0.0069). Infants who received low (<60 mM) LNFP III-containing milk were more likely to become affected with CMPA when compared with infants who received high LNFP III-containing milk (odds ratio: 6.7; 95% CI: 2.0–22.0).

There have been a series of studies carried out to evaluate the impact of adding HMO to infant

formula. To establish whether infant formula with HMO is well tolerated by healthy infants, Storm et al.[20] conducted a 6-week, randomised, controlled study of partially hydrolysed whey-based infant formula with 2'FL and *Bifidobacterium lactis* in healthy infants from 2 weeks of age. The control group received the same formula without HMO. Based on a gastrointestinal symptom questionnaire, outcomes were no different between groups. As already reported, the benefits of adding HMO to infant formula were studied by Puccio et al.,[12] who found that at the end of the study the formula +HMO (2'FL and LNnT) group showed lower risk from infectious diseases and related medication use up to 12 months of age. A recent multicentre, randomised trial has been conducted to test hypoallergenicity of whey-based extensively hydrolysed formula (EHF) containing 2'FL and LNnT in IgE-mediated CMPA. Full term infants aged 2 months to 4 years, not breastfed, and with physician-confirmed IgE-mediated CMPA, were enrolled. The test formula group received the whey-based EHF Althéra (Nestlé Health Science, Vevey, Switzerland) with reduced content of extensively hydrolysed whey protein (2.2 g/100 kcal) with added 2'FL and LNnT, while the control group received Althéra with extensively hydrolysed whey protein (2.5 g/100 kcal) without added HMO. The criterion for formula hypoallergenicity is defined by the American Academy of Pediatrics (AAP): at a minimum, it must be confirmed that 90% of infants with documented CMPA will not react with defined symptoms to the formula under double-blind, placebo-controlled conditions. Results showed that >90% of subjects tolerated the novel whey-based EHF with two HMO, confirming its hypoallergenicity.[21]

Finally, Prof Nowak-Wegrzyn proposed hypotheses that still need further study: whether EHF with HMO has the potential to accelerate tolerance development in infants with CMPA by modulating the gastrointestinal microbiome and directly influencing Treg development and function. Additionally, whether EHF with HMO might reduce the frequency of infections and related drug use in infants and children with CMPA requires further study.


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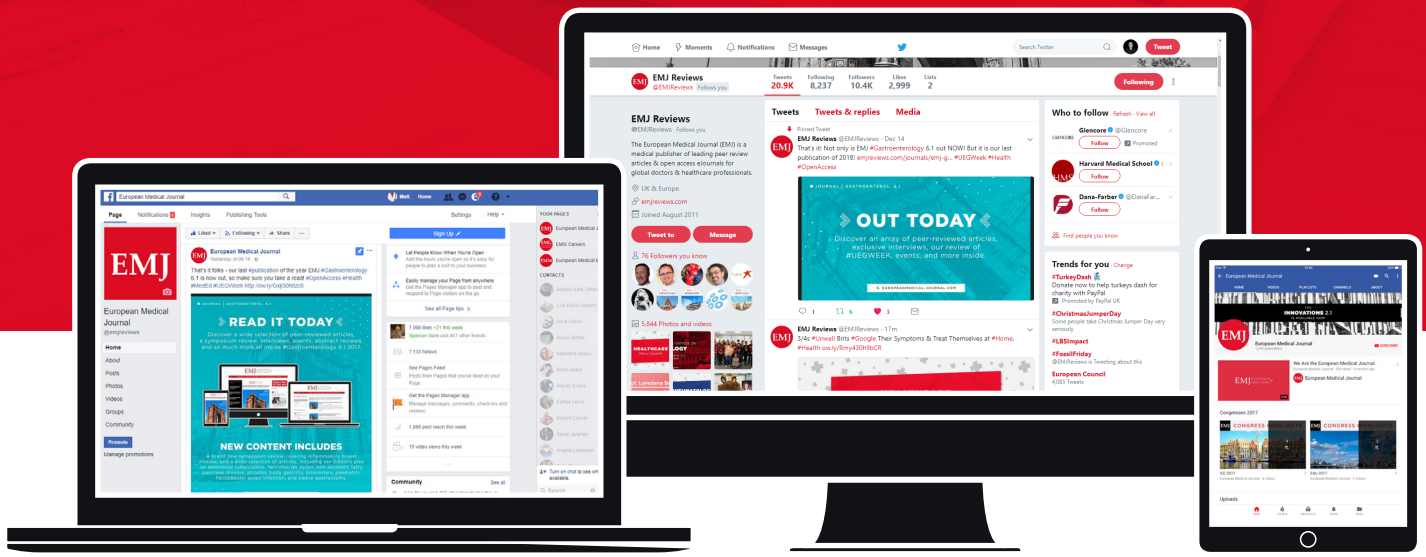


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# Abstract Reviews

Herein, we provide a collection of some of the top abstracts presented at this year's EAACI Congress, including a new application for allergic eye disease in children.

## *In Vitro* Silencing of Y-RNA: A Potential New Tool for Studying an Epigenetic Mechanism Recently Described in Allergy

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**Disclosure:** The authors have declared no conflicts of interest.

**Acknowledgements:** This work was supported by the Instituto de Salud Carlos III (ISCIII), cofounded by the European Regional Development Fund (Grant PI17/00818, PI: Ignacio Dávila), the Network for Cooperative Research in Health - RETICS ARADyAL (RD16/0006/0019, PI: Ignacio Dávila), Junta de Castilla y León Health Council (GRS1596/A/17, PI: María Isidoro), and Junta de Castilla y León Education Council (CAS086P17, PI: Ignacio Dávila).

**Keywords:** Allergy, Y-RNA, epigenetics, expression, *in vitro* study, Jurkat, regulation, sncRNA, Y-RNA.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:56-58. Abstract No AR1.

### BACKGROUND

Allergic diseases are becoming a public health concern, their frequency has notably increased in the last decades, which is likely related to changes in lifestyle and environment. In recent years, the epigenetic regulation has emerged as a pivotal group of mechanisms that can help explain the allergic disease emergence and allows us to understand their molecular basis.[1]

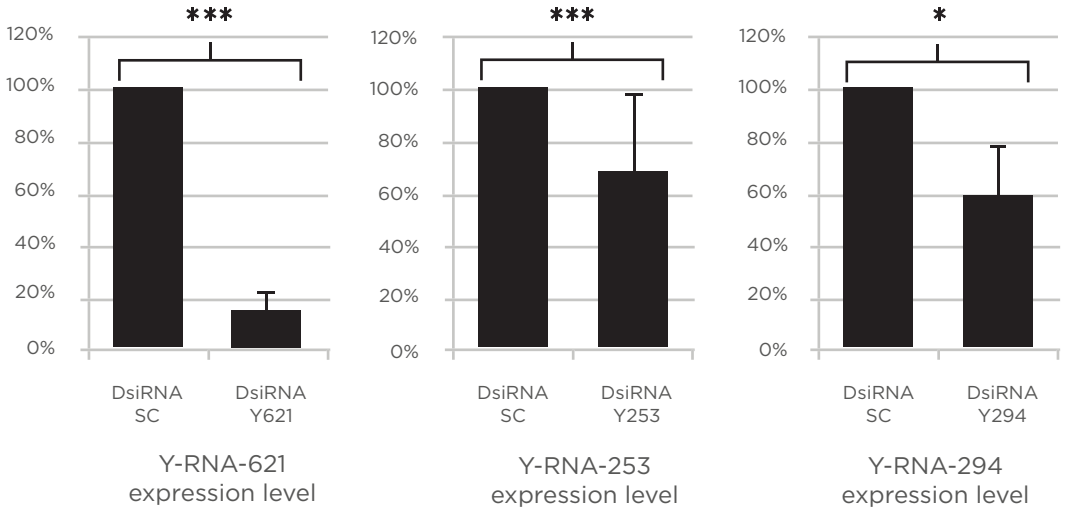


**Figure 1: Changes in Y-RNA levels after transfection with Dicer-substrate short interfering RNA.**

DsiRNA: Dicer-substrate short interfering RNA (***p<0.001; *p<0.05).

Some of these mechanisms of epigenetic regulation are conducted by small non-coding RNAs (sncRNA). Small cytoplasmic RNA, or Y-RNA, are a group of sncRNA of approximately 100 bp in length and are highly conserved from the evolutionary point of view and involved in the initiation of DNA replication and RNA stability that regulate gene expression.[2,3] Besides their cellular functions, their presence in the extracellular milieu, as part of ribonucleoprotein complexes or associated to extracellular vesicles, underscores their potential role in the modulation or amplification of different responses whether at local or at systemic level.[4] Thus, Y-RNA have been previously related to autoimmunity[5] and cancer,[6] and now to allergy. In this sense, our group has recently found differential Y-RNA expression profiles in allergic patients. In particular, the authors have shown a significant increase of certain Y-RNA in pollen allergic patients.[7]

The proper study of epigenetic regulation requires the implementation of the new methods for its research. To increase the repertoire of laboratory approaches to the disease, cell line studies need to be improved with new sophisticated techniques that mimic pathological states to deepen their molecular mechanisms. To unravel the function of these small RNA in allergy, we have developed a strategy for transiently silencing Y-RNA in cell culture. This will allow us to study the physiological effects behind different expression levels of the Y-RNA of interest in this particular cell line, as well as how it is related to signal transduction mediated by extracellular media.

## METHOD

Transient silencing of different Y-RNAs was performed in the cell line Jurkat, used as a model of T lymphocyte. Dicer-substrate short interfering RNA (DsiRNA) were used; these are 27mer duplex RNA that demonstrate increased potency in RNA interference compared to traditional, 21mer short interfering RNA[8] (provided by Integrated DNA Technologies, Inc., USA) designed against the Y-RNA of interest.

Cells were plated at a density of 2.5 x$10^5$ cells/mL and transfected with DsiRNA designed for each Y-RNA or the scrambled control at a final concentration of 50 nmol/L. Briefly, DsiRNA were diluted in of Opti-MEM® (Thermo Fisher Scientific, USA) and mixed with Lipofectamine® RNAiMAX (Thermo Fisher Scientific, USA) diluted in Opti-MEM®. The complexes of DsiRNA-Lipofectamine® RNAiMAX were added directly to the cells and mixed gently. Complexes did not have to be removed following transfection. Cells were incubated at 37 °C in a $CO_2$ incubator for 3 days post transfection before assaying for silencing gene expression.

## RESULTS

With this method successful and significant silencing of the different Y-RNA was obtained. When compared to the scrambled transfected control, the levels of the specific transcript were reduced between 32% and 85% (Figure 1).

## CONCLUSION

These findings have led to the development of an easy and affordable method to silence sncRNA in the cell line Jurkat, providing new perspectives in this cell line to study molecular mechanisms related to allergic diseases and its epigenetic *in vitro* control.


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# eQUICK - An Application for Reporting Symptoms of Allergic Eye Disease in Paediatrics


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**Disclosure:** The authors have declared no conflicts of interest.

**Keywords:** Allergic eye, paediatric, symptom reporting, vernal.

**Citation:** EMJ Allergy and Immunol. 2019;4[1]:58-59. Abstract No AR2.


Vernal keratoconjunctivitis (VKC) is a relatively rare but severe allergic eye disorder mainly affecting 5–17-year-old males. It generally resolves with puberty, and seasonal variability can be noted. Flares and quiescent phases are unpredictable and can affect both eyes. If VKC is not adequately managed during the active phase, sight can be threatened. Timely intervention and frequent monitoring of VKC are crucial as the consequences of poor management and/or treatment can be devastating to the patients

involved.[1-4] VKC is rare, affecting 1 in 10,000 children each year.

A VKC quality of life questionnaire exists that was developed and validated by Italian researchers for use with older adolescents (the QUICK questionnaire).[5] This was translated into English language with a pictorial representation of VKC symptoms for use by young patients in collaboration with the original authors of QUICK. Young VKC patients and unaffected individuals (recruited from the hospital Youth Forum) were asked to review the translated questions and provide opinions on whether the questions were easy to understand, and if the pictures represented the questions asked adequately.

Alterations were made based on this feedback and an electronic application compatible with the Apple iOS and Android platforms was created (eQUICK) linked to the hospital's electronic patient administrative system. This was made available to patients on an invitation only basis to access either during consultations in the hospital or remotely. Young patients were able to use it with ease, enabling them to easily report symptoms first-hand to clinicians during routine appointments and whilst at home to report exacerbations or improvements in their condition.

The authors compared the symptoms recorded on eQUICK to three parameters routinely recorded in their clinic. These were:

- An ocular examination score adapted from the Shoji et al.[6] scoring system.
- A treatment score which the authors have developed as a bespoke system.
- A rhino-conjunctivitis quality of life score adapted from the Juniper Adolescent Rhino Conjunctivitis Quality of Life[7] (RCQoL) scores.

Using data from 11 patients, synchronous changes were found in both parameters (RCQoL and eQUICK) indicating correlation of 0.75. eQUICK was more sensitive in identifying symptom changes in children whose presenting symptoms are predominantly ocular. There was an inverse relationship between the treatment score and the eQUICK score, and a proportional relationship between ocular finding score and the eQuick score. These results have been previously presented in a poster at the European Academy of Allergy and Clinical Immunology (EAACI) congress in Lisbon, Portugal, 2019.[8]

# Probiotic Micro-organism *Lactobacillus Reuteri* Impact on the Prevalence of Allergic Asthma and Atopic Dermatitis in Obese Slovenian Children

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**Disclosure:** The author has declared no conflicts of interest.

**Keywords:** Asthma, atopic dermatitis, children, obesity, prevention, probiotics.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:59-61. Abstract Review No. AR3.

## BACKGROUND

There is evidence of a positive association between asthma and obesity in children.[1] Perinatal probiotic supplementation has been shown to be effective in the primary prevention of atopic dermatitis (AD), although the long-term effects of probiotics on AD and allergic asthma is less certain. Many reports suggest that certain probiotic strains also have potent

immunomodulatory activity in allergic asthma. However, the underlying mechanism of action is still unclear.[2,3] Recent evidence suggests that gut microbiota are involved in the control of body weight and inflammation, and thus play a role in the pathophysiology of obesity.[4] The association between the gut microbiota and obesity in children is not fully studied.

## AIM

The aim of this study was to evaluate the efficacy of probiotic micro-organism *Lactobacillus reuteri* DSM 17938 (LR) in the prevention of the development of asthma and AD in obese, 7-year-old Slovenian children.

## MATERIALS AND METHODS

This prospective study included 904 maturely born children. All enrolled children were exclusively breastfed for ≥4 months. After the fourth month the same dietary intake for the child was recommended to all parents. A total of 238 children were breastfed with the addition of LR from the age of 4 weeks for 16 weeks. Every child was followed up by the same paediatrician until they were 7 years old. At the age of 7 years they were divided into groups: group A comprised 712 children with BMI <95 percentile, and group B comprised 192 children with BMI >95 percentile for age and sex. According to the addition of LR, diet group B was divided into subgroups: Bx contained 107 children exclusively breastfed for 4–6 months, and By contained 85 children breastfed with addition of LR. The prevalence of doctor's diagnosed asthma and atopic dermatitis at the age of 7 years, asthma course, and episodes of wheezing (lasting up to 5 days) in the first 5 years of life were observed. Allergic aetiology of asthma and AD was confirmed with specific IgE testing and positive skin prick tests performed by a physician who was unaware of the child's group allocation. Statistical analysis was performed with a PC using chi-square analysis with Yates' correction. p-values <0.01 were considered significant.

## RESULTS

In the study group, 10.4% of children had positive history of parental allergy, 10.4% had asthma, and 8.9% had AD. There was no significant difference between groups in the percentage of children with positive history of parental allergy (p>0.50). The prevalence of asthma (A: 7.6%; B: 20.8%) and AD (A: 6.2%; B: 19.8%) were significantly higher in obese children (group B), (p<0.001). No significance between group difference in prevalence of wheezing was confirmed (A: 16.7%; B: 25%), (p>0.01). The authors observed significantly lower prevalence of wheezing (Bx: 32.7%; By: 15.3%) and asthma (Bx: 28.0%; By: 11.8%) in the subgroup By (p<0.01). The prevalence of AD in this subgroup also tended to be lower (Bx: 26.2; By: 10.6%), (p=0.018). There was also a lower frequency and shorter duration of asthma attacks observed in subgroup By. No significant difference between subgroups in percentage of hospitalised children was observed, but frequency of hospitalisation was significantly lower in subgroup By (Figure 1).

| | Subgroup Bx | Subgroup By | |
|---|---|---|---|
| Frequency of asthma (attacks/asthmatic child). | 5.6 | 3.4 | t=2.803, p<0.010 |
| Mean duration of asthma attack (days/attack). | 8.8 | 6.9 | t=3.830, p<0.001 |
| Hospital admission of asthmatic children (percentage). | 55.5 | 40.0 | $\chi 2$=0.31, p>0.500 |
| Frequency of hospital admissions (no/asthmatic child). | 1.3 | 0.4 | t=5.190, p=0.010 |

**Figure 1: Data about frequency and episode duration and percentage of hospitalisations in each subgroup.**

## CONCLUSIONS

The authors demonstrated that the addition of LR to early child's diet has a preventive effect on asthma and AD development in obese children. It also has a strong beneficial impact on the asthma course.


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# "People Don't Know How Severe Some Of Them Can Be.": An Exploration of Beliefs and Attitudes in Adolescents with Food Allergy


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**Disclosure:** PhD study funded by Midlands Asthma and Allergy Research Association (MAARA).

**Acknowledgements:** The authors would like to thank the Midlands Asthma and Allergy Research Association (MAARA) who funded this research and the adolescents and their families who gave their time to participate.

**Keywords:** Adolescents, beliefs, food allergy.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:61-62. Abstract Review Number: AR3.


## BACKGROUND

Adolescents with food allergy (FA) are in an age group that is associated with higher rates of allergic and fatal allergic reactions,[1] potentially due to increased risk-taking behaviour.[2] This study explored attitudes and beliefs of adolescents aged 11–16 years with FA to better understand this population.

## METHOD

The study had a qualitative design using semi-structured interviews. Semi-structured interviews were conducted with 20 UK adolescents with FA aged 11–16 years old via phone, face-to-face at their home or Aston University, or Skype; 18 were recruited through Leicester Royal Infirmary and 2 were recruited via social media. Interviews were transcribed verbatim and analysed with thematic analysis.[3]

## RESULTS

Four themes were drawn from the data: 1) Nut allergies are treated more seriously than others: "I think everyone would take it more seriously."; 2) Adrenaline auto injectors (AAI) experience needle anxiety: "I don't like talking about the needle, it just scares me."; 3) The severity of FA symptoms affects beliefs: "They're like annoying, don't get me wrong, but they're not like super life-threatening."; and 4) Recent diagnoses present challenges: "I would like to have been born with it and grown up with it because then I would have understood it more and I wouldn't be so worried."

In these themes, it was highlighted that nut allergies were believed to be the most serious allergen. Multiple types of nut were considered more difficult to deal with due to lack of labelling

specificity, while common ingredients such as milk and egg had more dietary challenges. Challenges of AAI use included apprehension towards administration and anxiety over the needle. Carriage was inconvenient, however participants who had previously used their AAI were more positive about administering. Tester pens were considered useful, but emotional and psychological components were not mentioned in training. Participants who had not used an AAI felt their allergies were less severe and that an AAI would never be needed. They downplayed the severity of their FA in comparison to others, but believed peers should be aware of their food allergies. Participants with a history of anaphylaxis felt that food allergy was not understood, and that later diagnosis was seen as more challenging. It was believed to be easier when growing up with a food allergy as opposed to being recently diagnosed, as management becomes part of life. Participants with adolescent-age diagnosis all had allergens to nuts and felt the impact of their allergy to be small as they already disliked nuts.

## CONCLUSION

Adolescents with FA showed varying beliefs dependent on age, gender, allergen allergic to, severity of FA, and age of diagnosis. Concerns around AAI and dismissal of allergen labels support previous research, and all participants felt further education was necessary. Peer support was seen as important and should be incorporated into the development of interventions designed to help adolescents manage their food allergy safely. Beliefs around the seriousness of different allergens and beliefs associated with the age of diagnosis warrant further investigation.


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# Patient–Physician Discordance in the Assessment of Adherence to Inhaled Medication


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**Disclosure:** Dr Jácome is a post-doc fellow (SFRH/BPD/115169/2016) funded by Fundação para a Ciência e Tecnologia (FCT), reimbursed by Fundo Social Europeu and by national funds of MCTES. The additional authors have declared no conflicts of interest.

**Support:** This work was funded by the ERDF (European Regional Development Fund) through the operations: POCI-01-0145-FEDER-029130, cofunded by the COMPETE2020 (Programa Operacional Competitividade e Internacionalização), Portugal 2020, and by Portuguese Funds through FCT (Fundação para a Ciência e a Tecnologia).

**Keywords:** Asthma, discordance, logistic models, medication adherence.

**Citation:** EMJ Allergol Immunol. 2019;4[1]:62-65. Abstract Review No. AR4.

Assessing adherence to inhaled medication remains a challenge in clinical practice. Self-reports, although subjective, are still considered one of the preferred methods as they are simple, cheap, and minimally intrusive.[1,2] However, subjective methods may generate patient–physician discordance and impair the identification of patients with poor adherence. In turn, this might influence patient satisfaction, and compromise shared decision-making and therapeutic adjustments.[3] Evidence is lacking on the degree and characteristics of discordance between patients and physicians in relation to the assessment of inhaled medication adherence, but this knowledge is essential to delineate effective strategies to maximise patient–physician agreement and improve clinical decisions. Therefore, this study was conducted to compare patient and physician assessments of inhaled medication adherence and to identify predictors of patient–physician discordance.

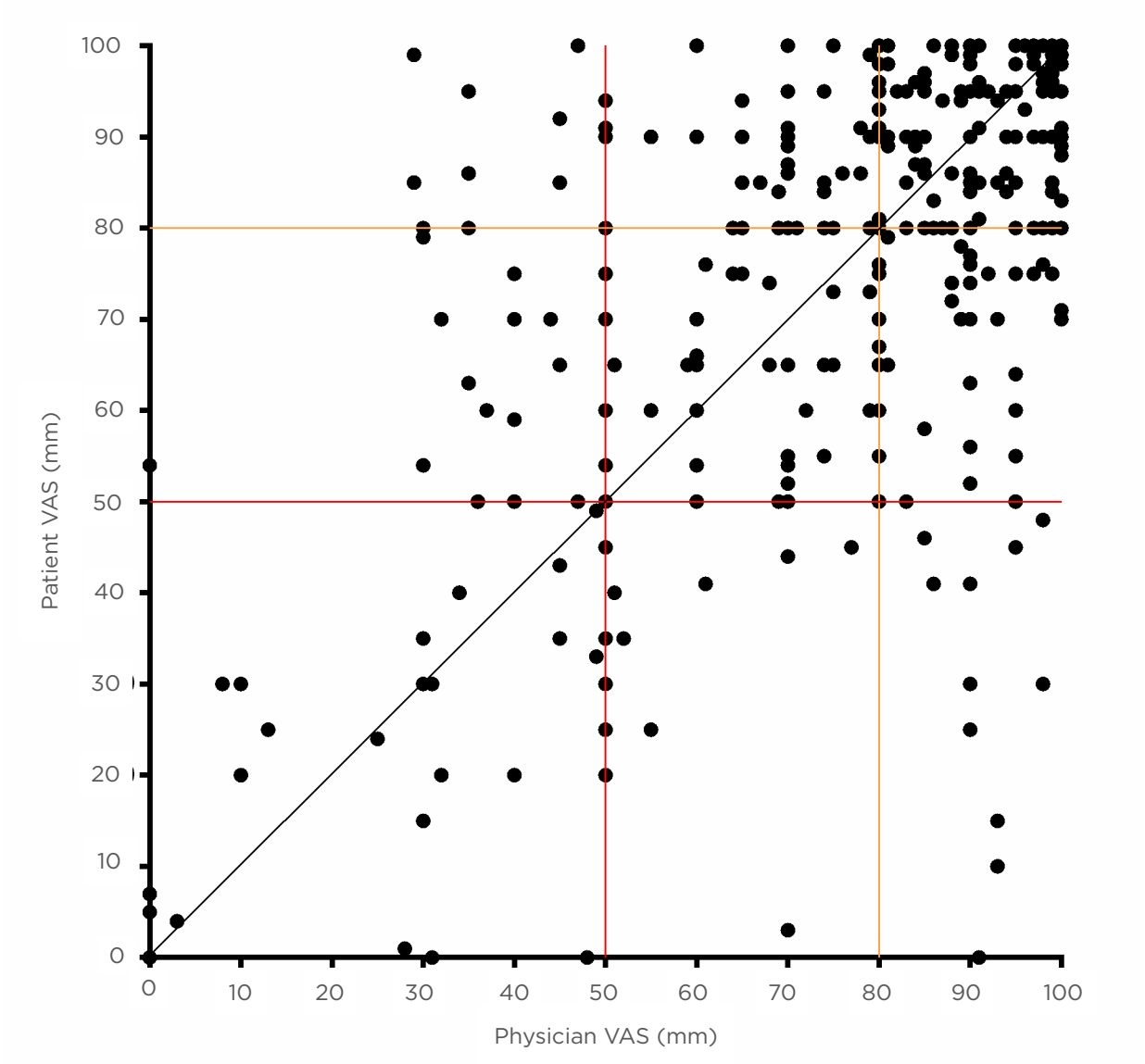


**Figure 1: Scatter plot showing the relationship between patient and physicians estimates of inhaler adherence (N=395).**

The black line represents perfect agreement; the red and orange lines represents the cut-offs of 50 and 80. In 40% of cases both patients and physicians classified adherence to inhaler treatments in the previous week as >80% in 15% of cases between 51–80%, and in 9% of cases <50%.

Adults and adolescents (≥13 years) with persistent asthma were recruited at 29 allergy, pulmonology, and paediatric secondary care outpatient clinics in Portugal, in the context of two observational prospective studies of the INSPIRERS project. This project addresses the topic of adherence to asthma inhalers among adolescents and adults with persistent asthma. Patients and physicians independently rated adherence to inhaled medication during the previous week, using a 100 mm visual analogue scale (VAS).[4] Demographic and anthropometric characteristics, patients' follow-up time, asthma characteristics and control according to Global Initiative for Asthma (GINA),[5] and details of current treatment were collected. VAS scores and categories (low: 0–50, medium: 51–80, and high: 81–100) were used in the analyses. The cut-offs of 50% and 80% are frequently used for differentiation of adherence groups.[6,7] Discordance was defined as VAS scores difference (VAS-d)≥10 mm or classification in distinct categories. Correlations with Spearman's rho ($r_s$) were used to explore the

association between patients' and physicians' VAS scores and Cohen's kappa to determine the agreement between categories. Multivariable logistic regression analysis was used to identify predictors of discordance (VAS-d≥10mm).

A total of 395 patients (61% female; 68% adults), with a median age (percentile 25 to percentile 75) of 28 years (16–46) years were analysed. According to the GINA classification, nearly half of participants had their asthma poorly controlled (n=184; 47%). Inhaler adherence was rated as high, both by patients (median: 85 mm [65–95 mm]) and physicians (median: 84 mm [68–95 mm]; p=0.707), with a median VAS-d of 10 mm (4–20). Correlation between patient and physician VAS scores was moderate ($r_s$=0.58; p<0.001) (Figure 1). Using VAS-d≥10 mm, patients and physicians disagreed in 53% of cases (n=211), with physicians overestimating adherence in 26% (n=102) of cases and underestimating it in 27% (n=109). Using VAS categories, disagreement occurred in 36% of cases (kappa 0.4; 95% confidence interval [CI]: 0.32–0.48), with physicians overestimating adherence in 17% (n=66) of cases and underestimating in 19% (n=76). Absence of asthma control (odds ratio [OR]: 3.05; 95% CI: 1.59–5.89) and short-acting β2-agonist prescription (OR: 2.69; 95% CI: 1.22–5.92) were associated with increased discordance, while having a written asthma action plan (OR: 0.38; 95% CI: 0.20–0.74) and hospital admissions in the past year (OR: 0.13; 95% CI: 0.03–0.52) were associated with reduced discordance. This model explained 20% of the variance in patient–physician discordance and correctly classified 63% of cases.

In conclusion, patients' and physicians' assessment of inhaler adherence were discordant in more than one third of cases and were only moderately correlated. These results highlight the shortcomings of global subjective measures of inhaler adherence. Implementation of objective adherence measures and effective communication are needed to improve patient–physician agreement. This study has also identified some predictors that can help to improve understanding of this discordance.


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# "I Look Disgusting": A Qualitative Enquiry into the Impact of Atopic Dermatitis on Quality of Life in Adults


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**Disclosure:** The authors have declared no conflicts of interest.

**Acknowledgements:** The authors would like to extend their appreciation to all participants who took the time to take part in the study.

**Keywords:** Atopic dermatitis, mental health, qualitative study, quality of life.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:65-66. Abstract Review No. AR5.

## BACKGROUND

Atopic dermatitis (AD) has been related to poorer health-related quality of life (HRQoL) in adults, but no qualitative research has been published to explore in depth how this long-term condition affects the lives of adults. Interviews with AD patients provide the opportunity to uncover issues that may not be addressed in quantitative research and allow a more in-depth exploration of the impact this condition has on patients' lives. The purpose of this study was to explore the impact of AD on HRQoL of adults.

## DESIGN

The study used a qualitative design using semi-structured interviews.

## METHODS

Adults with a clinical diagnosis of AD were recruited by advertising on a university campus and through social media sites. All participants completed a screening questionnaire on AD diagnosis, treatment, duration, and severity. Semi-structured interviews were conducted, audio-taped, transcribed verbatim, and analysed using thematic analysis.[1]

## RESULTS

Participants (N=19) consisted of 10 white and 9 black and ethnic minority participants, aged 19–52, 18 of whom were female.

Five super-ordinate themes emerged from the analysis. The first theme discussed the visibility of AD, which contributed to issues with body image and resulted in participants feeling stigmatised and judged; as a result, patients often attempted to cover their skin as much as possible. The second theme centred on threats to inner sense of self, which captured participants' accounts of the psychological impact of AD, such as impact on mood, clinical depression and anxiety, and issues relating to self-esteem and confidence. The third theme captured the threats to physical capacity due to pain and management. Within this theme, participants spoke about being unable to lead 'normal' lives due to the pain and itch of the condition. These physical symptoms affected their productivity at work, sleep, engagement in physical activity, social life, and, for many, affected them financially due to the cost of managing their condition. The fourth theme focussed on participants developing confidence in management of AD and used different methods of coping to improve or manage their daily QoL. Some used active strategies, such as avoiding triggers and having strict maintenance regimens, whereas others used distraction methods to cope with their condition. The fifth theme captured the contrasting reactions and support from others where most participants felt strongly that their general practitioner (GP) and primary care doctors did not understand the psychological impact that AD was having on them. There was a general idea amongst participants that their GP was not equipped to address and treat mental health issues that arose as a result of AD. There were qualitative differences in the narratives of those who were diagnosed with AD at an early age compared to a later age, and across ethnic groups.

## CONCLUSIONS

AD has a great impact on the QoL of adults. Participants in this study discussed issues that have not been reported in quantitative research, particularly the lack of understanding of the psychological impact of AD, and the stigma attached to AD. Consideration of these factors may enhance disease management and improve HRQoL. Understanding and recognition of AD as a complex long-term condition involving significant psychosocial impact is crucial. These findings suggest more integrated and accessible psychological support is required for people with AD. Men were under-represented in this study and further exploration of the impact on AD in men is needed.


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# Hereditary Alpha-Tryptasaemia Due to *TPSAB1* Gene Duplication is Associated with Multifocal Sclerotic Bone Disease

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**Disclosure:** The authors have declared no conflicts of interest.

**Keywords:** Hereditary alpha-tryptasaemia, KIT mutation, mast cell disorders, sclerotic bony lesions, TPSAB1 gene.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:67-69. Abstract Review No. AR6.

## INTRODUCTION

Hereditary alpha-tryptasaemia (HAT) affects 4–6% of the population and is caused by allelic replications tandem duplications of the alpha-tryptase encoding sequencing of the *TPSAB1* gene. It is typically associated with allergic-type skin and bowel symptoms, cardiovascular symptoms, and non-specific systemic symptoms.

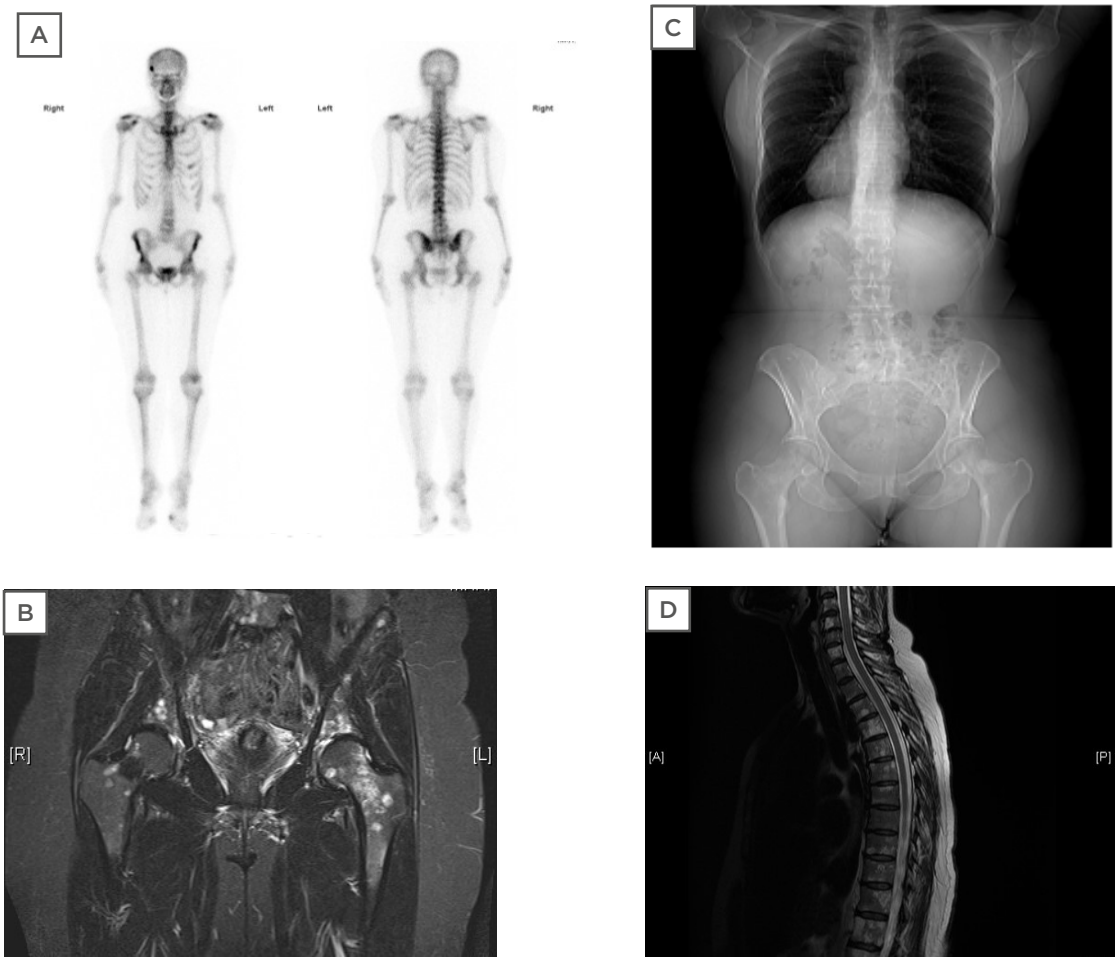


**Figure 1: A) TcMDP bone scan showing areas of increased activity; B) MRI-coronal section of the pelvis; C) CT scan showing sclerotic bones; D) MRI sagittal section of the spine showing multiple bony lesions.**

Generalised aches and pains are common, as is joint hypermobility; however, bone abnormalities have not been previously described. Bone abnormalities associated with HAT are described in this clinical case report.

## CASE DESCRIPTION

This 57-year-old female presented with numbness in her feet and intermittent bony pains at the age of 53. Her past medical history included: i) a previous reaction to oxytetracycline (skin rash), ii) a reaction to salmon (lip swelling and vomiting), and iii) a collapse episode, for which all investigations at the time were unremarkable. Investigations for the numbness in her feet and intermittent bony pains included pelvic MRI and nuclear medicine scans (Figure 1). These showed multiple sclerotic bony lesions in her pelvis, for which she was issued with zoledronic acid that resulted in a partial symptomatic response.

In addition to the bony lesions, she had experienced multiple episodes of urticarial rashes and flushes that responded to antihistamines, gastrointestinal symptoms in the form of indigestion, nausea, vomiting with no diarrhoea and which responded to regular ranitidine, and a few episodes of palpitations; however, haemodynamically she was stable and no treatment had been required for this so far.

Due to the widespread appearance of sclerotic lesions in the bone, she had undergone thorough assessment by the Manchester University NHS Foundation Haematology team, including bone marrow trephine biopsies twice which excluded malignancy. These however showed an increased number of non-spindle-shaped CD2-mast cell aggregates in the patient that accounted for approximately 15% of the total cellularity. Her baseline mast cell tryptase (MCT) was between 17.0 and 19.8 ug/L. C-KIT mutation screening was negative. Due to persistently raised serum MCT concentration, she was assessed for the recently described increase in copy number of the *TPSAB1* gene encoding alpha-tryptase, which is associated with an increase in basal serum tryptase levels in patients presenting with multisystem complaints.[1] A tandem duplication of the alpha-tryptase encoding sequencing of the *TPSAB1* duplication gene was identified in this patient by droplet digital PCR, performed at the Manchester Centre for Genomic Medicine, Manchester, UK.

## DISCUSSION

Lyons et al.[1] have described two large patient cohorts with elevated basal serum tryptase levels that were exclusively associated with increased copy numbers of the alpha-tryptase-encoding sequence of *TPSAB1,* caused by tandem duplications within the gene. Affected individuals reported multiple symptom complexes such as irritable bowel syndrome, cutaneous manifestations, connective tissue abnormalities, and dysautonomia.

Recently, there have been more patient cohorts with similar clinical phenotypes described and found to be associated with elevated basal MCT levels.[2,3]

Affected individuals had described various symptoms, including those often described as 'functional' symptoms, i.e., when all investigations have not revealed a pathological cause for their symptoms. The most common symptoms were:

- Gastrointestinal complaints, particularly irritable bowel syndrome and chronic gastroesophageal reflux.[3-5]
- Connective tissue disorders, congenital skeletal abnormalities, and retained primary dentition.[6] Interestingly, the patient presented with multiple sclerotic bony lesions and bone aches rather than the above described skeletal abnormalities.
- Autonomic dysfunction, including postural orthostatic tachycardia syndrome.[1]
- Recurrent cutaneous flushing and pruritus, which in some cases associated with urticaria and was concomitant with complaints of sleep disruption.[1]
- Systemic reaction to stinging insects such as Hymenoptera[7,8] was increased by 2–3-fold compared to the frequency of such reactions in the general population.[8]

## CONCLUSIONS

This case highlights that bony abnormalities may be associated with HAT. Further studies are required to substantiate this potential association.

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# IgE Signalling Repression by a Designer DNA Vaccine to Modulate Shrimp Hypersensitivity


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**Disclosure:** The authors have declared no conflicts of interest.

**Acknowledgements:** This work was fully supported by the Health and Medical Research Fund (02130206), Hong Kong. Dr Wai is supported by the AXA Postdoctoral Fellowships.

**Keywords:** Allergen-specific immunotherapy, DNA vaccine, hypoallergen, RNA-sequencing, shellfish allergy.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:69-71. Abstract Review No. AR7.


Shellfish is one of the most common food allergens worldwide, causing frequent anaphylaxis in both paediatric and adult populations.[1] Despite the identification of tropomyosin as the major cross-reactive shellfish allergen for over two decades,[2] strict avoidance and epinephrine injection remain the predominate preventive and therapeutic recommendations. To provide an allergen-specific immunotherapeutic option, the authors constructed a hypoallergen of shrimp tropomyosin, namely MEM49, by site-directed mutagenesis within the major IgE-binding epitopes.[3] The prophylactic and therapeutic values of a MEM49-encoding DNA vaccine (pMEM49) constructed with the mammalian expression vector pCI-Neo were further demonstrated using a well-established BALB/c mouse model of shrimp tropomyosin-induced hypersensitivity.[4-6] Specifically, pMEM49 immunisation resulted in the down-modulation of allergen-specific IgE synthesis, intestinal expression of Th2 cytokines, and infiltration of inflammatory effector cells accompanied with increased regulatory T cell frequency.

One of the most critical outcomes of pMEM49 vaccination is the suppression of tropomyosin-specific IgE synthesis. The authors hypothesise that such suppression can be achieved by restricting Th2 cell signalling via the induction of regulatory immune cells. In an attempt to explore the potential pathways leading to the induction of regulatory cells by pMEM49 immunisation, RNA-sequencing was performed using the Ion Torrent Proton Platform (BGI, Shenzhen, China) with ileum samples from the negative control, positive control (PBS), prophylactic (P-MEM49), and therapeutic (T-MEM49) groups

(Gene Expression Omnibus accession number: GSE86840). Differential gene expression profiles compared between each pair of animal groups were correlated to the immunological signatures available on ImmuneSigDB, PaGenBase, and the authors' in-house generated database by gene set enrichment analysis (GSEA),[7] to identify candidate pathways and key genes involved in the immuno-regulation by pMEM49.

The authors first identified significant enrichment of genes associated with the induction of tolerogenic dendritic cells (tDC), with gene signatures of P-MEM49 and T-MEM49 consistent with the RNA profile of tDC activated by vitamin D3. This includes heightened metabolic processes such as glycolysis and oxidative phosphorylation through stimulating the PI3K/AKT/mTOR pathway (Figure 1). pMEM49 also induced overexpression of *IDO1*, which was shown to maintain oral tolerance by supporting the TGF-β synthesis[8] that was also upregulated in the pMEM49-immunised animals. The authors thus speculate that this specific pathway is important to the inhibition of Th2 responses.

In addition to gene sets related to tDC induction, there was also significant enrichment of gene sets concordant to regulatory T cell activities, such as upregulation of *IL27*, *IL27R*, *CD39*, *CD73*, and *CTLA-4*. In addition, *IL21* also represented as a core enriched gene, which is well-illustrated as a key cytokine for the repression of IgE synthesis. This is achieved by inhibiting germ line transcription, partly through the diminishing of *PAX5* expression.[9] In fact, *PAX5* downregulation was validated in both P-MEM49 and T-MEM49 RNA samples. Notably, tDC-produced IL-27 is a potent transactivator of IL-21 production through induction of *c-Maf* and *ICOS* expression.[10] More strikingly, these key genes involved in this IL27-IL21 axis were all upregulated in our P-MEM49 and T-MEM49 samples compared to the control groups.

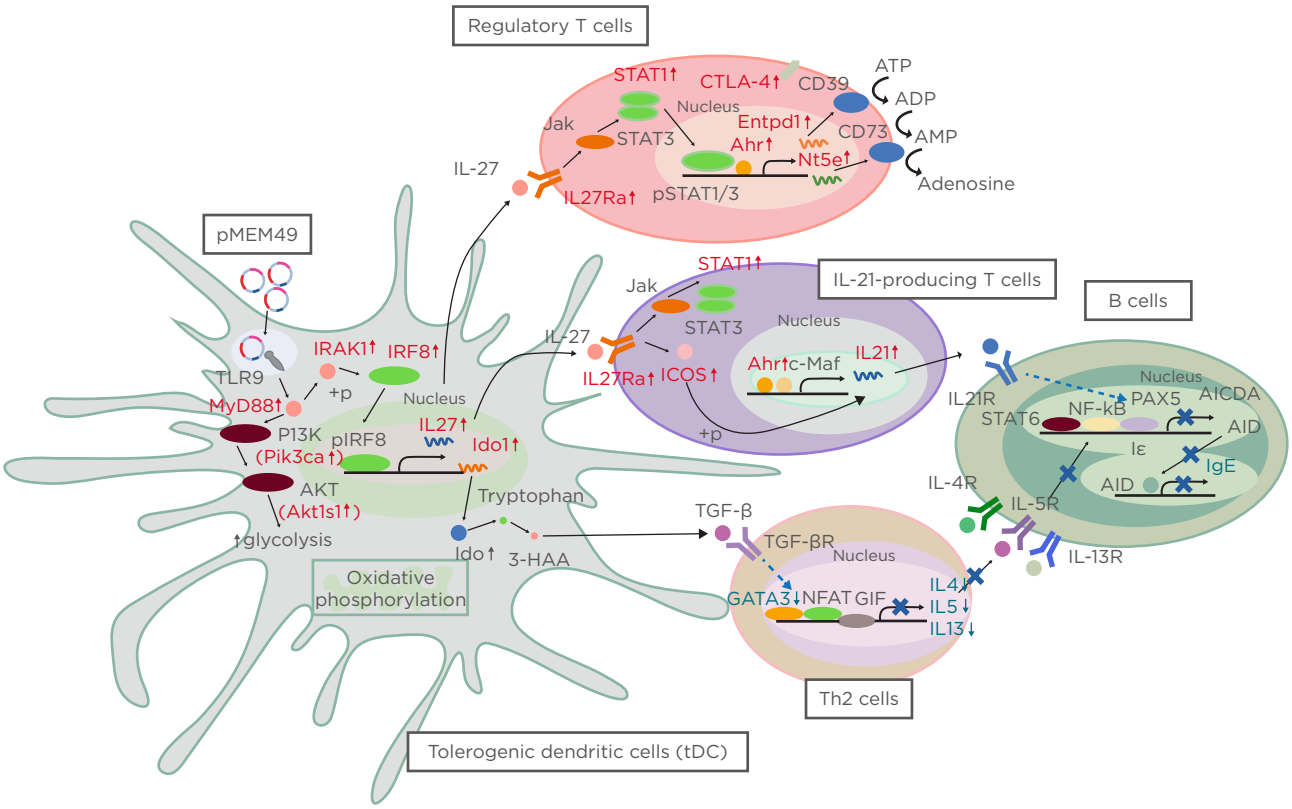


**Figure 1: Proposed model for the mechanistic action of pMEM49 immunisation in inducing tDC and Treg cells that repress IgE synthesis in B cells.**

tDC: tolerogenic dendritic cell; Th: T helper cell; Treg: regulatory T cell.

In summary, these data suggest the potential of pMEM49 in activating tDC that synthesise IL-27 to orchestrate the generation of IL-21-producing T cells. Together with the action of TGF-β, which limits the Th2 response, IgE synthesis is restricted via a *PAX5*-dependent pathway. Although the present study is limited by the lack of cell-specific analysis, the data provided herein sheds light on candidate molecules and immunological pathways involved in this platform of DNA vaccine-based modality.


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# Understanding the Anaphylaxis Campaign

## An Interview with CEO Lynne Regent

**Q1 The Anaphylaxis Campaign has been operating for 25 years now. What do you believe has been the biggest change in the way the organisation operates, and does this reflect changes in the general public's perception of allergy and anaphylaxis?**

The biggest change over the last 25 years for the Anaphylaxis Campaign would be the growth in digital media. Our website (www.anaphylaxis.org.uk) enables the allergic community to access up-to-date and research-based information about their allergies. Digital media has certainly allowed us and our supporters to positively increase awareness of the potentially fatal consequences for individuals living with severe allergies. Social media is a prime example, playing a huge role in enabling the allergic community the ability to communicate with us and others in the allergic population, providing them with immediate responses to questions and advice.

*"Digital media has certainly allowed us and our supporters to positively increase awareness of the potentially fatal consequences for individuals living with severe allergies."*

**Q2 The charity's main focusses are medical facts, food labelling, risk reduction, and allergen management: a diverse collection of priorities. How does the organisation identify, and subsequently act**

**on, specific areas within these focusses that you believe require attention?**

As a membership organisation, we are in direct contact with individuals with a variety of allergy types, including food, drug, venom, and latex, and additionally interact with their families every day. Food businesses and healthcare professionals can also become members, giving them direct access to our services. The queries made via our national helpline also ensure that we are aware of what issues are impacting the daily lives of the allergic community.

## Q3 Are there any specific areas of nationwide allergy and anaphylaxis management where you feel funding is lacking or where increased awareness is needed?

While public awareness and understanding of allergies has grown, there is still a misconception that only peanut allergy can be serious and fatal. This lack of understanding is not only irritating for non-food allergic individuals, but potentially dangerous for food allergic individuals who may not be taken as seriously by, for example, restaurant staff. The general public that are not affected by allergies or do not know anyone with an allergy may not realise how serious some allergies can be, and that the number of allergens are on the increase.

*"While public awareness and understanding of allergies has grown, there is still a misconception that only peanut allergy can be serious and fatal."*

The community pharmacist plays a vital role in ensuring that their patients know how to use their adrenaline auto-injector each time it is dispensed, and also to ensure the patient knows how to store and check the expiry date. We have a specific online training course for community pharmacists to support them for this purpose (www.allergywise.org.uk).

## Q4 How important to your mission is the UK-wide network of volunteer-led peer support groups that work with the charity?

We are a small, non-government funded charity, so our volunteers allow us to support allergic individuals and their families all across the UK. These volunteers are vital to the support we can offer and help raise awareness and understanding of severe allergies and anaphylaxis to the public.

## Q5 It appears that the education system, particularly in schools, provides the perfect platform for increasing awareness and providing support to allergic children. What is the long-term goal of targeting this younger demographic?

We hope that if school-aged children are educated about allergies, they will be able to support their allergic peers who may suffer from the associated psychological impacts. We hope they would be able to do this by being more understanding and to respond positively in the event of an emergency. This will also lead to the next generation growing up with a better understanding of the challenges faced by the allergic community.

To help educate this generation, we have resources available for children and young people at primary and secondary schools. This can be implemented as a whole school resource in an assembly-type platform, or alternatively across smaller groups in the classroom. Through our website we have a suite of online training resources. These training courses enable schools to ensure their staff are trained in the understanding of allergies and how to treat a child experiencing an anaphylactic reaction.

## Q6 The UK has a broad mix of different ethnicities, cultural beliefs, and socio-economical groups. Do you adapt the approaches you take to disseminating information based on the demographic that you are targeting, or is this perhaps more related to the age of the audience you are looking to engage?

We certainly adapt the information and support we provide based on the age of our audience: for example, we have a lot of resources targeted at

teenagers and young adults because they are a high-risk age group.

The local support groups target local communities, and therefore tailor their strategies to different populations.

Furthermore, most of our information and resources are free as we are keen for these to be widely accessible. Resources are available via our website and also our information office for those people who feel more comfortable either calling or emailing for further information.

*"We hope that if school-aged children are educated about allergies, they will be able to support their allergic peers who may suffer from the associated psychological impacts."*

Q7

**Raising awareness must be a key objective of any charity, but it is also hard to measure. What are some of the metrics that you can use to determine the overall impact of the charity?**

Fundraising is vital, but as well as this we actively monitor the engagement that visitors have on our website, our resources downloaded and requested, the number of registrations on our online training courses, our national helpline enquiries, and attendances of our local support groups.

Q8

**The restaurant sector has come under fire in the past for incidences of misinformation and negligence. Is raising awareness and providing support through charitable means enough to make a noticeable difference, or is fundamental regulatory change needed?**

We believe that regulation change is only as good as the enforcement. In the UK, trading standards and the environmental health teams who enforce the current legislation are underfunded.

We work directly with the Food Standards Agency (FSA), the Codex Alimentarius Commission, and the Environmental Health and Trading Standards to support implementation of food standard regulations. We are also key partners with government working parties that influence regulatory change.

Q9

**Is collaboration with other private or public bodies something that your charity is currently doing or actively seeking to do in the future to help you achieve your goals?**

Collaboration with both public and private bodies is central to much of our work, for example, working with the Civil Aviation Authority (CAA) to influence safe airline travel for the allergic community. We also work alongside many major food outlets to influence the application and management of the regulatory FSA standards. We also collaborate with other charities and societies to influence the rising cost of healthcare, for example, the Prescription Coalition.

Q10

**What are some of the major challenges facing your charity, and are there plans in place to tackle them?**

All charities face the challenge of future funding and the Anaphylaxis Campaign is no different, as we do not receive any government funding. Our main challenge is how to ensure we promote our work in an effective way to interested parties that might be willing to contribute to our services. Because of this, our marketing and fundraising teams are critical to our future.

*"We believe that regulation change is only as good as the enforcement. In the UK, trading standards and the environmental health teams who enforce the current legislation are underfunded."*

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# Well Known and Unknown Asthma Phenotype: Allergic Asthma

EDITOR'S PICK

I personally choose this article this year because, as we know, it is now very important to make a better treatment selection for our asthmatic patients, by going deeper to determine their own specificity related to many aspects. This way will help us to create some patient subtypes, whom could be more sensitive in responding to a particular class of drug compared to another.

It is exciting to realise that some new treatment options are now, or will be in the near future, available and this article gives us a good review of the different types of patients according to their potential, either non-pharmacological or pharmacological, responses.

Enjoy reading!

**Prof Dr Jacques Bouchard**

Université Laval, Canada, and CIUSSS Capitale-Nationale, Canada

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**Disclosure:** The authors have declared no conflicts of interest.

**Received:** 15.02.19

**Accepted:** 09.05.19

**Keywords:** Allergic asthma, asthma, asthma phenotypes, atopy.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:76-87.

## Abstract

Allergic asthma is the most common phenotype of asthma and presents with various clinical subtypes and clusters, emphasising the importance of personalised treatments in its management. The disease has an IgE-mediated inflammatory course that may be triggered by many agents, such as pollens and nonsteroidal anti-inflammatory drugs. The allergic asthma patients are relatively young, with early-onset asthma and frequent exacerbations. The primary goal of this literature review is to provide a deeper insight into different patient groups and allergic asthma phenotypes, as well as to discuss treatment options accordingly. Triggering factors and clinical presentation of patient groups are also covered in this study.

## INTRODUCTION

Asthma is a common disease in both children and adults, and it is estimated that there are >300 million people with asthma worldwide.[1] Due to the heterogenous origin of the disease, various asthma phenotypes have been identified which can be broadly classified as allergic and non-allergic. The World Asthma Phenotypes (WASP) study started in 2016 and includes five centres, one from each of the UK, New Zealand, Brazil, Ecuador, and Uganda. The findings of this study will be released soon and will help the scientific community to understand the characterisation and the distribution of asthma phenotypes in different geographic areas.[2] However, allergic asthma is still known to be the most common phenotype of asthma and its prevalence is increasing, while the prevalence of non-allergic asthma remains stable.[3]

Th2-related, IgE-mediated eosinophilic airway inflammation due to allergen exposure is the classical pathway of allergic asthma.[1] Atopy is common in patients with allergic asthma and the prevalence of both allergic rhinoconjunctivitis and atopic dermatitis are also high.[4] It is also seen more commonly in the young male population.[4] There are several atopic clusters identified under atopic asthma and several triggers, such as pollens and aspirin, which are related to distinguished forms of allergic asthma.[4] Asthma treatment is recommended to be made in accordance with the phenotypic characteristics of each patient. Therefore, these differences in allergic asthma phenotypes are important for proper management of the disease. The analysis of the phenotypes suggest personalised novel therapeutic strategies, such as omalizumab for allergic asthma management. These new treatments are shown to be beneficial in slowing down the allergic inflammation. However, there is no definitive treatment to halt the allergic inflammation in asthma. This review aims to provide analyses of the literature on allergic asthma phenotypes, mainly focussing on the adult population. The effect of biological therapies will also be discussed.

## CLINICAL ALLERGIC ASTHMA PHENOTYPES

In phenotyping studies, clustering of asthma phenotypes requires certain variables related to clinics, such as demographics, natural history, and airway inflammation. A recent study using data from ADEPT and U-BIOPRED cohorts identified four phenotypes.[5] Among these four groups, the Phenotype 2 was a 'moderate, hyper-responsive, eosinophilic' phenotype with moderate asthma control, mild airflow obstruction, and Th2 predominant airway inflammation. This group was highly atopic and mostly consisted of moderate persistent asthmatics with high hyperreactivity.[5] Cluster analysis of the COREA cohort identified four asthma phenotypes: Clusters 1, 2, 3, and 4, which are smoker's asthma, severe obstructive asthma, early-onset atopic asthma, and late-onset mild asthma, respectively.[6] Atopy rates in these clusters were 34.0% in Cluster 1, approximately half of the patients in Cluster 2, and about two thirds of the patients in Cluster 3. Mean forced expiratory volume in 1 second ($FEV_1$) was the lowest in Cluster 2 and mean age at onset was lowest in Cluster 3.[6] Haldar et al.[7] performed k-means cluster analysis in a mild-to-moderate asthma population managed in primary care and a severe asthma population managed in secondary care. Cluster analysis of both populations described a subgroup with early-onset atopic asthma. The subgroup was associated with a significantly greater number of previous hospital attendances, asthma exacerbations requiring oral corticosteroids, and eosinophilic airway obstruction. Siroux et al.[8] identified four asthma phenotypes from cluster analysis of two large European population-based epidemiological studies. Phenotypes defined as 'active treated allergic childhood-onset asthma' and 'inactive/mild untreated allergic asthma' were characterised with atopy, airway hyperreactivity, and active disease at the time of examination. Boudier et al.[9] investigated asthma phenotypes and the transitions across these phenotypes via cluster analysis of three big European 10-year follow-up cohorts. Two atopic phenotypes were identified in the study: the first cluster was composed of asthma patients with allergic sensitisation and no or few respiratory symptoms, while the second cluster

was composed of allergic, highly symptomatic asthma patients with high hyperreactivity. The probabilities of being in the same phenotype group at each time point varied from 54% to 88% across phenotypes. During the 10-year period, transitions between phenotypes occurred rarely. Results of all these studies show that allergic asthma patients are clinically young, have early-onset mild-to-moderate asthma with high bronchial hyperreactivity, airway eosinophilic inflammation, and frequent asthma exacerbations.

Recent studies have suggested that allergic asthma prevalence in patients aged >55 years might be more common than reported.[10] The study by Ozturk et al.[11] showed that the prevalence of atopy was 21% in an elderly population with asthma; these patients were most commonly sensitised to *Dermatophagoides pteronyssinus* (44.4%). In the study by Park et al.,[12] four clusters were identified in elderly patients enrolled from the COREA cohort. Atopy rate was higher in the elderly cohort compared to the primary younger cohort (31.8% versus 18.9%). Atopy rate among clusters was between 15.6% and 26.4%. The highest atopy rate was detected in Cluster 3, which included patients with high smoking rates and reduced lung function. Sano et al.[13] classified elderly asthmatic patients into three clusters: asthma-predominant, asthma-obstructive airway disease overlap, and asthma–emphysema overlap. The overall group atopy rate was 46% and the asthma–emphysema overlap group had the most frequent atopic status. Ozyigit Pur et al.[14] found that older patients with allergic asthma had worse asthma control and lower values for $FEV_1$ compared to younger ones. In the study by Lombardi et al.,[15] sensitisation to at least one allergen was observed in 52.4% of elderly asthma patients and their results were similar to those of Ozturk et al.,[11] who found that house dust mites were the most common allergens in elderly patients with asthma.

Phenotypic characteristics of severe asthma are usually defined as low lung function and high exacerbation rate with no atopy.[16] However, recent studies defined allergic phenotype in severely asthmatic patients.[17,18] In the study of Shaw et al.,[17] the European U-BIOPRED adult severe asthma cohort was classified into four groups; atopy rates were 78.3% in non-smokers with severe asthma, 71.3% in smokers and ex-smokers with severe asthma, 92.3% in non-smokers with mild-to-moderate asthma, and 46.2% in healthy controls who were non-smokers. Patients with severe asthma had more symptoms and exacerbations with lower lung function and higher eosinophil count, despite the treatment with higher doses of inhaled or oral corticosteroids, compared to patients with mild or moderate disease. In the study by Wu et al.,[18] six subject clusters were identified; four of these clusters had skin test reactivity to multiple allergens. Severe asthma patients were in Clusters 3 and 6. Patients in Cluster 3 had frequent symptoms, normal $FEV_1$ values, little inflammation, and a high degree of allergic sensitisation. Patients in Cluster 6 had early-onset asthma and were more symptomatic with the lowest lung function, frequent asthma attacks, and more sinusitis incidence. The cross-sectional analyses of the Belgian severe asthma registry showed that most severe asthmatics are female and atopic.[19] Eosinophilic asthma was the predominant phenotype and the most common comorbidities were rhinitis and chronic rhinosinusitis in the severe asthma group.

Borders of asthma phenotypes are drawn by clinical findings such as age at disease onset, severity of disease, and presence of comorbid conditions. However, present cluster studies are using the presence of atopy as the sole discriminating criterion of allergic asthma causing overlaps between allergic and non-allergic phenotypes. To elucidate the association between allergic phenotype and symptoms, severity, comorbidities, and response to therapy, allergic sensitisation characteristics, and allergenic triggers of each patient should be determined. To discuss the issue further, trigger-induced asthma phenotypes should be examined and, in the following section, specific trigger-induced asthma phenotypes, such as aspirin-exacerbated respiratory disease (AERD) and pollen asthma, will be discussed.

## TRIGGER-INDUCED ASTHMA PHENOTYPES

### Pollen Asthma

Pollen allergy has been found in 80–90% of childhood asthmatics and 40–50% of adult-onset asthmatics.[20] Thunderstorm asthma epidemics

that are triggered by grass pollen rupture in the atmosphere have been mentioned in the literature.[20] Pollen exposure causes an increase in airway inflammation during pollen season, especially in central airways that do not affect the degree of bronchial obstruction.[21] However, pollen exposure also affects small airways. Patients who were mono-sensitised to weed pollen were found to have different degrees of airway wall thickness and small airway obstruction.[22] Airway wall thickness is negatively correlated with airway obstruction and positively correlated with the duration of rhinitis.[22] It has been reported that asthma-related emergency department visits have been increasing due to high atmospheric pollen and mould levels during spring.[23] Although early asthmatic responses were similar, the study of Boulet et al.[24] showed that late asthmatic responses induced by house dust mites were stronger than those induced by pollens.

Allergic clinical symptoms and their severity may vary according to the type of allergen. Findings of the PERFILAR I and II studies indicated that clinical characteristics of asthma could be statistically different depending on the allergen type to which the patient is sensitised.[25] Seasonal allergens are found to be associated with a longer duration until the development of asthma. *Parietaria* is more important than *Olea* and *Gramineae* as a risk factor for developing nonspecific bronchial hyperresponsiveness.[26] In a recent study, cough was detected as a predominant symptom in adults with pollen-induced asthma.[27] Celikel et al.[28] analysed the records of 922 patients diagnosed with seasonal allergic rhinitis retrospectively to determine the risk factors for asthma in adults. In the study, the patients with seasonal allergic rhinitis were divided into four groups: no sensitisation, mono-pollen sensitisation, poly-pollen sensitisation, and mite sensitisation. When compared with the poly-pollen sensitisation group, no sensitisation and mite sensitisation groups had a higher risk of asthma, whereas the mono-pollen sensitisation group was unlikely to have any other allergic disease including asthma.

## Aspirin-Exacerbated Respiratory Disease

The prevalence of AERD in adult-onset asthma patients is 7.4%.[29] Its prevalence is twice as high in patients with severe asthma.[29] Aspirin intolerance can be detected in 20% of patients with adult-onset asthma without any sinus disease.[29,30] In this phenotype, patients have both nasal polyps and sinusitis with an allergic reaction involving upper and lower respiratory tracts after ingestion of aspirin or any other nonsteroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase-1.[30] Differing from the typical characteristics of allergic asthma phenotype, AERD is more common in females, rarely clusters in families, and develops in the third decade of life. However, two thirds of the patients with AERD have a history of atopy.[30] In a recent study, the atopy rate among the patients with AERD was detected to be 84%, which was higher than the atopy rate in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) and CRSwNP-asthma overlapping patients.[31] It was also reported that patients with both CRSwNP and asthma had significantly more severe sinonasal inflammation and were significantly more likely to have oral corticosteroid-dependent severe asthma.

Some AERD subclasses have been reported in the literature and this heterogeneity is similar to that of allergic asthma patients. Bochenek et al.[32] identified four classes within the AERD phenotype: Class 1 is asthma with a moderate course, intensive upper airway symptoms and blood eosinophilia; Class 2 is asthma with a mild course, relatively well controlled, and with low health care use; Class 3 is asthma with a severe course, poorly controlled, and with severe exacerbations and airway obstruction; and Class 4 is poorly controlled asthma with frequent and severe exacerbations in female subjects. In this study, atopy rate was 52.2%. However, atopic status did not affect distribution of patients among classes. Karakaya et al.[33] defined two phenotypes in aspirin intolerant patients. Asthma patients are found to be associated more with the presence of a nasal polyp and/or rhinosinusitis, smoking history, and food allergy or food intolerance. In another study of the same group, asthma in patients with NSAID hypersensitivity was associated with female sex, sinonasal polyposis/polyp surgery, rhinitis or rhinosinusitis, NSAID-induced rhinitis, asthma or a blended reaction pattern, immediate reaction following NSAID intake, self-reported history of food allergy, and family history of asthma.[34]

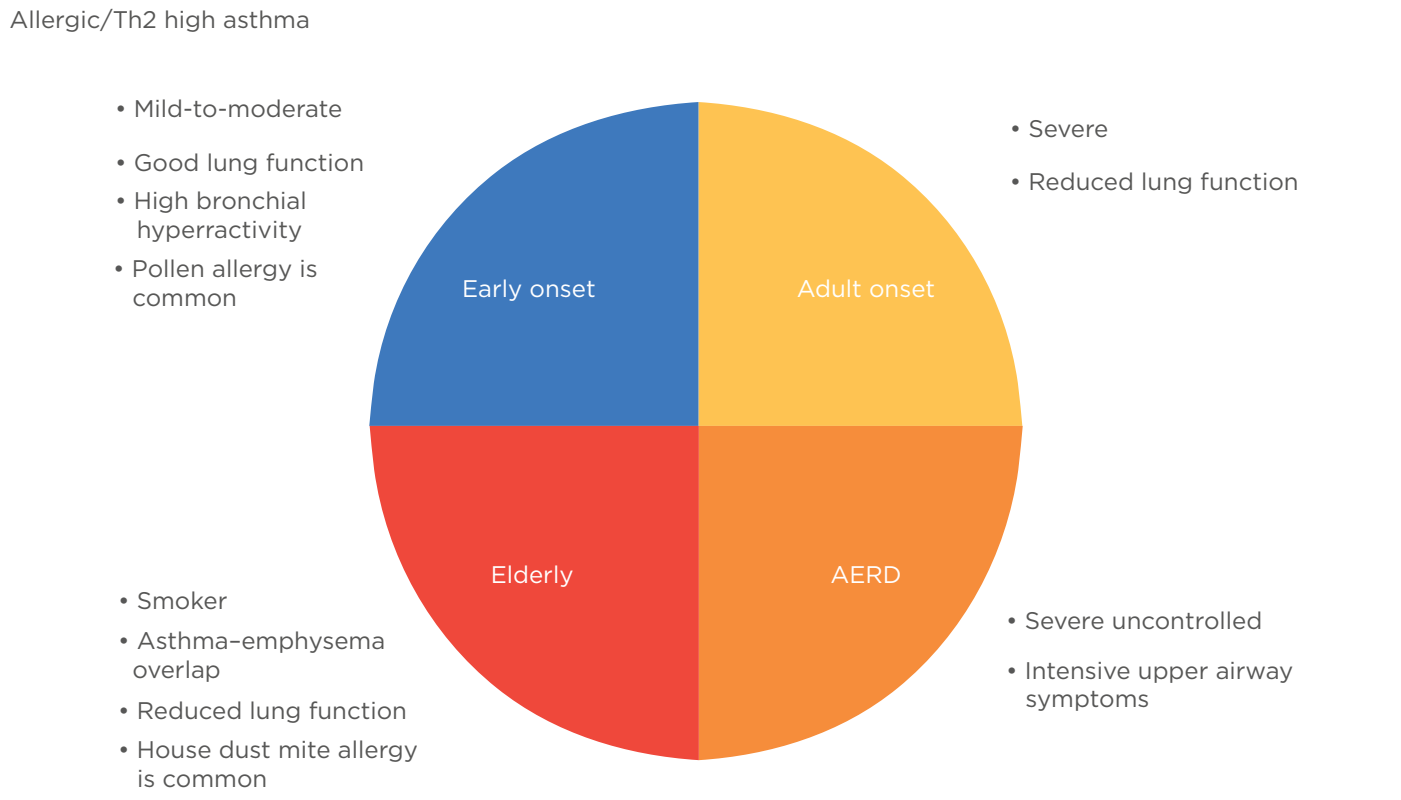


**Figure 1: Clinical characteristics of different asthma subgroups.**

AERD: aspirin-exacerbated respiratory disease; Th2: T-helper 2 cell.

However, atopy was not associated with asthma in patients with NSAID hypersensitivity. Clinical characteristics of different asthma subgroups are summarised in Figure 1.

## BIOLOGICAL THERAPIES IN ALLERGIC ASTHMA

Since allergic asthma is a Th2-related, IgE-mediated disease, therapies targeting type 2 cytokines may have an effect on allergic asthma patients. Following consistent positive efficacy results of clinical trials on biological treatments, therapies targeting IgE, IL-5, IL-13, IL-4 receptor A, and thymic stromal lymphopoietin (TSLP) are either approved now or will be approved soon for allergic asthma.[35] Revised Global Initiative for Asthma (GINA) 2019 guidelines recommend an add-on anti-IgE treatment for severe allergic asthma and anti-IL5, anti-IL-5R, or anti-IL-4R for severe eosinophilic asthma.[1] In the following section, biologicals, including omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab, will be discussed in terms of their effects on different allergic asthma phenotypes.

## OMALIZUMAB

Omalizumab is an anti-IgE monoclonal antibody that was approved by the US Food and Drug Administration (FDA) in 2003 and by the European Medicines Agency (EMA) in 2005 as an add-on treatment for patients aged >12 years of age with severe persistent allergic asthma.[36] It is the first biological treatment for allergic asthma that selectively binds to circulating IgE and suppresses asthma symptoms by preventing the binding of IgE to effector cells.[36]

Since 2003, consistent evidence on the effectiveness of omalizumab has been presented and now it is in use for patients aged >6 years old who have severe uncontrolled asthma. Its efficiency rate in severe allergic asthma is approximately 61%.[37] Within this phenotype, multi-sensitised allergic asthma patients with high baseline eosinophil levels before

the treatment seem to respond better to omalizumab therapy.[38] Allergic asthma patients who have high FeNO level, sputum, and nasal mucosa eosinophilia may also be positive responders to omalizumab.[39] However, STELLAIR study found the response rate to be similar in both subgroups of severe allergic asthma patients with high (≥300 cells/µL) and low (<300 cells/µL) eosinophil counts.[40] A recent study suggests that the patients with higher baseline serum IgE levels, shorter disease duration, and higher blood eosinophils may have a delayed benefit from omalizumab therapy and current non-responders can be evaluated again after between 16–32 weeks of treatment.[41]

Huang et al.[42] showed that the beneficial effect of omalizumab was high in severe allergic asthma patients who have IL-33, IL-25, and TSLP aggravated type 2-high endotype. Responsive patients were mostly females and non-smokers who had higher eosinophilic airway inflammation and FeNO levels, worse asthma control, and lower forced vital capacity when compared with the non-responsive ones. Clinical efficacy of omalizumab is also high in severe persistent allergic asthma patients aged ≥50 years.[43] A post hoc exploratory analysis of the PROSPERO study[44] demonstrated that the patients with asthma–chronic obstructive pulmonary disease overlap (ACO) and a smoking history had similar improvements in asthma control when compared to patients without ACO when treated with omalizumab over 48 weeks. The optimal duration of omalizumab treatment is unclear. Findings of a recent study show that therapeutic effects of omalizumab use for 6 years may persist after discontinuation of therapy in 60% of patients for at least 4 years.[45] However, after the reintroduction of omalizumab, some patients may not respond to treatment and 20% of the previous responders may fail to respond to the reintroduction of omalizumab.[46]

## Mepolizumab

Mepolizumab is a fully humanised monoclonal IgG1 antibody that binds IL-5 and prevents it from binding its receptor. It was approved by the FDA and EMA in 2015 as maintenance treatment for severe eosinophilic asthma in patients aged ≥12 years.[1] In 2007, the first large-scale, multicentre, double-blind, placebo-controlled trial using 250 or 750 mg intravenous (IV) mepolizumab at monthly intervals failed to demonstrate any positive clinical endpoint including morning peak expiratory flow, $FEV_1$, daily β2-agonist use, symptom scores, exacerbation rates, and quality of life measures in moderate persistent asthma.[47] In this study, 85% of the patients had allergic asthma that was confirmed by skin prick test positivity to animal dander, mite, or pollen. Subsequent studies including Dose Ranging Efficacy and Safety with Mepolizumab (DREAM)[48] and Mepolizumab as Adjunctive Therapy in Patients with Severe Asthma (MENSA)[49] investigated the efficacy of mepolizumab in eosinophilic asthma patients and determined exacerbation rate to be the primary outcome. With the change of inclusion criteria and primary endpoint, DREAM study demonstrated the positive effect of mepolizumab on the frequency of asthma exacerbations in patients with severe, exacerbation-prone, and eosinophilic asthma with a blood eosinophil count ≥300 cells/µL. Cluster analysis of the DREAM study showed that the reduction in exacerbations was significantly greater in patients who received mepolizumab with raised eosinophils and low airway reversibility.[50] The patients in these clusters were more atopic and had more comorbid sinusitis and nasal polyposis.

The MENSA study confirmed the positive efficacy of mepolizumab on the reduction of asthma exacerbation rates in severe eosinophilic asthma. Relevant reductions in exacerbation frequency in patients with a blood eosinophil count ≥150 cells/µL were also noted.[51] A significant glucocorticoid-sparing effect of mepolizumab in severe eosinophilic asthma was shown by the Steroid Reduction with Mepolizumab Study (SIRIUS).[52] Post hoc analyses of the MENSA and SIRIUS studies were performed to evaluate the effect of mepolizumab in patients with severe eosinophilic asthma previously treated with omalizumab.[53] In MENSA, mepolizumab reduced the rate of exacerbations by 57% (prior omalizumab) and 47% (no prior omalizumab) versus placebo. In the SIRIUS, reductions in oral corticosteroid use was found to be significant and independent of prior omalizumab use. These post hoc analyses indicate that patients with severe allergic asthma with elevated blood eosinophilia respond positively to mepolizumab regardless of prior use of omalizumab.

## Reslizumab

Reslizumab is a humanised anti-IL-5 IgG4 monoclonal antibody that was approved by the FDA and the EMA in 2016 as add-on maintenance treatment for severe asthma patients with an eosinophilic phenotype and aged ≥18 years.[54] The first large Phase IIb study[55] of reslizumab evaluated the clinical efficacy of reslizumab at 3 mg/kg and 4 weekly IV doses in patients who had refractory eosinophilic asthma. Enrolled patients had confirmed airway reactivity, induced eosinophil sputum counts of ≥3%, and were on a high-dose inhaled corticosteroid and a second controller. Patients receiving reslizumab showed significant reductions in sputum eosinophils, improvements in airway function, and a trend towards better asthma control than those receiving placebo. Improved asthma control was significant in patients with nasal polyps. However, comorbid aspirin sensitivity rate was very low (6%) among this subgroup.

Analysis of two key Phase III multicentre studies reported a significant, larger reduction in asthma exacerbations in late onset (≥40 years) asthma patients than early-onset disease after 52-week reslizumab therapy.[56] Compared to early-onset asthma patients, late-onset asthma patients were less atopic and had more nasal polyps. A single-blind, placebo-controlled sequential trial investigated the treatment response of weight-adjusted IV reslizumab in patients previously treated with 100 mg subcutaneous mepolizumab monthly for at least 1 year. Ten prednisone-dependent patients with asthma (five males; mean age: 50.9±7.6 years; mean BMI: 28.9±4.9) who presented with elevated blood and sputum eosinophilia (sputum eosinophils ≥3% and blood eosinophils ≥300 cells/µL) were included in the study. Interestingly, the authors found that the weight-adjusted IV reslizumab was superior to the fixed-dose subcutaneous mepolizumab in controlling asthma. Although the interpretation of this data is limited, study results suggest that biologicals targeting IL-5 may have different effects in different subgroups of severe eosinophilic asthma, such as prednisone-dependent asthma.

## Benralizumab

Benralizumab is a humanised IgG1 monoclonal antibody that binds to the IL-5 receptors, leading to the apoptosis of basophils and eosinophils. Following three Phase III trials, SIROCCO, CALIMA, and ZONDA, benralizumab was approved in the USA and Europe in 2017 for patients aged ≥12 years with severe asthma who have an eosinophilic phenotype.[57-59] The SIROCCO[57] and CALIMA[58] trials assessed the effect of benralizumab on asthma exacerbations over 48 and 56 weeks, respectively. Subjects were randomised to receive 30 mg of subcutaneous benralizumab every 4 weeks, or every 4 weeks for the first 3 doses then every 8 weeks, or placebo. When compared to placebo, both benralizumab dosages significantly lowered exacerbation rates and improved $FEV_1$ in patients with a baseline blood eosinophil count of at least 300 cells/µL. Efficacy and safety of benralizumab for eosinophilic asthma patients with low blood eosinophil counts (≥150 cells/µL) were also shown in a sub-analysis of SIROCCO and CALIMA studies.[60]

The subgroups analysis of SIROCCO and CALIMA was according to age (<18 years, 18–65 years, and ≥65 years), sex, BMI (≤35 kg/$m^2$ or >35 kg/$m^2$), baseline oral corticosteroid use, number of exacerbations in the previous year (two, three, or four), race, nasal polyposis, atopic status, and smoking history.[61] This showed that larger reductions in exacerbation rates and greater improvements in $FEV_1$ were associated with a history of more frequent exacerbations. It is also found that larger $FEV_1$ improvements were associated with oral corticosteroid use and history of nasal polyposis.[61] However, decreases in exacerbation rates were found to be similar in high or low IgE and atopic or nonatopic groups.[62] The third trial, ZONDA,[59] investigated steroid sparing effect of benralizumab and showed that 28-week benralizumab use decreased median final oral glucocorticoid dose by 75% compared to baseline dose.

Mepolizumab, reslizumab, and benralizumab seem to have similar effects on symptom control and exacerbation rate reduction in patients with severe eosinophilic asthma when they are used in correct doses.[63] However, GINA guidelines recommend assessing the response to biological therapy after 4 months and switching between different Th2 targeted therapies when little or no response is observed with a previous one.[1]

**Table 1: Eligibility criteria, suggested initial dose, route and trial, and common side effects of biological treatments for allergic asthma.**

| Biological treatment | Eligibility criteria | Suggested dose and route | Suggested initial trial | Side effects |
|---|---|---|---|---|
| Omalizumab | • ≥6 years of age<br>• Sensitisation to inhaled allergens on skin prick testing or specific IgE | SC, every 2–4 weeks, with dose based on weight and total IgE | At least 4 months | • Injection site reactions<br>• Anaphylaxis is rare |
| Mepolizumab | • ≥12 years of age<br>• Frequent severe asthma attack history<br>• Oral corticosteroid dependent asthma<br>• Blood eosinophil count ≥300 cells/µL | SC, 100 mg, every 4 weeks | At least 4 months | • Injection site reactions<br>• Anaphylaxis is rare |
| Reslizumab | • ≥12 years of age<br>• Frequent severe asthma attack history<br>• Oral corticosteroid dependent asthma<br>• Blood eosinophil count ≥300 cells/µL | SC, 30 mg every 4 weeks for 3 doses, then every 8 weeks | At least 4 months | • Injection site reactions<br>• Anaphylaxis is rare |
| Benralizumab | • ≥18 years of age<br>• Frequent severe asthma attack history<br>• Oral corticosteroid dependent asthma<br>• Blood eosinophil count ≥300 cells/µL | IV, 3 mg/kg every 4 weeks | At least 4 months | • Injection site reactions<br>• Anaphylaxis is rare |
| Dupilumab | • ≥12 years of age<br>• Frequent severe asthma attack history<br>• Oral corticosteroid dependent asthma<br>• Blood eosinophil count ≥300 cells/µL | SC, 200 mg or 300 mg every 2 weeks | At least 4 months | • Injection site reactions<br>• Transient blood eosinophilia |

*Adapted from the GINA 2019 Difficult to Treat and Severe Asthma Pocket Guide.*[68]

IgE: immunoglobulin E; IV: intravenous; SC: subcutaneous.

## Dupilumab

Dupilumab is a human IgG4 antibody against the IL-4 receptor α-subunit, blocking the signalling of IL-4 and IL-13.[64] In the USA, the FDA approved dupilumab for the treatment of moderate-to-severe atopic dermatitis in adults that is uncontrolled with topical medications in March 2017. Then, through the results of three randomised, double-blind, placebo-controlled trials with about 2,800 patients who were >12 years of age and presented with uncontrolled persistent asthma, dupilumab received further approval by the FDA. It was thereby allowed for use in an add-on maintenance therapy in patients with moderate-to-severe asthma aged ≥12 years with an eosinophilic phenotype or with an oral corticosteroid-dependent asthma in October 2018.[64-67] The first of these trials[65] enrolled 769 patients who were receiving treatment with medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist. It randomised them 1:1:1:1:1 to receive either 200 mg or 300 mg every 2 weeks or every 4 weeks, or placebo. Treatment with dupilumab for a period of 12 weeks and 24 weeks resulted in significant

increases in $FEV_1$ and decreases in severe asthma exacerbations compared to placebo. Percentage of patients with nasal polyposis were higher in the subgroup with eosinophil counts >300 cells/µL. However, dupilumab significantly improved lung function and reduced the rate of severe exacerbations independently from baseline eosinophil counts.

The second trial[66] enrolled 1,902 patients who were ≥12 years of age with uncontrolled asthma. The patients were randomised 2:2:1:1 ratio to receive add-on subcutaneous dupilumab at a dose of 200 or 300 mg every 2 weeks or matched-volume placebos for 52 weeks. At Week 12, increased $FEV_1$ levels and decreased severe asthma exacerbation rates were observed in the dupilumab receiving group. The mean age of the entire group was 47.9±15.3 years. Ongoing atopic or allergic condition rate was 80% and greater benefits were detected in patients with higher baseline levels of eosinophils.

In the third trial,[67] 210 patients with severe asthma were randomised to receive add-on treatment with dupilumab or placebo. The dosing was 300 mg or placebo every 2 weeks for 24 weeks. At Week 24, 48% of patients in dupilumab group had completely stopped using oral corticosteroids, compared to 25% of those in the placebo group. Dupilumab use also had positive effects on decreasing severe asthma exacerbations and improving lung function. Greater benefits were observed in patients with higher baseline blood eosinophil counts.

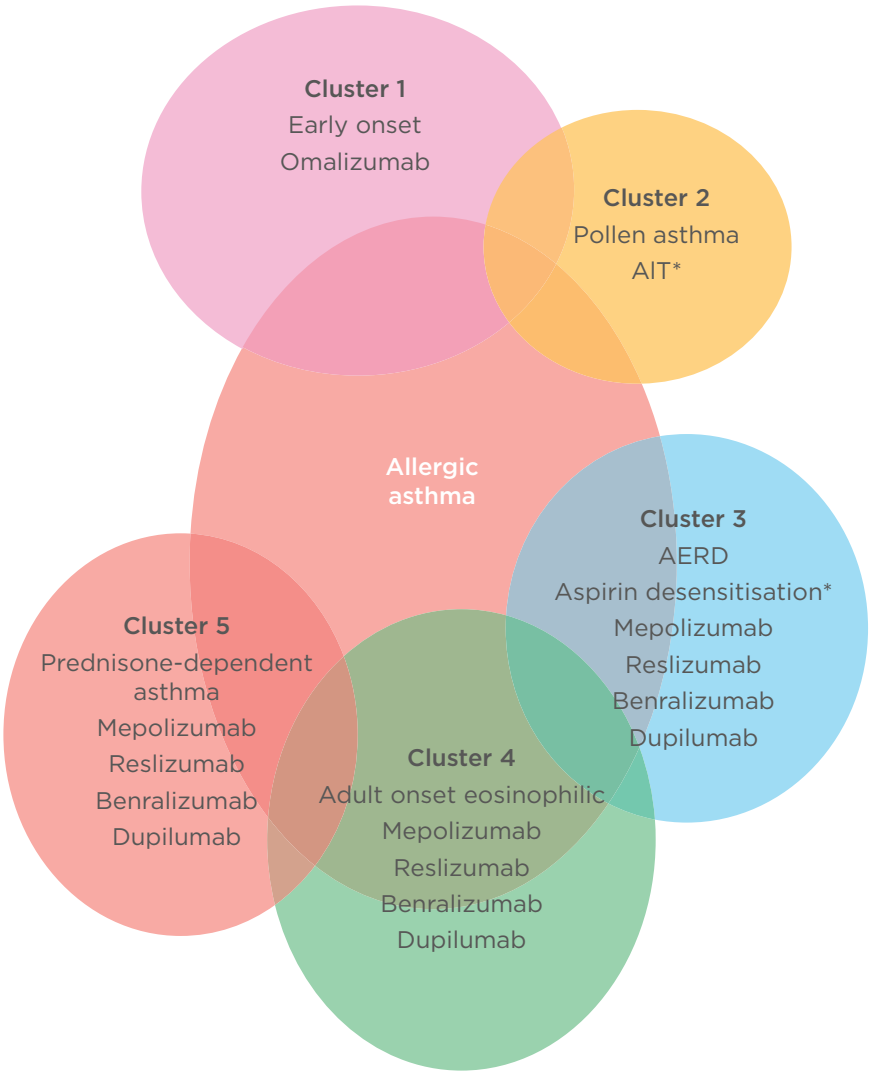


**Figure 2: Different treatment options in different allergic asthma clusters.**

*Forced expiratory volume in 1 second ratio >70% predicted.

AERD: aspirin-exacerbated respiratory disease; AIT: allergen immunotherapy.

As supported by the increasing evidence found in different phenotypes of allergic asthma, new molecules and approaches other than anti-IgE, anti-IL-5, and anti-IL-4R will also shape the future of allergic asthma management, as seen with anti-TSLP, anti-IL-33, and anti-IL-25. However, which clusters in allergic asthma phenotypes are responders to these treatments is still an open question and further studies are needed.

Eligibility criteria, suggested initial dose, route and trial, and common side effects of biological treatments are summarised in Table 1 and different treatment options in different allergic asthma clusters are shown in Figure 2.

## CONCLUSION

The data on allergic asthma is rapidly increasing. However, there are still missing pieces in the puzzle of allergic asthma. Research will help to find those pieces and to understand the clinical and biological differences in patients with allergic asthma. This will lead to the development of novel therapeutic drugs aiming to stop allergic airway inflammation. Some of the drugs that are currently in use, including omalizumab, have proven to be beneficial in the treatment of allergic asthma phenotype. Some of those drugs, such as mepolizumab, reslizumab, benralizumab, and dupilumab, need time to prove beneficial effects in different allergic asthma phenotypes.

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# Novel Insights on Interleukin-10 Functions: A Manipulative Tool for the Deviation of Immune Response and Disease Outcome

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**Disclosure:** The authors have declared no conflicts of interest.

**Acknowledgements:** Both authors contributed equally to this work.

**Received:** 05.02.18

**Accepted:** 27.03.18

**Keywords:** Disease outcome, immunity manipulation, infection, IL-10, physical processes, T helper 1 profile.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:88-94.

## Abstract

It is known that IL-10 plays a critical role in the resolution of inflammation or tissue damage and is the most widely studied anti-inflammatory cytokine, as discussed in different reviews. Since its initial discovery, IL-10 production has been observed in an array of leukocytic cell types and some non-immune cells. Considering recent findings, this review discusses the role of IL-10 in different pathological contexts. In this respect, IL-10 may be considered a manipulative tool that suppresses the much more effective T helper 1 profile which is produced upon the influence of infective agents. The increased IL-10 concentration, which persists for a period of days to a few weeks, is associated with influencing various diseases' outcomes, and its implications are observed in different tissues and processes, including infections, traumas, regeneration, or hyperthermia during physical activity. These findings reinforce the concept that IL-10 should be used in association with co-stimulatory effectors as necessary to exert the appropriate influence during the management of inflammatory or infective pathologies. Hopefully, further findings can open new avenues to study the biology of this cytokine and its therapeutic potential.

## INTRODUCTION

IL-10 plays an important role in the attenuation of inflammation or tissue damage and has been found to be produced by an array of white blood cell types, including lymphocytes, monocytes, and granulocytes, as well as non-immune cells such as epithelial or neuronal cells.[1-4] IL-10 acts through a trans-membrane receptor complex, which is composed of IL-10R1 and IL-10R2, and regulates the functions of lymphocytes, macrophages, and various other cells.[5,6] Several infection studies support the idea that IL-10-producing cells, including T regulatory cells (Treg), macrophages, and dendritic cells (DC), are a major subset of immune cells, possessing potent suppressive properties directed at T effector cells.[1] Additional

IL-10-producing cells are polymorphonuclear cells, natural killer cells (NK), and B regulatory cells (Breg), which are involved in infective, autoimmune, and neoplastic diseases, as well as tolerance induction.[1,5,6]

Inducible IL-10-secreting Breg have also been demonstrated to contribute to allergen tolerance through suppression of effector T cells and selective induction of IgG4 isotype antibodies.[1,7,8] The allergen-tolerant state after exposure to high concentrations of pathogen-associated molecular patterns is associated with the local and systemic induction of distinct populations of allergen-specific T regulatory lymphocytes, including IL-10+ Treg, TGF-β+ Treg, and FoxP3+ memory Treg.[9,10] The protective and recovery-promoting effects of IL-10 during autoimmune diseases (mainly produced by Breg or DC) include a reduction in peripheral T-cell proliferative responses via the modulation of antigen-presenting cell function, a decrease in pro-inflammatory cytokine secretion, and a preferential inhibition of T helper (Th)17-mediated neuro-inflammation; the defective expression of Breg combined with impaired Treg and enhanced Th17 cells play an important role in the development of autoimmune pathologies.[1,11,12]

Notably, several studies on infective diseases show IL-10 to be a crucial factor in inhibiting the harmful effects of the innate pro-inflammatory immune response in a Th1-dominated milieu only, but not if the balance is shifted towards a Th2 response.[1,13-15] The induced manipulation of T cells and B cells toward the Th2 profile and macrophage/monocytes activation can lead to impaired resistance, therefore assuring chronicity and an increased survival potential for different infective agents, such as viruses, chlamydia, protozoans, and parasites.[13,14,16-19]

IL-10 production also induces extensive changes in gene expression and cytokine release, which also lead to endotoxin tolerance and deviations in cellular function, maintenance, growth, and proliferation, as well as coagulation and fibrinolysis, cell–cell signalling or interaction, and cellular movement.[1,20,21] In this review, the authors discuss the role of IL-10 in different situations in light of recent findings.

## INFECTIVE PATHOLOGIES, IL-10 TRANSIENT INCREASE, AND THE SWITCH OF IMMUNE RESPONSE

There have been numerous regulatory effects of IL-10 described, predominately related to infective pathology regulation (Table 1).

**Table 1: The role of interleukin-10 in infective pathologies (2016–2018).**

| Reference | Pathologic condition/ intervention | IL-10 | Source(s) | Primary immune effects | Immune and non-immune consequence(s) |
|---|---|---|---|---|---|
| 3 | *Plasmodium chabaudi* | ↑ | Murine spleen CD19+Breg | IgG production | ↑ transient infection susceptibility |
| 4 | Viral laryngotracheitis | ↑ | Chicken trachea, Harderian gland | ↑ IL-1β, IL-13, IFN-γ | ↑ inflammatory cell involvement/tissue damage, ↓ virus replication |
| 22 | H1N1 vaccine | ↑ | NK, CD4+/CD8+ T cells, monocytes | ↑ IL1β, IL-6, IL-12, TNF-α, IFN-γ | Humoral response |
| 23 | Mycobacterial hyper-sensitivity, pneumonitis | ↑ | Murine bronchoalveolar lavage | ↑ IL-1β, IL-6, TNF-α | Granulomatous inflammation, neutrophil/ lymphocyte infiltration |
| 24 | *Shigella flexeneri* vaccine | ↑ | Human serum, lymphocyte supranatant | ↑ IL-2, IL-17, TNF-α, IFN-γ, ↓ IL-8 | Humoral response |
| 25 | *Brucella pinnipedialis* | ↑ | Atlantic cod, blood, internal organs (spleen, liver, etc.) | ↑ IL-1β, IFN-γ | Humoral response, bacterial load, mononuclear cell invasion |

**Table 1 continued.**

| Reference | Pathologic condition/ intervention | IL-10 | Source(s) | Primary immune effects | Immune and non-immune consequence(s) |
|---|---|---|---|---|---|
| 26 | Orf virus, pustular dermatitis | ↑ | Mice, blood, skin, lymph nodes | ↓ monocytes, DC, MC recruitment or activation | ↓ inflammatory cell infiltration in the skin |
| 27 | Chronic hepatitis C/antiviral treatment | ↑/↓ | Human serum | ↑/↓ IL-1β, IL-4, IL-15, TGF-β, IFN-γ | ↑/↓ cirrhosis, fibrosis |
| 28 | HIV and HCV coinfection | ↑ | Human plasma, liver | ↑ CD4+ Treg, memory Treg, IFN-γ, ↓ IL-2, IL-17 | Liver stiffness, immunosuppression |
| 29 | HIV and meningitis | ↑ | Blood, CSF | ↑ WBC, TNF-α, RANTES | CNS immunologic stimulation |
| 30 | *Streptococcus suis* | ↑ | Murine splenocytes | ↑ IL-6, ↓ IFN-γ, TNF-α, CD4+ splenic depletion | Lack of DC activation, impaired antibody response |
| 31 | *Staphylococcus aureus* | ↑ | Tolerogenic DC | Treg and TLR2 activation | Th1 response impairment, immune evasion |
| 32 | Malaria and non-typhoidal salmonella vaccine | ↑ | Mouse serum | ↓ CD4+ and CD8+ affectivity, ↓ IFN-γ | Immune protection loss (restored after IL-10 blockade) |
| 33 | *Trypanosoma cruzi,* Chagas cardiomyopathy | ↑ | Murine myocardium, CD4+ T cells | ↓ M1 bactericide macrophage, ↑ M2 anti-inflammatory macrophage | Local parasitic persistence |
| 34 | Lymphocytic chorio-menginitis virus | KO | Murine CD8+ T cells | ↑ immune response | ↑ organ damage, mortality |
| 35 | Varicella-Zoster virus vaccine, murine IL-10 inhibition | ↑/↓ | Human/murine serum | ↓ T cell response/ ↑ T cell response | Reduced immune protection/normal immune protection |
| 36 | Herpes virus uveitis | ↑ | Macaque, neural retina | ↑ IL-6, NFκB activation | Determination of disease's severity |
| 37 | *Escherichia coli* | WT/KO | Murine intestine, mesenteric lymph nodes (B cells, CD4+ T cells) | ↓IFN-γ/↑IFN-γ | Mucosal homeostasis/ chronic colitis |
| 38 | Dengue virus | ↑ | Human serum, CD14+ monocytes | ↑ IL-6, ↓ Th1 response/↑ IL-8 | Thrombocytopenia, severe disease, delayed recovery/ recovery |

/: versus; Breg: B regulatory cells; CD: cluster of differentiation; DC: dendritic cells; HCV: hepatitis C virus; HIV: human immune-deficiency virus; H1N1: swine flu; KO: knock out; IFN: interferon; CNS: central venous system; CSF: cerebrospinal fluid; Ig: immunoglobulin; M: macrophage type; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; NK: natural killer; RANTES: regulated on activation, normal T expressed and secreted; TGF: transforming growth factor; Th: T helper; TLR: toll-like receptor; TNF: tumour necrosis factor; Treg: T regulatory cells; WBC: white blood cell; WT: wild type.

Accordingly, spleen analysis of the CD19+ Breg cell response on *Plasmodium chabaudi*-infected BALB/c mice reinforced the observation of its regulatory role during an infection-related phenotype shift, represented by a strong IL-10 production.[3] Similarly to infections, vaccination with monovalent H1N1 influenza is associated with a broad spectrum upregulation of inflammatory and regulatory biomarkers, such as IL-1β, IL-6, IL-10, and IL-12 derived from NK, CD4+, and CD8+ T-cells.[22] This immune response relies on antibody production to provide persistent immune protection against the influenza antigen that subjects are exposed to.

Previous findings regarding functions of the mentioned cells indicate that IL-10 is mainly produced during transient immune conditions and that the persistent IL-10-related effect may be the effectuation of the switching immunological response.[1] These effects are demonstrated during exposure to infective agents, immunotherapy to different allergens, and the development of autoimmune pathologies, in which the implication of Th1, Treg, or Breg cells lead to the production of specific antibodies.[1,3-21] Recent evidence appears to support IL-10 being a switcher of immune response.[4,23-27] Thus, the peak of pro-inflammatory (IL-1β, IFN-γ) and anti-inflammatory (IL-10, IL-13) cytokine gene transcription, 5 days post-infection with an infectious laryngotracheitis virus strain in chickens, coincided with an increased recruitment of inflammatory cells, extensive tissue damage, and limiting of virus replication in the trachea.[4] Early responses in an acute model of mycobacterial hypersensitivity pneumonitis in mice revealed a time and dose-dependent increase in a range of cytokines, including TNF-α, IL-1β, IL-6, and IL-10, followed by subsequent granulomatous inflammation.[23] Inactivated whole-cell *Shigella flexneri* 2a-vaccine also induced a transient increase of different cytokines, including IL-2, IL-10, IL-17, IFN-γ, and TNF-α.[24] *Brucella pinnipedialis* additionally stimulates IL-10 or IFN-γ production, as observed from the 1st—28th day of experimental challenge in Atlantic cod, whereas anti-*Brucella* antibodies were detected from Day 14 onwards.[25] A transient increase of IL-10 production during skin orf virus infection in mice was associated with recruitment limitation and trafficking inhibition of certain white blood and connective tissue cell subpopulations.[26] In contrast, hepatitis C virus (HCV)-infected subjects show IL-10 reduction to normal levels only after successful antiviral treatment.[27] In concert, these observations suggest that the increased IL-10 concentration under the influence of infective agents persists during the period of immune switch or modulation.[1] The post-infective balance stabilisation for immune response compounds could correspond with the restoration of the lower (normal) IL-10 concentration to pre-infection levels.

## INFECTIVE AGENTS, IL-10, AND MANIPULATION OF IMMUNE RESPONSE OR DISEASE OUTCOMES

Infectious agents are believed to be manipulators of immune response due to implications for IL-10 production, as reported in several studies. In this respect, HIV/HCV-co-infected patients have shown an immunosuppressive profile compared to healthy controls and HIV-mono-infected patients.[28] White blood cell and inflammatory responses in cerebral liquor during asymptomatic bacterial meningitis in HIV-positive subjects has suggested that the central nervous system immune response in patients with HIV infection was independent of the systemic immune response.[29] The manipulation of immune-responsiveness is also observed during *Streptococcus suis* infection, showing a modulation of DC functions.[30] *S. suis* mouse splenocytes produced different cytokines, such as IL-6 and IL-10, but the level of Th1 cytokines TNF-α and IFN-γ were very low. Altogether, these results suggest *S. suis* interferes with the adaptive immune response.[30] *Staphylococcus aureus* uses highly efficient immune evasion strategies to cause immune tolerance and results in a wide range of pathologies; the central mechanism corresponds to DC-related production of high amounts of IL-10, which is associated with an impaired Th1 response.[31] Similarly to bacterial agents, malaria-related IL-10-production inhibited protection against an attenuated non-typhoidal *Salmonella* vaccine in mice co-infected with malaria in a transient manner.[32] The infection-related Th1 response impairment may be an adaptive mechanism by the infective agent, because the Th1 immune profile is much more effective against infections than the Th2 isoform.[1]

Recent clinical and experimental studies on infective diseases also report the outcome deviation caused by IL-10, such as the failure to eliminate Varicella-Zoster virus, the deviation of parasite load within the myocardium during the acute phase of Chagas cardiomyopathy, or immunopathology exacerbation in select organs, ranging from transient local swelling to an increased risk for mortality during acute primary infection with the lymphocytic choriomeningitis virus.[33,34,39] Gershon et al.[35] specified that significant humoral immunity boosting after zoster vaccine only occurred in patients with a low constitutive IL-10 levels, while Jacobshagen et al.[34] highlighted the physiological role of IL-10 in the regulation of a balanced T-cell response, also limiting the immunopathological damage. Moreover, the modulation of immune response and disease outcome, under variations of IL-10 level in a murine model of infection by *Trypanosoma cruzi*, is associated with a sudden switch from the classical M1 macrophage (microbicidal) phenotype toward an alternative M2 (repairing/anti-inflammatory) phenotype that occurred within the myocardium very shortly after infection.[33] Considering that parasite persistence within myocardium is a necessary and sufficient condition for the development of the chronic myocarditis, Ponce et al.[33] discovered that transient inhibition of the aforementioned macrophage switch enhanced the microbicidal M1 subset predominance, diminished IL-4 and IL-10-producing CD4+ T cells, promoted a pro-inflammatory cytokine milieu, and reduced parasite load within the myocardium during the acute phase.

IL-10 involvement with disease outcomes during infective pathologies is also observed in herpes simplex-infected neuronal retina, and mesenteric lymph nodes infected with *Escherichia coli*.[36,37] According to Wu et al.,[37] production of IFN-γ rapidly and progressively declined after colonisation of wild-type but not IL-10-deficient mice. CD4+ and B cell-related IL-10 in wild-type mice peaked at Day 4 and subsequently declined, suggesting that *E. coli* may deviate the profile of the effector immune system in normal hosts for their own purposes, in parallel with induction of IL-10 that subsequently suppresses this response to mediate mucosal homeostasis. Additionally, severe dengue cases had low Th1 cytokines and a concurrent increase in inflammatory mediators such as IL-6, IL-8, and IL-10, which originate from CD14+ cells. The reduction in the levels of IL-8 and IL-10 were identified as the most significant markers of recovery from severe disease.[38] Aside from demonstrating the cytokine's manipulative abilities, these studies reinforce the finding that the immunoregulatory cytokine IL-10 can suppress Th1-cell immunity.[34]

The increased IL-10 production can be associated with deviations in infectious disease outcomes and may be influenced by the infective agents themselves.[13] The suppression of the Th1 response (and development of other less effective anti-infection profiles), as well as the deviation of the disease's outcome towards the less aggressive anti-inflammatory phenotypes may be a result of interactions between the host immunity and the infective agent.[1] This could lead to lack of infection eradication (disease chronicity), because, apart from the point-of-view of host-related beneficiary purposes, the anti-inflammatory and a less aggressive response may allow the infective agent to persist longer or under more favourable conditions within the host (avoiding the early death for both organisms) to fulfil its life cycle. In this context, the increased level of IL-10 seems to be necessary to mediate the switch of immune response.[1,13,40]

## IL-10, TRAUMA, PHYSICAL STRESS, AND REPAIRING PROCESSES

Recent publications report the IL-10 involvement in traumatic or physical stress situations, showing some similarities with pure infective conditions. Thus, the transient early implication of IL-10 in repairing processes after traumatic situations is also shown in the microglia of epileptic subjects, during the recruitment of macrophages accompanying the shortening of the early phases of skeletal muscle regeneration in mice, in the T cell response after stroke, or in response to intensive exercise in hot environments.[41-44] In addition, Lentivirus-related IL10-production and the consequent reduction of the neuroinflammatory response among spinal cord-traumatised mice reduced neutrophil infiltration at both Day 7 and Day 28 of experimental trauma. Similarly to *T. cruzi* infection, this effect correlated with skewing of the macrophage population toward an anti-

inflammatory M2 phenotype and improvement of motor function, suggesting reduced secondary damage and increased sparing.[33,45] Bodaan et al.[46] reported that delayed healing of equine limb wounds was characterised by intensified and extended pro-inflammatory signalling and an exacerbated innate immune response, concomitant with the absence of anti-inflammatory IL-10.[46] Short-term treatment of these wounds with orf virus IL-10 has dampened inflammation and promoted repair processes without accelerating closure. Taken together, these results indicate that localised expression of anti-inflammatory factors, such as IL-10, can modulate the inflammatory response following various traumatic injuries, and may be a key component of a combinatorial approach that targets the multiple barriers to regeneration and functional recovery.[45] However, any manipulation of the IL-10 response for treatment purposes should be considered very cautiously due to its potential hazards to the immune system.[1]

## CONCLUSION

In light of recent findings, IL-10 (as a potential switcher of the immune response) may be a manipulative tool that, when produced due to the influence of infectious agents, suppresses the much more effective Th1 profile. Lasting transiently for a period of days or weeks, the presence of increased IL-10 is associated with deviations in disease outcomes, and its implications are observed in different tissues (e.g., muscles and nerves) and processes (e.g., infections, traumas, regeneration, or physical stress). These observations emphasise that IL-10 should be used in association with the necessary co-stimulatory immune effectors to determine the appropriate deviation during the treatment of respective pathologies.[1] Hopefully, further findings can open new avenues to study the biology of this cytokine and its therapeutic potential.

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# Leukocyte Adhesion Deficiency Type 1: A Case Series and Review of the Literature

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**Disclosure:** The authors have declared no conflicts of interest.

**Received:** 12.01.19

**Accepted:** 18.02.19

**Keywords:** Infections, leukocyte adhesion deficiency type-1, neutrophilia.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:95-100.

## Abstract

Leukocyte adhesion deficiency type-1 (LAD-1) is a rare primary immunodeficiency that is characterised by compromised neutrophil adhesion and transmigration to infection or inflammation sites. In this article, the authors report the cases of five patients with LAD-1 deficiency. The aim of this study is the demonstration of the wide variety of manifestations in these patients with a review of the literature. Patients were both male and female, with their ages ranging from 1 month to 10 years old. Omphalitis was the most common presentation in this series, followed by pneumonia and gingivitis. Impaired wound healing and cellulitis were other common findings in these patients. Two of the patients did not show delayed separation of the umbilical cord. The findings indicate that LAD-1 remains a life-threatening condition with omphalitis, oral, skin, respiratory tract, and ear infections as the most common complications. Early identification of these patients is essential in ensuring a definitive diagnosis and early implementation of haematopoietic stem cell transplantation.

## INTRODUCTION

Leukocyte adhesion deficiency type-1 (LAD-1) is a rare autosomal recessive primary immunodeficiency caused by mutations in the *ITGB2* gene, which encodes the CD18 subunit of the $\beta_2$ integrins, leading to compromised neutrophil adhesion and transmigration to infection or inflammation site.[1,2] LAD-1 severity is related to the degree of CD18 deficiency.[3] Leukocyte trafficking from circulation to tissues, as a critical response to integrins, serves as an essential dynamic process in immune surveillance and is mediated with a set of integrin molecules on leukocyte cell surfaces.[4,5] Genetic alterations in the numbers or activation of leukocyte integrins may lead to the development of LAD-1 syndromes.[6,7] Members of the $\beta_2$ subclass of integrins are essential for normal adhesion-dependent functions of

polymorphonuclear leukocytes and monocytes by providing normal integrin dimerisation.[8-11] Deficient expression of each of the following four members of the $\beta_2$ integrin subfamilies leads to LAD-1 syndrome: $\alpha_L\beta_2$(CD11a/CD18 and LFA-1), $\alpha_M\beta_2$ (CD11b/CD18, MAC-1, and CR3), $\alpha_X\beta_2$ (CD11c/CD18, and p150/95), and $\alpha_D\beta_2$ (CD11d/CD18). The $\beta_2$ integrins are also expressed by other immune effector cells, including macrophages and lymphocyte subsets.[12-15] $\alpha_M\beta_2$ and $\alpha_L\beta_2$ serve as the C3b receptor in myeloid and lymphoid cells[16] and $\alpha_D\beta_2$ is restricted to macrophage subsets in mice and humans.[12,17,18] The *ITGB2* gene encoding for the $\beta_2$-integrin component CD18[1,2,11,19] is located at the long arm of chromosome 21q22.3,[3] and has >80 mutations identified so far. The severity of the clinical manifestations is directly related to the degree of CD18 deficiency,[3] with the mild-to-moderate types characterised by 2–30% CD18-expressing neutrophils and severe LAD-1 classified as <2% CD18-expressing neutrophils.[11,19,20]

Patients with LAD-1 typically present with recurrent indolent necrotic infections of the skin and mucosal surfaces, which usually occur during the neonatal period with omphalitis and delayed separation of the umbilical cord.[3] Additionally, failure to thrive, severe malnourishment, and delayed wound healing are common, while colitis may only occur in rare cases.[21] Many patients may develop infections before they are diagnosed with LAD-1 because many physicians are unaware of the disease.[22] Here, the authors describe five cases admitted to Mofid Children's Hospital, Tehran, Iran, with different presentations and prognoses to emphasise the importance of disease awareness and timely diagnosis in the successful management of the disease.

## METHODS

The patients were evaluated with a detailed history, physical examination, and immunologic screening tests at the time of their first admission to hospital. Complete blood counts (CBC) were performed using an automated blood counting machine (Sysmex XE-2100 [Sysmex, Kobe, Japan]). Haemagglutinins were quantified by CA 1600 and immunoglobulin levels were evaluated by ELISA. Review of CD markers was conducted by flowcytometry analysis (PAS system [Partec, Munich, Germany]). The study was approved by the ethics committee of Mofid Children's Hospital. The authors also established clear policies to secure patients' privacy.

## CASE 1

A 45-day-old full-term infant was admitted due to fever, poor feeding, and vomiting. She was first referred to the authors' hospital at this age due to refractory sepsis that did not respond to antibiotics. The infant was a consanguineous product, born to a 28-year-old mother by normal vaginal delivery, and she presented with normal Apgar scores and appropriate weight for gestational age. At the time of admission, she was sick and febrile (39 °C). The umbilical cord had been separated but the parents reported a significant delay in its detachment (at 30 days). Following physical examination, periumbilical erythema with serous discharge, suggesting omphalitis, was detected. Laboratory investigations revealed significant leukocytosis (a leukocyte count of 59,900 $mm^3$ with 78% polymorphonuclears). Immunophenotyping performed by flow cytometry analysis reported low CD18 (1.0%), CD11a (2.0%), CD11b (3.0%), and CD11c (0.9%) antigens, which is consistent with LAD. Haematopoietic stem cell transplantation (HSCT) has not been conducted because a matched donor has not yet been found. However, several follow-up visits have been conducted until the present day. She is currently aged 3 years.

## CASE 2

A 40-day-old female infant, the only child of consanguineous parents, was admitted to hospital three times. Her third admission was due to fever, omphalitis without pus, and poor wound healing at the site of a previous intravenous line from her last hospital admission. Her past medical history revealed two previous admissions, with the former due to omphalitis and the latter due to soft palate lesions mimicking fungal infections. She had a history of delayed umbilical detachment (at 23 days of life). The laboratory tests at the time of admission revealed leukocytosis (80,700 $mm^3$) with neutrophil predominance (72%) that persisted throughout the course of hospitalisation despite appropriate antibiotic

therapy. Flow cytometry analysis detected low levels of CD11a (0.70%), CD11b (0.35%), CD11c (0.23%), and CD18 (0.10%). She had follow-up visits regularly for 9 years, but, because of the lack of a matched donor, she received HSCT at the age of 9 years at a late stage when she had nearly experienced the late sequela of the disease. She died 1 month after the transplantation due to infectious complications.

## CASE 3

A 10-year-old girl, the product of a twin pregnancy from unrelated parents, was admitted to the authors' hospital due to necrotising skin lesions on the left inguinal region 3 years ago at the age of 7 years. There were also multiple scarring lesions on both extremities due to recurrent cellulitis beginning from 3 years before admission. She has a history of both delayed umbilical cord detachment in her neonatal period and also delayed wound healing since infancy (at the age of 8 months). She had recurrent attacks of gingivitis. The laboratory tests revealed moderate leukocytosis (34,800 $mm^3$) with neutrophil predominance (67.0%) at the time of admission. Flow cytometry analysis detected low levels of CD18 and CD11a (9.1% and 0.1%, respectively). A successful human leukocyte antigen-identical HSCT was performed from her healthy twin sibling last year. She has had several follow-up visits after HSCT for 1 year until recently and she is responding well.

## CASE 4

In 2017, a 7-year-old boy, who was the only child of unrelated parents, presented with recurrent gingivitis, but responded well to oral antibiotics and mouthwash every time. His past medical history was indicative of omphalitis in the neonatal period. The umbilical cord separation was not delayed. His family history revealed that his cousin had LAD at an early age and died as a result. A CBC at the time of admission showed 30,700 white blood cells (WBC) with neutrophil predominance (71%). Flow cytometry analysis detected CD11a: 0.1%, CD11b: 92.0%, CD11c: 6.0%, and CD18: 0.1%. HSCT has not been performed for him. However, he is still alive and has been undertaking follow-up visits for the last 2 years.

## CASE 5

A 2-year-old boy from consanguineous parents was admitted with a history of recurrent bilateral otitis media and mastoiditis from early infancy (age of 2 months). At the time of his first admission in our hospital at the age of 2, he presented with left leg cellulitis, which had not been responsive to several courses of oral antibiotic therapy. At 3 years old, he had a perianal abscess and has had recurrent buttock cellulitis since then. CBC revealed leukocytosis (WBC count: 28,000 $mm^3$) with neutrophil predominance (75%). Flow cytometry analysis showed low numbers of CD11a (1.5%), CD11b (1.0%), CD11c (4.0%), and CD18 (2.0%). A matched donor was not available for him and he died because of pneumonia and fulminant sepsis from his last admission at another hospital. He had been participating in follow-up appointments for 6 years before his death at the age of 8 years.

## RESULTS

Patients' age, sex, clinical features, parental consanguinity, laboratory findings, treatment, and outcome are summarised in Table 1. Omphalitis was the most common presentation in this series, followed by pneumonia and gingivitis. Impaired wound healing and cellulitis were other common findings in these patients. One patient presented with fungal infection. Laboratory evaluation was indicative of severe LAD (according to CD18 <2% in flow cytometry analysis) in four of the patients.

HSCT was not available for all of the patients as a curative therapy. It has been performed in two patients, which led to death in one of them due to infection and sepsis because of the immunosuppressive state after receiving HSCT. The procedure was carried out in a tertiary centre for transplantation. Therefore, the authors do not have detailed information regarding post-transplant patient history.

## DISCUSSION

LAD syndromes are rare genetically determined immunodeficiencies with challenging clinical manifestations.[8] Here, the authors presented five cases with LAD with different presentations, management, and prognoses.

**Table 1: Clinical features, laboratory findings, treatment, and outcome in five patients with leukocyte adhesion deficiency type-1.**

| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 |
|---|---|---|---|---|---|
| WBC counts ($mm^3$) | WBC count: 59,900<br>PMN: 78% | WBC count: 80,700<br>PMN: 72% | WBC count: 34,800<br>PMN: 67% | WBC count: 30,700<br>PMN: 71% | WBC count: 28,000<br>PMN: 75% |
| Clinical features | • Omphalitis<br>• Sepsis | • Omphalitis<br>• Impaired wound healing<br>• Soft palate fungal infection | • Necrotising fasciitis<br>• Impaired wound healing<br>• Gingivitis | • Omphalitis<br>• Gingivitis | • Otitis media<br>• Perianal abscesses<br>• Cellulitis (leg and buttock)<br>• Pneumonia |
| Umbilical cord detachment delay | Yes | Yes | Yes | No | No |
| Related parents | Yes | Yes | No | No | Yes |
| Flow cytometry results | CD11a: 2.00%<br>CD11b: 3.00%<br>CD11c: 0.90%<br>CD18: 1.00% | CD11a: 0.70%<br>CD11b: 0.35%<br>CD11c: 0.23%<br>CD18: 0.10% | CD11a: 0.10%<br>CD11b: 7.00%<br>CD11c: 12.00%<br>CD18: 91.00% | CD11a: 0.10%<br>CD11b: 92.00%<br>CD11c: 6.00%<br>CD18: 0.10% | CD11a: 1.50%<br>CD11b: 1.00%<br>CD11c: 4.00%<br>CD18: 2.00% |
| HSCT | No | Yes | Yes | No | No |
| Outcome | Alive | Dead | Alive | Alive | Dead |

HSCT: haematopoietic stem cell transplantation; PMN: polymorphonuclears; WBC: white blood cell.

Jasani et al.[23] reported a case of severe LAD-1 presented during the neonatal period and considered LAD-1 a rare neonatal presentation, with only two cases reported in the literature prior to 2014. Neonatal presentation of the disease is a function of disease severity. However, the age at presentation in the patients reported here was within the neonatal period in almost all of the cases (four at the age of 1 month and one at the age of 2 months).

Recurrent, severe, and difficult-to-treat bacterial infections[4] are the predominant clinical manifestation of these patients, sometimes presenting as life-threatening infections, such as septicaemia, bronchopneumonia, and aseptic meningitis.[24] Skin and mucosal surface infections may present as indolent and necrotic lesions that can enlarge and recur, commonly leading to systemic spread of infections.[3] Infections usually appear first in the neonatal period as omphalitis and delayed umbilical detachment.[5,25] Umbilical cord complications were more frequent in patients with severe LAD, as identified in a comprehensive review performed by Novoa et al.,[11] and they reported a significant correlation between lack of these complications and survival to 2 years of age.

After infancy, gingivitis, periodontitis, and impaired wound healing[4,26] are characteristic of LAD. Absence of pus formation is significant[24] due to the impaired migration of leukocytes and defect in extravascular accumulation of polymorphonuclears and monocytes. Failure in clearing apoptotic polymorphonuclears and generating inflammatory modulator signals leads to impaired wound healing due to dysregulated macrophage–neutrophil interactions.[27,28]

Novoa et al.[11] reported infections in 77% of their patients with severe LAD, of which the majority were due to respiratory infections, sepsis, and otitis media. Those with less severe deficiencies (CD18 ≥2%) in the previous study presented with periodontal infections, otitis media, and sepsis.[11] Dababneh et al.[29] reported periodontal findings, including intense redness and inflammation of the gingiva, primary teeth, and permanent dentition involvements, leading to rapid periodontal destruction refractory to conventional non-surgical therapies. However, the most frequent infections in our patients who had predominantly presented with severe LAD were omphalitis and delayed umbilical separation followed by oral ulcers indicating

gingivitis. The authors could not find a significant relationship between the WBC counts and the severity of the disease (CD18 <2%) in the patients in this study.

The most frequently isolated micro-organisms from LAD patients are *Staphylococcus aureus* and Gram-negative bacteria.[3] Other micro-organisms, such as fungal infections, although unusual, have also been reported.[3,16,30] Lymphocyte extravasation is not affected; therefore, defences against viral infections are usually maintained.[3] Despite severe infections in the patients in this study, the authors could not isolate micro-organisms from the patients because they had received long-term antibiotic therapies due to their severe infections every time before their admission. Fungal infection in the second case is assumed to be the result of phagocytic dysfunction. In this case, a positive fungal culture was not obtained. However, after an infectious consultation and because of the pattern of the lesions, the authors conducted an empirical therapy with an antifungal agent (amphotericin), which was accompanied by a good response to the therapy and the lesions disappeared.

Persistent neutrophilia in the absence of infections and dramatically increased myeloid leukocyte counts in the presence of infections are characteristic.[4,31] Although a higher median WBC count were reported by Novoa et al.[11] in 143 cases with CD18 <2% (48 versus $30x10^9$/L), a limited correlation was found between CD18 expression and WBC counts for the entire cohort.

Patients with severe LAD-1 can present with a failure to thrive due to general malnourishment and sometimes colitis.[3] In this study, the failure to thrive observed in children was because of malnourishment and recurrent infections. A rare mutation with non-functional although normal $\beta_2$ integrins expression has been reported.[32-34]

Patients with moderate LAD mostly survive childhood.[11] Severe LAD-1 is associated with significant mortality (reported as 75% by the age of 2 years)[20,35] in those who do not receive allogeneic HSCT. Four of the patients reported, except one, fell into the classification of severe LAD-1. Two of these patients with severe LAD-1 are still alive while the other two died. HSCT was not available for all of the patients and one of the two patients who underwent HSCT died as a result of complications of the procedure. The children were not transplanted in the same centre where their disease was diagnosed; they were referred to a tertiary centre for bone marrow transplant. The cause of death in the transplanted child was fulminant sepsis. Unfortunately, due to the prolonged time taken searching for a matched donor, the patients experienced the complications of a severe chronic disease with recurrent infections, which significantly affected their prognoses even after receiving HSCT and was fatal for one of the patients.

Although *ITGβ2* gene sequencing and mutation analysis may confirm the diagnosis and is useful for genetic counselling,[35,36] none of the patients in this study had a mutation analysis and all of them were diagnosed based on their typical clinical manifestations and *in vitro* diagnostic tests, including CBC and blood flow cytometry.

## CONCLUSION

The findings reported indicate that LAD-1 remains a life-threatening condition with omphalitis, oral, skin, and respiratory tract and ear infections being the most common complications. The most important issue in the management of these patients is to control infections. Early identification of these patients is essential in definitive diagnosis and early implementation of HSCT. However, HSCT was not associated with a favourable response in one of the two patients who received this treatment in the series.

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# The Intensity of Physical Activity in Asthmatic Children During Active Video Game Playing

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**Disclosure:** The authors have declared no conflicts of interest.

**Received:** 23.11.18

**Accepted:** 04.02.19

**Keywords:** Active video game, asthma, children, exercise intensity.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:101-107.

## Abstract

**Background:** The use of virtual reality and video games has gained traction in physical rehabilitation medicine. In respiratory rehabilitation, their use is more recent, and for the application of this tool in this area it is necessary to examine the intensity of the effort reached in the activity. To evaluate the intensity of effort achieved in the 'Reflex Ridge' Xbox 360™ (Microsoft, Redmond, Washington, USA) game compared to the current gold standard treatment, the cardiopulmonary exercise test (CPET), in asthmatic children.

**Methods:** A cross-sectional study evaluating children participating in a CPET and the Reflex Ridge active video game (AVG), played using an Xbox 360 Kinect,™ over two different days.

**Results:** Thirty children who had a mean age of 7.86±1.9 years were evaluated. The maximum heart rate using the AVG reached 87% of the maximum predicted value and 93% of that obtained in the CPET. The intensity of the exercise using AVG reached 8.46±1.86 MET; this corresponded to 81% of the intensity reached in the CPET. There were no episodes of exercise-induced bronchospasm.

**Conclusion:** Using AVG can promote high intensity physical exercise. AVG playing may be considered a safe mechanism for the physical treatment of asthmatic children.

## INTRODUCTION

Physical exercise is an important non-pharmacological component of clinical asthma control, especially in children.[1] However, physical exercise can lead to induced bronchospasm, which affects 40–90% of asthmatic children.[1] In addition, physical exercise may not be motivating, and therefore these aspects may lead these children to adopt a sedentary lifestyle, which can worsen their clinical condition.[2] Alternative forms of exercise, such as playful physical activities (e.g., interactive video games), can be beneficial as long as the patients are properly monitored and the activities are safe.

It is understood that playful physical activity provides physiological benefits for children with asthma provided that the activity fulfils three conditions: involves large muscle groups; is of moderate intensity, reaching at least 3–6 MET; and has a minimum duration of 20 minutes.[3] This activity should also be applicable in clinical practice.[4] It is important to emphasise that a playful and motivational physical activity may include active video games (AVG). Although the number of published studies involving asthmatic children is small, there are publications that show the potential of AVG in asthma treatment protocols.[5-7]

AVG has been highlighted as a form of physical activity with adequate intensity in the literature since Del Corral et al.[8] compared the 6-minute walk test (6MWT) to three AVG and found that two of the three AVG imposed a metabolic demand that was similar to the 6MWT. A previous study[9] has shown that AVG played by children with asthma led to a higher energy expenditure than achieved by aerobic testing on a treadmill.

There are few commercial computer programs designed exclusively for rehabilitation. In the vast majority of studies, and even in clinical practice, AVG originally designed for entertainment are used as a rehabilitation tool. Based on the scientific literature and the authors' belief of its potential to provide multi-articular activity with a great variety of movement of the lower and upper limbs, the 'Reflex Ridge' AVG was selected for study.

The hypothesis of this study was that this game could reach high levels of intensity. The objective of the present study was to evaluate the intensity of the activity achieved by asthmatic children who played the Reflex Ridge AVG in comparison to the intensity of the activity achieved while undertaking cardiopulmonary exercise tests (CPET) on a treadmill.

## MATERIALS AND METHODS

This was a cross-sectional study conducted at the Respiratory Functional Assessment Laboratory, Nove de Julho University, São Paulo, Brazil. The Ethics and Research Committee of the same institution approved the study, protocol number 907236/2014, according to resolution 466/2012 of the National Health Council. All persons responsible for minors provided informed consent for the inclusion of the minors in the study and all the children accepted the respective consent form.

### Inclusion Criteria

Inclusion criteria for this study were children aged 5–11 years with a diagnosis of asthma who did not practice regular physical activity. The children were evaluated by pulmonologists.[10]

### Exclusion Criteria

Children whose condition worsened within 30 days before testing and those who had a respiratory infection up to 2 months before testing were excluded. Additional exclusion criteria included children who had received theophylline or aminophylline and oral corticosteroids in the last 30 days; who were unable to perform any of the tests; who carried cardiac diseases of inflammatory, congenital, or ischaemic origin; and who had an infectious disease, with fever, at the time of the of the experiment.

### Experimental Procedures

#### Screening the Asthmatic Children

Thirty-four asthmatic children were assessed and screened at Mandaqui Hospital, São Paulo, Brazil, and their parents were invited to refer them to and accompany them at the Respiratory Functional Assessment Laboratory, where the children were assessed and completed an AVG activity and a CPET. The order in which the children completed the two tests was randomised, and the two tests were performed on different days, as shown in Figure 1.

#### Anthropometric Measurements and Body Composition

All the children were individually assessed in the afternoon and anthropometric measurements, including height, weight, and tetrapolar bioimpedance[11] (using a Biodynamics™ model 310, Biodynamics Corporation, Seattle, Washington, USA), were taken. The AnthroPlus software (World Health Organization [WHO]) was used to set the Z score according to the determination established by the WHO.[12] The Z score was used to classify the child as obese or eutrophic (normal body weight) by keeping values between 2 and -2, depending on the Z score.

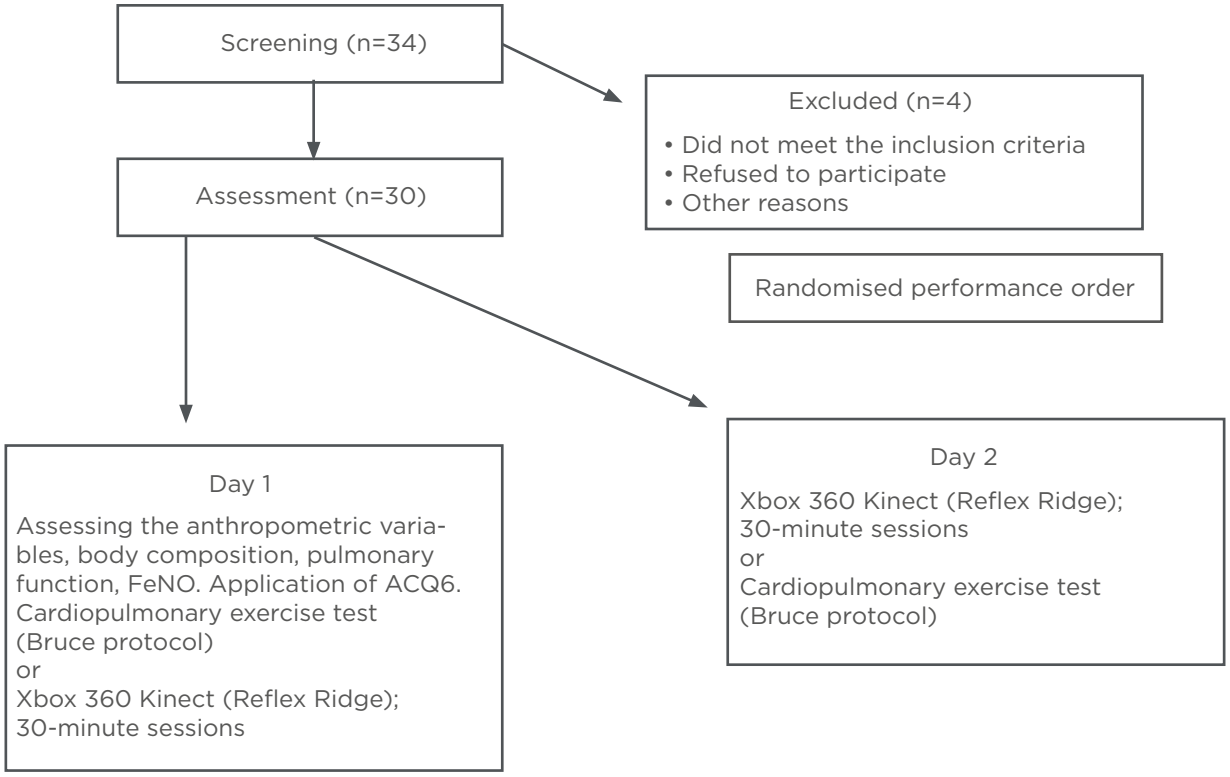


**Figure 1: Flowchart showing the setup of the study.**

ACQ6: Asthma Control Questionnaire 6; FeNO: fractional exhaled nitric oxide.

## Pulmonary Function

The pulmonary function of all the children was assessed through spirometry, using the Easy One™ Air (ndd Medical Technologies, Andover, Massachusetts, USA). The spirometer was calibrated according to the Polgar and Promadhat[13] reference values suited for children, prior to use in an acclimatised room. Three reproducible and technically accepted tests of forced vital capacity were recorded according to the criteria of the American Thoracic Society (ATS).[14]

## Degree of Airway Inflammation

The degree of airway inflammation was measured through fractional exhaled nitric oxide (FeNO) and followed the ATS criteria.[15] All the children performed a test using the NIOX Mino® (Circassia AB, Uppsala, Sweden) in the sitting position. This test was performed with the children exhaling and emptying their lungs as much as possible, up to the level of expiratory reserve volume, to decontaminate the respired gasses. Subsequently, a deep inspiration was recorded, and all the air was exhaled through the mouth, with constant flow through the equipment for at least 6 seconds. One minute and forty seconds later, the FeNO reading in parts per billion was recorded.[16]

## Cardiopulmonary Exercise Test

The Bruce Test[17,18] was the protocol used during the CPET and it was interrupted when the child reported or presented fatigue or when they reached the maximum heart rate (HRmax), which was set at 208 bpm minus (0.7 x age of the participant [years]).[19] Blood pressure was recorded, as well as the peripheral oxygen saturation and data from the electrocardiogram.

The fatigue in the inferior limbs and the dyspnoea at effort and at rest were recorded through the modified Borg scale.[20]

## Active Video Game

The children completed a 30-minute AVG playing session using an Xbox 360 Kinect™ (Microsoft, Redmond, Washington, USA). The game used

was Reflex Ridge from the Adventure Kinect video game catalogue.[9]

Three maximum peak flow measures were collected before and after the AVG session and CPET completion, to monitor the possible presence of exercise-induced bronchospasm (EIB); the children were kept in the standing position and used a nose clip. These measures were performed using the Assess™ (USA) equipment. Bronchospasm was characterised by a ≥20% reduction in the peak flow.[21]

A warm-up was performed on the treadmill for 10 minutes at a speed of 2 km/hour before the AVG session. The AVG playing section lasted 30 minutes and was composed of 10 matches (3 minutes long each) of growing effort intensity, with a mean interval of 30 seconds between matches. Increased intensity demanded a larger number of jumps, squats, and lateral displacements, as well as movements of the upper limbs.

## Energy Spending

The energy spent (ES) was measured to find the MET and the calories per minute (cal/minute). This was completed using the portable SenseWear® (BodyMedia, Pittsburgh, USA) equipment algorithm v5.2 in software v.8.1, which assesses the skin temperature, as well as the galvanic skin resistance. The monitor was attached with the elastic strap around the non-dominant arm and collected data was processed in software 8.1 using measured weight and height, together with information about age, sex, and dominant arm. The movement was measured using a biaxial accelerator to calculate the energy expenditure in MET and in cal/minute.[12,22]

## Statistical Analysis

The CPET was used as the gold standard and a comparison was made between the HRmax rate achieved during the CPET and the percentage of that HRmax rate achieved during AVG. A paired t-test was used to compare the activities. The SPSS 20.0 software was used in the analyses and the data were subjected to a normality test through visual graphic inspection and the Shapiro–Wilk test. A p value of <0.05 was considered significant.

**Table 1: Overall features of the 30 asthmatic children included within the sample population of this study.**

| Variables | Sample (n=30)* |
|---|---|
| Age (years) | 7.86±1.92 |
| Sex (female/male) | 13/17 |
| Weight (kg) | 29.94±13.11 |
| Score Z weight (mean) | 1.30 |
| Height (m) | 1.29±0.12 |
| Score Z height (mean) | 0.67 |
| BMI (kg/m$^2$) | 17.27±4.42 |
| Score Z BMI (mean) | 1.09 |
| Lean mass (%) | 81.05±8.68 |
| Fat mass (%) | 19.12±8.56 |
| FeNO (ppb) | 29.26±17.81 |
| $FEV_1$ (%) | 77±12.91 |
| $FEV_1$/FVC (%) | 84.37±12.66 |
| ACQ6 | 1.21 (1.15–2.30) |

*Thirty-four children started the assessment procedures but only 30 completed them.

ACQ6: Asthma Control Questionnaire 6; FVC: forced vital capacity; FeNO: fractional exhaled nitric oxide; $FEV_1$: forced expiratory volume in 1 second.

**Table 2: Data comparing the ergometric test with active video game playing.**

| | AVG | CPET |
|---|---|---|
| HRres | 100.5±5.03 | 98.8±9.31 |
| HRmax | 176.8±18.27 | 192.1±18.81* |
| HRmax/HRpred | 87% (±0.08) | 95% (±0.09) |
| $HR_{AVG}/HR_{CPET}$ | 93% (±0.14) | |
| $METS_{max}$ | 8.46±1.86 | 11.07±1.89* |
| $METS_{AVG}/METS_{CPET}$ | 76.42% | |
| $VO2_{PEAK}$ | 25.5±1.06 | 39.00±7.03* |
| $VO2_{AVG}/VO_{2CPET}$ | 65.4% | |
| Borg LL | 3.5 (3.0–6.5) | 4.75 (2.5–7.5)* |
| Borg dysp | 3 (1.5–3.5) | 2.5 (1.0–5.0) |

*p<0.05 paired t-test.

AVG: Active videogame; BORG dysp: effort perception dyspnea; BORG LL: effort perception in lower limbs; CPET: cardiopulmonary exercise test; HRmax: maximum heart rate achieved in each activity; HRpred: predicted max heart rate for age (208-[0.7*age]); HRres: heart rate at rest; METSmax: maximum metabolic equivalent measured in each activity.

## RESULTS

Table 1 shows the data from the assessed children; 34 children started the assessment sections, but only 30 children completed the tests. Four children did not complete the protocol due to transportation difficulties reaching the evaluation site.

The children in the sample consisted mostly of moderate and severe asthmatics according to GINA criteria,[10] with adequate height, percentage of body fat <20%, and FeNO levels >20 ppb (Table 1).

There are differences between the intensities of effort reached in CPET compared with AVG, with CPET achieving more intense activity than the AVG, but the level of effort reached when playing the AVG Reflex Ridge can still be considered moderate-to-intense.

The HRmax reached following the AVG session was 93% of that reached in the CPET, and 87% of the maximum predicted HR. An ES >6 MET is considered intense effort and >9 MET is considered a very intense effort. The perception of exertion in the lower limbs was higher during CPET compared with AVG, but there were no differences in the sensation of dyspnoea. Table 2 presents the intensity and ES data following CPET and AVG use.

The mean pre-AVG playing peak flow was 188.33±91.42 L when the mean of the greater three measures was considered, whereas the post-AVG playing peak flow was 194.66±86.70 L; thus, there was no significant difference. Just 2 out of the 30 children (6.6%) presented with a reduction >20% between the mean pre and post-physical activity peak flow during AVG playing; these children required a bronchodilator. The same children who had a reduction >20% in the peak flow in the AVG session also presented with this reduction after the CPET.

The predicted mean peak flow value of a population of children with asthma, assessed by height (cm) and recorded at sea level, would be 250.20±66.05 L according to Polgar and Promadhat.[13]

## DISCUSSION

According to the results presented here, the intensity of the physical activity during AVG sessions (more specifically, when playing Reflex Ridge) is high for asthmatic children. If regularly achieving this intensity is maintained

for a period >8 weeks, it can lead to an increase in aerobic capacity. The children in this study reached, on average, 87% of the HRmax expected for their age group, and this was similar to the results found in previous studies,[9] which have shown that 90% of expected HRmax was reached.

The literature has shown that exergames can lead to a greater energy expenditure and a more enjoyable experience than traditional physical activities, which makes children more motivated to play them. However, it is important to study different clinical conditions and age groups. McDonough et al.,[23] when evaluating the intensity reached during Reflex Ridge in young adults (mean age: 23.6 years±4.1 years), found that activity intensity reached a moderate effort level, which is 3–6 MET. Traditional exercise intensity reaches an intense level >6 MET. In addition, it is thought that undergoing an interval-based physical activity is safer than continuous aerobic activity regarding the induction of bronchospasm.[24]

These results presented in this study are quite close to those found by Holmes et al.,[25] who found individuals playing the Your Shape Xbox Kinect game achieved 86% of the HRmax achieved by individuals undergoing a CPET; however, that study recruited adults with cystic fibrosis.

AVG has been used in other populations of patients and the results appear to be promising when it comes to physical training, mainly when they are compared to results of a physical performance test. Del Corral et al.[8] compared different AVG, using a Wii (Nintendo, Kyoto, Japan) device, with the 6MWT and found that some games provided an effort intensity higher than that of the 6MWT.

Another important result in the current study regards the metabolic consumption, as the children reached 8.4 MET. It is worth highlighting that peaks >6 MET are considered to be intense. Therefore, such a result is a good indicator that AVG can provide positive physiological effects in this population if carried out periodically with adequate intensity and over regular time intervals. Other studies have shown the success of AVG use, including Holmes et al.,[25] who noted an exercise intensity of 6.1 MET using the Xbox Kinect in a population of adults with cystic fibrosis, and Le Gear et al.,[26] who noted 3.9 MET using the Wii in adults with chronic obstructive pulmonary disease who presented with a Borg Scale of approximately 3–5.

It is possible that such results were achieved because AVG allows breaks between bouts of physical effort. Thus, video game playing is more suitable to the physiology of children, who find performing continuous and lengthy activities more difficult.[27,28] Besides this, it is known that intense activities (which induce a 80–90% of HRmax response) and those with a 6–8-minute duration may trigger EIB in 40–90% of children with eosinophilic asthma (most of the children in this study were of this phenotype).[25] Just 2 (6.6%) of the children in this study presented with some degree of respiratory discomfort during the time of the activity. This reinforces the safety of using AVG as a physical activity.

The activities performed on the treadmill are continuous, while video games, including the one used in this study, are interval-based. In this study, each Reflex Ridge match had a 3-minute duration, with a 30-second interval between matches, and the participants reached a mean HR of 70% of HRmax with a peak of 93%. The children usually hit this intensity in the third or fourth match out of ten in total.

According to Sidiropoulou et al.,[25] regular interval-based physical activity performed by asthmatic children may reduce the risk of EIB. It is worth highlighting that the activity should be low-duration and high-intensity.

There has been growing interest in activities with AVG or exergames over recent years using both the Wii and Xbox 360. These were originally developed as entertainment systems, but this study and others like it present the potential for these systems to be therapeutic resources. However, it should be emphasised that this type of physical activity presents with certain limitations, such as the inability to establish an individual intensity for each child.

Regarding energy expenditure, Sensewear may underestimate the intensity of activities >10 MET, but it is accurate to be equivalent to calorimetry measurements at moderate activity levels as was the case in this study. The algorithm of version 8.1

of Sensewear has good applicability to evaluate energy expenditure in children, which makes it feasible for this type of evaluation.[29,30] A limitation of the study was that calorimetry could have been used to record an additional measure of energy expenditure in the activity; future studies may consider this possibility.

## CONCLUSION

These results show that high-intensity physical activities using AVG can ensure an exercise intensity similar to that of exercises performed on ergonomic treadmill to be drawn. AVG playing can be considered a safe, motivating, and efficient treatment modality for asthmatic children.

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# Natural Killer Cells and Their Role in Immunity

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**Disclosure:** The author has declared no conflicts of interest.

**Received:** 05.03.19

**Accepted:** 04.04.19

**Keywords:** Cancer, immune response, memory, natural killer (NK) cells, viral infection.

**Citation:** EMJ Allergy & Immunol. 2019;4[1]:108-116.

## Abstract

Natural killer (NK) cells are effector lymphocytes that play protective roles against both infectious pathogens and cancer. Although NK cells contribute to the innate immune system, they have a number of similarities to cells of the adaptive immune system, including T and B cells. Recent discoveries have also shown that NK cells are capable of adapting and developing into long-lived memory cells, providing new functional insights into the roles of innate immune cells. In this article, the author provides an overview of human and murine NK cell development, function, and memory, as well as their role in viral infection and cancer.

## INTRODUCTION

Natural killer (NK) cells were originally described in the 1970s as large granular lymphocytes able to develop natural cytotoxicity against tumour cells without a prior encounter.[1-3] They play an invaluable role in early defence against invading pathogens and cancer and are able to produce an array of cytokines and chemokines to help regulate an immune response.[4] NK cells make up 5–15% of human peripheral blood and 2–3% of murine splenocytes.[5] They are found in both primary and secondary immune compartments, with the majority of cells being localised in the spleen, lymph nodes, bone marrow, and peripheral blood to carry out immunosurveillance throughout the vasculature. They are also found in mucosal tissues, including the lungs, small and large intestines, and colon.[6] Along with their roles in immune defence, NK cells play significant roles during pregnancy. In response to sex hormones, there is a substantial increase in uterine NK cells, which are thought to promote placental growth and provide maternal–fetal immunomodulation;[7] however, both peripheral NK cells and uterine NK cells have been associated with infertility and miscarriage.[8] Almost a decade ago, NK cells were recognised as a member of the lymphocyte family known as innate lymphoid cells (ILC). ILC were classified into three main groups based on their cell surface marker expression, functionality, and transcriptional regulation.[9] Group 1 ILC, which originally included ILC1 and NK cells, were distinguished from other ILC groups by their constitutive expression of *TBX21* and its protein product T-bet, and the production of IFN-γ following IL-12 stimulation.[10] However, recently the ILC family has been reclassified into subsets based on their development from the common lymphoid progenitors (CLP) and their immune functions. In these subsets, NK cells are no longer grouped with ILC1.[9] This article will review the development, function, and memory capacity of NK cells, along with their roles during viral infection and cancer.

## NATURAL KILLER CELL DEVELOPMENT

NK cells have been classified as components of the innate immune system; however, they have also been shown to possess numerous developmental and functional characteristics similar to cells of the adaptive immune system, including T and B cells. These include the development from the CLP in the bone marrow, expression of the recombination-activating genes during ontogeny, the need for common γ-chain-dependent cytokines (including IL-15) during development and homeostasis, and an education process analogous to T cell development in the thymus.[11,12] It has also been proposed that NK cells may develop in both the thymus and the liver.[13] Furthermore, much like T and B cells, which use their activating receptors (T cell or B cell receptor, respectively) to recognise antigens, NK cells express germline-encoded activating receptors that are able to bind directly to stress-induced or pathogen-derived antigens.[14]

Developing from haematopoietic stem cells, CLP in murine bone marrow differentiate into pre-NK precursors with a Lin-CD117$^{lo}$CD127+ phenotype and express a number of NK cell-specific receptors including NKG2D and CD244,[15] but during this stage the cells are negative for classical markers such as NK1.1 and CD49b. Following the expression of the β-chain receptor for IL-15 (a cytokine required for NK cell development), these cells are now classed as NK precursors. Once CD122 is expressed, they become responsive to IL-15 and develop into immature NK cells, observed by CD11b$^{lo}$ and CD27 surface expression.[16] At this point, a number of activating and inhibitory receptors are also beginning to be expressed on the surface of the developing NK cells.[17] CD11b and CD27 expression defines murine NK cells in four stages of maturation, which correspond with their cytolytic activity and production of inflammatory cytokines (Figure 1). During maturation, immature NK cells progress to CD11b$^{lo}$CD27$^{hi}$, then CD11b$^{hi}$CD27$^{hi}$, and finally CD11b$^{hi}$CD27$^{lo}$.[18] In humans, NK cells also develop from haematopoietic stem cells and through a CLP. During five stages of development, there are a number of changes in expression levels of CD56, CD94, and CD16, and, much like for mice, human NK cells become responsive to and require the cytokine IL-15. Maturing human NK cells can differentiate into CD56$^{bright}$ cells, which usually remain in the lymphoid tissue to interact with dendritic cells (DC) and CD56$^{dim}$ cells that return to circulation via the lymphatics.[15] Human NK cells are considered fully mature when they have high cytolytic activity and are able to produce large amounts of IFN-γ.[17]

Along with the expression of various cell surface markers and receptors, there are complex networks of transcription factors that can help dictate lymphocyte lineage commitments and give rise to distinct cell fates. Thymocytes can be diverted into an NK cell-like lineage if Bc111b, a Notch-1-dependent transcription factor, is ablated during T cell development.[19] Furthermore, NK cells and other helper ILC, but not T or B cells, require Id2 and Nfil3 for their development.[16]

## NATURAL KILLER CELL FUNCTION

The effector function of NK cells is determined by an integration of numerous signals. To sense their environment, NK cells use a tightly regulated balance of activating and inhibitory germline-encoded receptors, and initiation of an NK cell response is dependent on signalling via these receptors (Figure 2). Under physiological conditions, circulating NK cells are mostly in a resting state; however, activation by an array of cytokines can lead to the infiltration of these cells into pathogen-infected or cancerous tissues.

Healthy cells express major histocompatibility complex class I (MHC I) molecules that act as ligands for inhibitory receptors on NK cells and contribute to the 'self-tolerance' of these cells. Killer cell immunoglobulin-like receptors in humans or members of the Ly49 family in mice make up the main inhibitory receptor profile of NK cells that bind MHC I molecules and maintain a tolerance for healthy host cells.[20] However, during viral infection and tumour development, MHC I molecules are usually downregulated, lowering the inhibitory signalling threshold of the NK cell and leading to cell activation.[21] Cellular stresses associated with infection or cancer growth, such as DNA damage responses and the expression of tumour suppressor genes, cause the upregulation of activating receptors on NK cells.

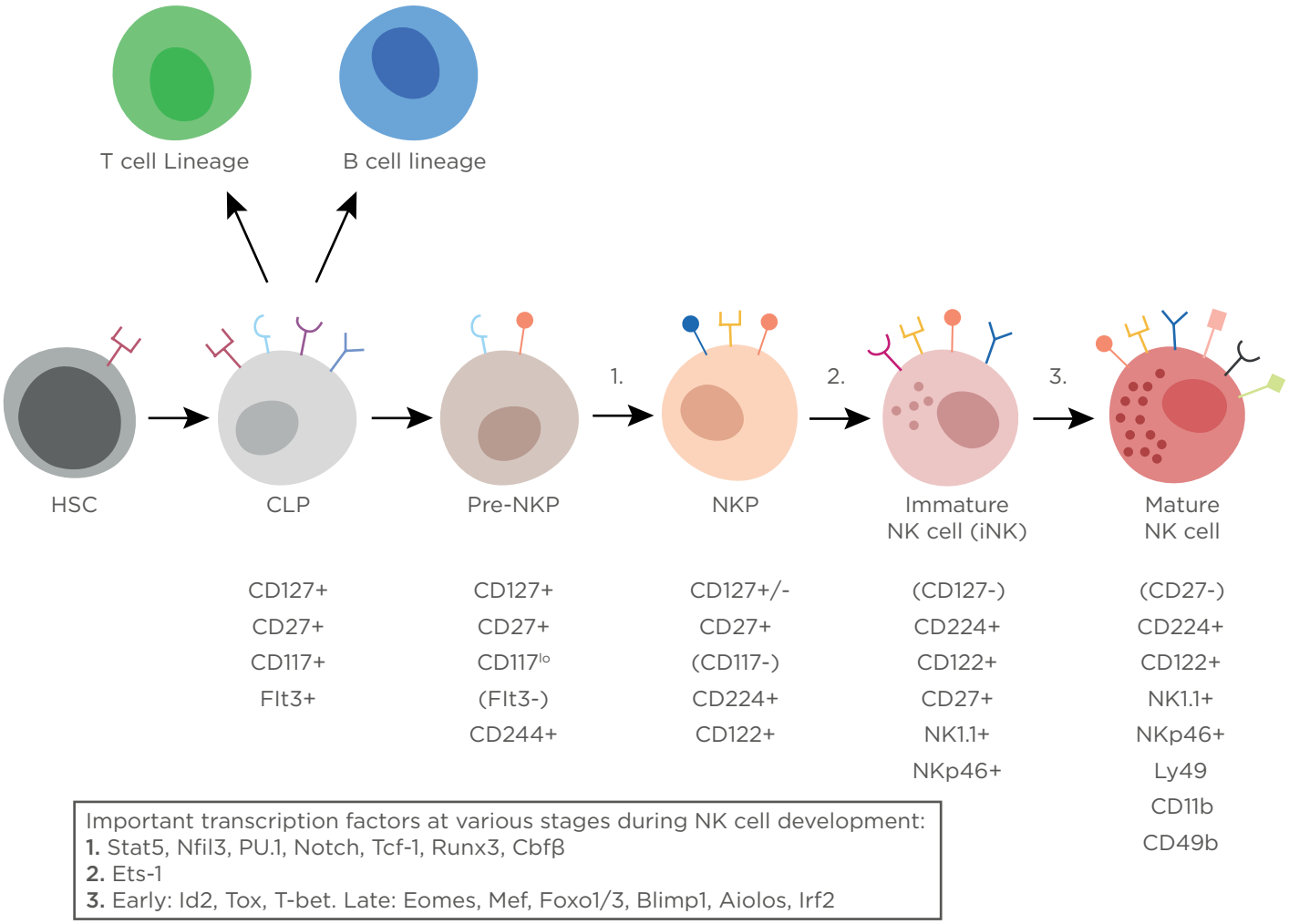


**Figure 1: An overview of natural killer cell development.**

NK cells derived from the CLP differentiate into a pre-NKP population, identified by its expression of CD117 and lack of CD122 expression. After becoming an NKP, the cells start expressing NK cell markers (NK1.1 and NKp46) and are considered to be immature NK cells at this stage. As they mature further, they acquire CD49b and CD11b expression and lose the expression of CD27. Fully mature NK cells are able to express cytolytic molecules and cytokines (including IFN-γ). Transcription factors also play important roles in governing lymphocyte fate from the CLP. A simplified list of transcription factors driving the NK cell lineage is shown in the box and the numbers on the diagram indicate where they have been identified at the different stages during development. 'Early' and 'Late' indicates when they are thought to be important during the maturation process.

CLP; common lymphoid progenitor; HSC: haematopoietic stem cell; NK: natural killer; NKP: NK cell precursor.

In most cases, NK cells are governed by a number of receptors and regulated by the integration of co-activating (NKp46, NKG2D, CD16, and LFA-1) and co-inhibitory (NKG2A, KLRG1, and TIGIT) signals via surface receptors that recognise the appropriate ligand.[22] Signalling via the activating receptors on NK cells causes a shift in the balance towards activated NK cells that are able to directly eliminate target cells through NK cell-mediated cytotoxicity, or indirectly via the secretion of pro-inflammatory cytokines. There are also cytokine receptors that transmit activating (including IL-2, IL-12, IL-18, Type 1 IFN, and TNF-α) or inhibitory signals (TGF-β) during NK cell activity.[22] Additionally, ligand interactions with cell–surface receptors on NK cells can lead to the secretion of pro-inflammatory cytokines, including IFN-γ.[23]

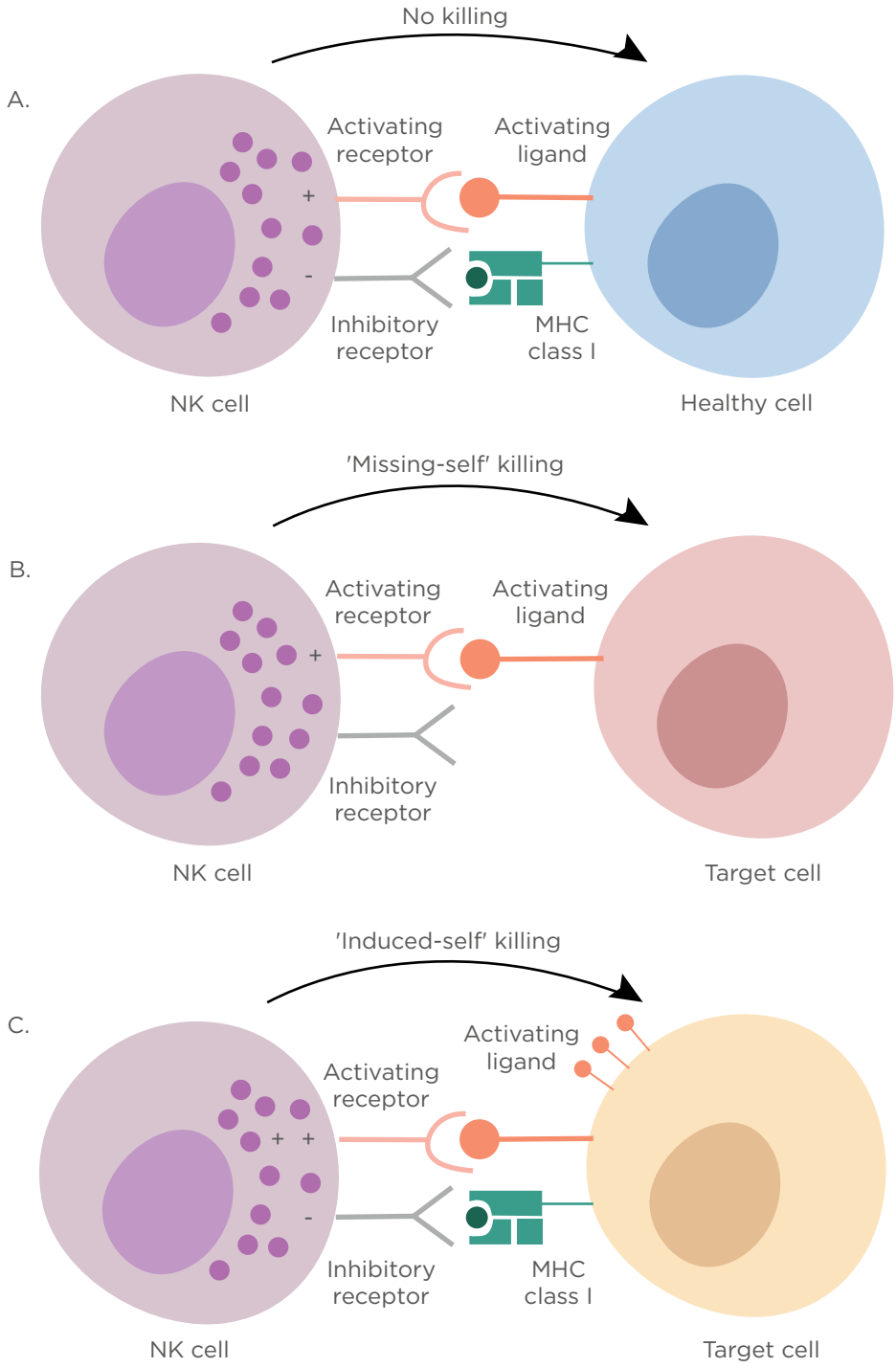


**Figure 2: Schematic of natural killer cell function during 'resting' and 'killing' states.**

NK cells are able to recognise and kill target cells by an integrated balance of activating and inhibitory signals, which allow them to distinguish between healthy cells and target cells (those virally infected or transformed). A) 'No-killing' NK cells have balanced activating and inhibitory signals when recognising healthy cells. The inhibitory signals are delivered by self-MHC class I in this setting. B) Target cells, such as those infected or transformed, often downregulate or lose MHC class I molecules on their surface and these cells are detected by the NK cells as 'missing-self' leading to killing of the target cell. C) Tumour cells and virally infected cells often overexpress activating ligands on their surface, which are recognised by activating NK cell receptors; this process overrides any inhibitory signals triggering 'induced-self killing' and lysis of the target cell.

A '+' symbol indicates activating signal and a '-' symbol indicates inhibitory signal.

MHC: major histocompatibility complex; NK: natural killer.

## NATURAL KILLER CELL MEMORY

The quick response capabilities and enhanced host protection against a previously encountered pathogen make up the classical definition of immunological memory.[24] Much like T cells, NK cells are able to acquire functional qualities associated with immunological memory in both non-infection settings and in response to pathogens.

During different educational routes, the formation of NK cell memory can occur in two ways: via antigen-dependent (virus or hapten-induced) or antigen-independent (cytokine-induced) mechanisms. Sensitising mice with haptens (molecules able to stimulate the production of antibodies) in the presence of the pro-inflammatory cytokines IL-12, IFN-α, and IFN-γ leads to hapten-specific memory NK cells.[25,26] Long-lived memory cells are generated following an infection and show a heightened response upon secondary challenge with the same pathogen. The memory formation process in T cells has been well characterised and is usually distributed into three main phases.[27] Upon cognate antigen exposure, naïve T cells clonally expand and differentiate into effector T cells during the 'expansion' phase. This is followed by a second 'contraction' phase, in which most of the effector cells undergo apoptosis, leaving a small pool of stable T cells that can enter the 'memory' phase. These memory T cells persist throughout the organs of the host and maintain their longevity through self-renewal until they encounter their cognate ligand, where they display enhanced host protection and effector function.[28] As cells of the innate immune system are unable to undergo somatic rearrangement of their receptor genes, it was thought that these cells, including NK cells, lacked antigen specificity and were, therefore, unable to develop classical immunological memory.[14]

However, in the common inbred laboratory mouse (C57BL/6), the activating receptor, Ly49H, which is expressed on approximately 50% of NK cells, binds with precise specificity to the mouse cytomegalovirus (MCMV)-encoded glycoprotein m157 expressed on infected cells to drive the expansion of virus-specific NK cells during the acute phase of MCMV infection.[29,30] Once the infection is under control, expanded effector NK cells undergo a contraction phase to form a pool of long-lived, self-renewing 'memory' or 'adaptive' antigen-specific NK cells, a response similar to that observed in T cells. These NK cells can be recovered months after the initial infection in a number of peripheral tissues.[31] The expansion and memory formation of virus-specific NK cells is dependent on an interaction with the viral antigen, as MCMV lacking the m157 glycoprotein does not induce Ly49H NK cell expansion or the development of memory.[31] Previous studies have also shown that memory NK cells have a unique transcriptional signature when compared to naïve NK cells[24] and possess functional attributes commonly associated with memory T cells, including secondary expansion, enhanced effector function, and increased protection against viral challenge.[31] However, until recently, little was known about how transcription is controlled at an epigenetic level in NK cells while they transition between naïve, effector, and memory states. Chromatin accessibility analysis by assay for transposase-accessible chromatin using high-throughput sequencing (ATAC-seq) and transcriptional profiling via RNA-seq showed that, during MCMV exposure, NK cells undergo dynamic changes in chromatin architecture and that NK cells and CD8+ T cells share common epigenetic and transcriptional programmes as they transition from naïve to memory cells.[32]

## NATURAL KILLER CELLS IN VIRAL INFECTION

NK cells play an important role in viral clearance but their responses were initially thought to be non-specific and lacking an immune memory response.[33] However, it is now understood that NK cells are able to respond specifically to an infection and, in many cases, are able to develop memory recall responses.[33] Inflammatory states can cause NK cells to enter the lymph nodes and influence T cell responses by promoting Th1 cell polarisation through the release of IFN-γ or restricting the expansion of T cells by killing activated cells.[34] In healthy individuals, NK cells contribute to controlling several viral infections, including cytomegalovirus (CMV), influenza, hepatitis C, and HIV-1.[35] Humans with complete or partial impairment in NK cell numbers or function have also been shown to have increased susceptibility to viral infections,

including herpes simplex virus, CMV, varicella zoster virus, and human papillomavirus.[35]

During viral infection, NK cells use a number of approaches to sense inflammatory signals and express receptors for cytokines, including IFN-α, IL-12, IL-15, and IL-18, whose expression is greatly upregulated during early infection, providing a vital role in the activation of NK cells and host protection.[36] Many cytokine receptors are uniformly expressed by NK cells, suggesting cytokines are able to signal to most NK cells and in some cases activate the entire NK cell compartment.[4] IL-12, IL-15, and IL-18 also provide important stimuli for the expression of IFN-γ, a hallmark of NK cell expansion in humans seropositive for human CMV (HCMV).[37] Type I interferons, IFN-α and IFN-β, have also been shown to play an important role in increasing the cytotoxicity of NK cells[38] while protecting NK cells from fratricidal killing, leading to enhanced defence and cellular expansion.[39] Furthermore, mouse IL-12 induces epigenetic remodelling in regulatory regions of genes encoding transcription factors such as *Zbtb32, Runx1,* and *Runx3*, thereby contributing to NK cell expansion during MCMV.[40] Along with the expression of cytokine receptors, NK cells express low-affinity Ig-G receptor FcRIII (CD16), allowing NK cells to bind to and become activated by antibody-coated target cells, a process known as antibody-dependent cellular cytotoxicity.[41] This antibody-dependent activation of NK cells has been identified in response to a number of viruses, including influenza, HIV-1, and CMV.[42-44]

As discussed previously, NK cells possess a large number of activating and inhibitory receptors that are known to play important roles in controlling viral infection. The majority of NK cells express the activating receptors NKG2D, DNAM-1, NKp46, and, in humans, NKp30. The ligand for NKG2D is upregulated following environmental cues, such as cellular stress caused by viral infection, and this 'induced-self' recognition allows for NK cells to broadly survey for stressed cells and remove unhealthy or harmful host cells.[45] NKG2D also provides a co-stimulatory signal to enhance proliferation and effector responses of NK cells during MCMV infection; however, NKG2D alone is unable to drive a robust expansion.[46] Furthermore, ligands for DNAM-1 are upregulated during cellular stress but, again, this signal alone is not sufficient to expand NK cell subsets.[47] Thus, co-stimulatory functions are needed for optimal expansion and differentiation of Ly49H+ NK cells during MCMV. Along with stress-induced receptor signals, other activating receptors are expressed to precisely sense viral signals including NK1.1, which is found on the majority of NK cells and recognises the MCMV encoded protein m12,[48] or NKG2C (human) and Ly49 family (mouse) receptors on specific subpopulations of NK cells that are activated by an interaction with their cognate viral ligands.[11] During HCMV infection, viral peptides derived from UL40 and presented on HLA class I histocompatibility antigen, alpha chain E and recognised by NKG2C, inducing population expansion of NKG2C+ NK cells.[49] During MCMV infection in mice, the viral protein m157 is expressed on the surface of infected cells and recognised by the Ly49H receptor.[50]

## NATURAL KILLER CELLS IN CANCER

NK cells were first identified for their ability to kill tumour cells without prior sensitisation. They are able to directly kill tumour cells through the release of cytotoxic granules containing granzyme and perforin.[51] NK cells and cytotoxic CD8+ T cells work together to generate an immune response against viruses and tumour cells; however, tumours often downregulate MHC I, making them unrecognisable to cytotoxic T cells, leading to a failure to initiate adaptive immune response functions.[52] The lack of MHC I expression or an upregulation of NKG2D ligands or CD70 (the ligand for CD27) can still render tumour cells susceptible to NK cell-mediated lysis.[53] NK cells play vital roles in eradicating tumour cells and numerous studies have shown this *in vivo* by implanting tumour cells into mice genetically lacking NK cell function or by the administration of antibodies to deplete NK cells.[54] In most cases, eliminating NK cells in these mice led to more aggressive tumour growth and metastasis.[54] NK cells are also able to exert cytotoxicity against an array of malignancies, including acute myeloid leukaemia, acute lymphocytic leukaemia, and multiple myeloma, along with many solid tumours, including ovarian and colon tumours, and neuroblastomas.[49,55]

NK cells can be activated by various stimuli, including contact with DC. DC are the main

antigen-presenting cells of the immune system and play a fundamental role in sensing pathogens and initiating an immune response. A bi-directional crosstalk between DC and NK cells has been observed in secondary lymphoid tissues and in the periphery via cell–cell contacts or the release of soluble factors.[56] These interactions can result in cellular activation and maturation, and the production of cytokines by both cell types.[57] Additionally, NK cells are able to directly or indirectly regulate T cell responses because NK cell-mediated killing can cause the release of antigens, which are able to enhance CD8+ T cell activation by cross-presentation on DC.[58] The tumour microenvironment contains transformed cancer cells along with stromal cells that are able to control tumour progression. Recent findings have shown that human NK cells directly identify the ligand platelet-derived growth factor DD (PDGF-DD) by using the activating receptor NKp44, which, in turn, enables NK cells to limit tumour growth.[59] This PDGF-DD–NKp44 interaction stimulates NK cell secretion of IFN-γ, TNF-α, and chemokines. The cytokine production triggers tumour cell–cycle arrest.[59] Conversely, cancer cells are able to evade the immune response and NK cells by using a number of mechanisms; these include increasing MHC I molecules to inhibit NK cell function, decreasing NKG2D ligand expression to impair NK cell recognition, and upregulating levels of inhibitory cytokines (such as IL-10 or TGF-β) in tumours following secretion by the tumour itself, regulatory T cells, or myeloid derived suppressor cells.[60]

Although it has been known for some time that NK cells play a key role in fighting tumour development and progression, in more recent years NK cell-based immunotherapy has become a novel and promising approach to treating tumours.[61] Preclinical and clinical studies have focussed on host NK cells and their antitumour function, and administration of activating cytokines (IL-2 and IL-15) have shown mixed results.[62] IL-2 treatment in mice was shown to be efficacious in improving antitumour responses and was approved for clinical use in a number of human cancer types;[63] however, following injections of IL-2, tumour relapse and survival rates were unaltered in some settings.[64] IL-15 treatment became an alternative to IL-2, and using IL-15/IL-15R complexes to reflect trans-presentation of IL-15 physiologically,[65] and, in combination with other immunotherapies, have also become a main focus of many studies.

## CONCLUSION

NK cells are a key component of the immune response and play vital roles in controlling and eliminating both virally-infected and cancer cells. Although our knowledge of basic NK cell biology and innate immunity continues to grow rapidly and many studies have shown that the development and function of NK cells is highly dynamic, there is still much to be investigated. The effector function of these NK cells must be further studied, with a predominant focus on immunotherapies along with the prevention of infectious diseases and cancer. Furthermore, the discovery of NK cell immunological memory and epigenetic reprogramming during infection has led to many thought-provoking and exciting questions regarding both innate and adaptive immune responses.

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# HIV Co-Receptor Usage, Broadly Neutralising Antibodies, and Treatment

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**Disclosure:** The authors have declared no conflicts of interest.

**Received:** 09.02.19

**Accepted:** 04.04.19

**Keywords:** Broadly neutralising antibodies (bnAb), clinical use, co-receptor, envelope, HIV.

**Citation:** EMJ Allergy Immunol. 2019;4[1]:117-125.

## Abstract

The discovery of a new generation of highly potent broadly neutralising antibodies (bnAb) has provided a new weapon in the fight against HIV-1. It is envisioned that multiple bnAb or a single bnAb in conjunction with antiretrovirals (ARV) can be used to treat HIV infection, especially individuals harbouring extensively drug-resistant virus or those that require regimen simplification. Furthermore, it is believed that bnAb may eliminate latently infected cells through antibody-mediated cellular cytotoxicity, and this functionality may induce virus remission. BnAb epitopes and HIV envelope determinants for CCR5 and CXCR4 usage often overlap, and this provides the basis for believing that there is a relationship between receptor utilisation and bnAb sensitivity. This review highlights the important intersection between HIV co-receptor usage and bnAb therapy. Compared to CCR5-using strains, CXCR4 strains are generally more resistant to bnAb that target the V1-V2 apex and V3 N332 glycan, but not the other envelope domains. This association between bnAb sensitivity and co-receptor usage can be leveraged both to develop pre-treatment assays to identify resistant strains, as well as to anticipate potential adverse outcomes with future HIV antibody-based therapeutics.

## INTRODUCTION

Combination antiretroviral therapy (cART) suppresses HIV-1 replication below detectable levels in the plasma, delays the onset of AIDS, and prolongs the lifespan of infected individuals.[1] Current therapies, however, must be taken on a daily basis and continued indefinitely. Whenever antiretrovirals (ARV) are stopped, the virus begins replicating again, and the march toward AIDS resumes for the patient. Virus replication re-emerges because of the presence of infectious virus in latently infected cells which are not eliminated by cART.[2] Chronic cART use can also be complicated by drug-associated adverse events and emergence of drug resistance. Thus, novel therapies are needed to overcome these shortcomings of current cART.

Passive infusions of some broadly neutralising antibodies (bnAb) or vaccination with vectors that generate antibodies have been shown to decrease virus replication in animal models[3-7] and human trials.[8-13] Antibody-based therapies provide an attractive new anti-HIV-1 weapon because of dosing simplification and activity against multidrug-resistant HIV. Furthermore, it has been

hypothesised that antibody-associated effector functions, such as natural killer cell-mediated antibody-dependent cellular cytotoxicity, can eliminate latently infected cells, which could potentially induce virus remission in the absence of ARV.[14,15] Often, the same HIV envelope glycoprotein domain contains both a bnAb epitope and the determinant for receptor usage that is necessary for host cell entry. This overlap provides the scientific basis for speculating that there is an association between the receptors viruses use to enter cells and bnAb sensitivity. Although there have been a number of recent reviews on bnAb, there has been relatively sparse discussion of the intersection between HIV-1 receptor utilisation and bnAb sensitivity.[16-19] The primary purpose of this review is to discuss the association between receptor usage and neutralising antibodies (nAb) and to highlight possible clinical implications for future antibody-based treatments.

## BROADLY NEUTRALISING ANTIBODIES TARGETING ENVELOPE VARIABLE LOOP DOMAINS MAY IMPACT RECEPTOR USAGE

Developing strategies that obviate the need for daily ARV, either through a sterilising cure that eliminates all latently infected cells or a functional cure that prevents virus re-emergence after cART discontinuation, are top priorities in the HIV-1 field.[2] The isolation of potent and broad nAb from HIV-1-infected individuals has energised this endeavor.[16-19] Even though no immunogen has been designed that can elicit bnAb, repeated passive bnAb infusions or vaccination with a vector that produces an antibody are being proposed as feasible alternative strategies to generate therapeutic levels that suppress plasma viraemia and induce antibody-mediated cellular cytotoxicity that can potentially eliminate latently infected cells. In contrast to the current ARV, bnAb can be given monthly or at even longer intervals. Furthermore, bnAb have been shown to enhance the anti-HIV-1 humoral immune response in animal models; this augmented immunity could yield virus remission in absence of ARV.[14]

In order to enter cells, HIV must bind the CD4 receptor and then a co-receptor: either CCR5 or CXCR4.[20] Early after infection the majority of HIV-1-infected individuals harbour variants that bind the CCR5 receptor (termed R5). Over time, some individuals can develop a dual-mixed (DM) virus population containing a mixture of R5 variants, viruses that are capable of using both the CCR5 and the CXCR4 (R5X4), or exclusively the CXCR4 (X4) receptor.[21-23] The evolution to CXCR4 usage over the course of HIV infection primarily depends on sequence evolution in the HIV envelope glycoprotein variable loop 3 (V3). Envelope glycoprotein V3 loop modifications are often accompanied by modifications in the variable loop 1 and 2 (V1-V2) region due to fitness constraints.[24,25] Although the HIV envelope glycoprotein sequence changes over time in all infected individuals, not everyone develops CXCR4-using virus. In addition, the percentage of individuals that eventually develop a DM population varies by HIV-1 subtype.[21-23] Among the most prevalent HIV-1 subtypes circulating in the world, people infected with HIV-1 subtype B (HIV-1B) and D (HIV-1D) are more likely to have CXCR4-using virus as compared to those infected with subtypes A (HIV-1A) and C (HIV-1C). In addition to having differences in the frequency of CXCR4-usage, various subtypes also have varying susceptibilities to different bnAb directed against the envelope.[26] Envelope glycoprotein differences among the various HIV-1 subtypes contribute to the variation in both bnAb sensitivity and frequency of CXCR4-using variants. The overlap between the location of the antibody epitopes and the co-receptor usage determinants potentially provides a rationale for these observations.

The majority of nAb against HIV-1 attach to the HIV envelope and prevent host cell entry by restricting receptor attachment and subsequent fusion. Currently the isolated bnAb target diverse envelope regions, including 1) the CD4 binding site (bs); 2) glycosylation-dependent epitopes in the V1-V2 loops; 3) a glycan patch encompassing the envelope V3 loop; 4) membrane proximal external region on the envelope gp41 domain; 5) glycan-dependent epitopes that bridge gp120 and gp41 including the fusion peptide; and 6) the silent face.[27-34] The V1, V2, and V3-directed bnAb target a glycosylated asparagine (N) at envelope position 160 and 332 respectively, and thus they are often also referred to as N332 and N160 bnAb, respectively. While the activity of the N160 and N332 bnAb primarily depend on the

presence of these glycans, other amino acids, especially those in and around the V1-V2 and V3 envelope regions, also impact neutralisation. For instance, the N332 bnAb PGT121 interacts with residues at the tip of the V3 loop, including the crown and amino acids directly before and after it. Another N332 bnAb, 10-1074, interacts with residues towards the base of the V3 loop.[35] Involvement of these other envelope V1-V2 and V3 amino acids, similar to those identified as key determinants for co-receptor usage, provides the basis for speculating that co-receptor usage may be associated with bnAb activity.

When considering this association, it is crucial to understand the differential neutralisation sensitivity of CCR5 and CXCR4-using viruses. Earlier studies implied that CXCR4-using viruses were more antibody neutralisation-sensitive compared to CCR5-utilising strains. In an animal model, it was demonstrated that the emergence of CXCR4-using simian HIV was temporally related to the loss of humoral immunity.[36] Antibodies that were present prior to the emergence of the CXCR4-using virus potently neutralised the X4 strain. This suggested that antibodies had prevented the emergence of a CXCR4-using virus and the X4 strain appeared as an escape variant only after humoral responses diminished with progressive immunodeficiency. The earliest human studies also deemed CXCR4-utilising strains more neutralisation-sensitive compared to CCR5-utilising strains. These investigations primarily compared the neutralisation susceptibility of either co-circulating or heterologous CCR5 and CXCR4-using strains to heterologous antibodies, such as first-generation bnAb, which have relatively limited potency and breadth compared to second-generation bnAb.[37-40] More recent studies have provided some conflicting data. First, the administration of second-generation N332 bnAb (PGT128) after established infection in humanised mice generated resistant virus.[6] Importantly, envelope sequence but not phenotypic analysis predicted that a higher proportion of the emerging resistant virus used the CXCR4 as opposed to the CCR5 receptor.[41] This implies neutralisation-resistant CXCR4-using variants emerged with N332 bnAb, PGT128, administration. In contrast to earlier human studies which never assessed sensitivity to second-generation bnAb, X4 as compared to R5 strains are less neutralisation-sensitive to N160 (PG9 and PG16) and N332-directed bnAb (PGT128 and CAP8 serum).[41,42] It should be noted that these studies have not evaluated the possibility that a greater frequency of X4 as compared to R5 isolates lack the N160 or N332 glycan.

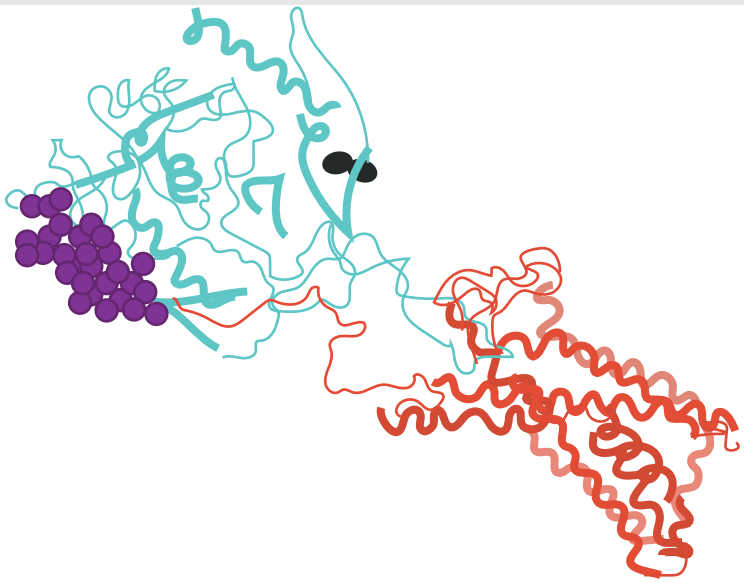

**Figure 1: Structural model of HIV-1 envelope and CCR5 receptor.**

Structural model shows HIV-1 envelope (cyan) and CCR5 receptor (red). Amino acid 332 is highlighted in black. The V1-V2 loop is shown is purple. The original structure lacked the envelope 160 amino acid. The published structure (PDB 6meo) was manipulated in PyMol for this figure.[43]

This is unlikely, however, because the absence of N160 and N332 have never been demonstrated to be predictive of CXCR4 usage. Furthermore, recent structural data demonstrates that the envelope 332 amino acid makes no contact with the CCR5 receptor (Figure 1).[43] The structure also suggests that the envelope 160 amino acid does not directly interact with the CCR5 receptor although the solved structure lacked this V1-V2 amino acid. It has been suggested that X4 as compared to R5 strains have a more open trimer structure, and this change may prevent N160 bnAb from binding because these antibodies depend on a quarternary epitope.[28-44] The X4 and co-circulating R5 variants, however, have similar susceptibility to CD4 bs (VRC01 and VRCPG04) and membrane proximal external region (10E8)-directed antibodies. Furthermore, co-circulating X4 as compared to R5 strains have been shown as more neutralisation-resistant to contemporaneous autologous and heterologous plasma.[42] In aggregate, the earliest and newer studies suggest that X4 and R5 variants have different neutralisation sensitivities. Importantly, the most recent studies have shown that X4 compared to R5 strains are more resistant to the second-generation bnAb that target the N160 and N332 envelope glycans.

## HUMORAL IMMUNE PRESSURE MAY SELECT FOR CXCR4-USING VIRUS

The mechanism for the evolution of DM virus population and the difference in the frequency for the emergence of CXCR4-using variants in various subtypes remains uncertain. Although the distinction between CCR5 and CXCR4-utilising strains has been known for over 25 years, surprisingly, previous studies have not elucidated a definitive mechanism for the emergence of CXCR4-using viruses over the course of infection. One hypothesis suggests that CXCR4-using viruses potentially emerge because of inadequate CCR5 receptor levels or limited availability of CCR5 bearing target cells.[22] Indeed, individuals with heterozygous CCR5 delta 32 genotype, who have lower levels of the CCR5 receptor on their cells, have an increased likelihood of harbouring CXCR4-using viruses compared to people with both wild-type alleles.[45] It should be noted, however, that after CXCR4-using strains emerge, R5 strains often persist, implying that co-receptor switching does not always occur in response to the limited availability of CCR5 receptor positive-susceptible cells.[21,23] As stated above, the observations from the non-human primate studies argue another possible hypothesis; CXCR4-using variants arise because of random mutations, and these new viruses persist because declining adaptive and innate immune responses fail to clear the less fit dual and/or X4 HIV-1.[22,36]

Lastly, newer observations suggest yet another possibility, that humoral immune pressure may play a role in the emergence of CXCR4-using viruses. While it is true that all HIV-1-infected individuals develop nAb against their autologous circulating viruses, the envelope region targeted by the autologous antibodies differs based on the characteristics of the circulating strains.[46-50] Indeed, only a small proportion of HIV-1-infected individuals develop a broad and potent humoral immune response, and the evolution of a bnAb is directly dependent on the antigenic properties of the circulating viruses' envelope glycoprotein.[51-54] The authors speculate that some infected individuals likely develop a unique type of antibody, termed CXCR4-inducing antibody (CXCR4-iAb) (Figure 2). The emergence of a CXCR4-iAb only occurs in the presence of specific HIV envelope glycoproteins and predominate only in certain HIV-1 subtypes. Because glycosylation modifications in the envelope variable loops have been associated with the R5–X4 transition, it is possible that CXCR4-iAb target glycan-rich epitopes in the envelope V1-V2 or V3 regions.[21,24,25] To escape these nAb pressures, some R5 variants could evolve CXCR4 usage while other co-existing R5 viruses that are not susceptible to CXCR-iAbs retain the same co-receptor phenotype. This speculative model predicts that individuals with CXCR4-iAb should have X4 variants that are more neutralisation-resistant compared to co-existing R5 variants. As stated above, previous studies have already demonstrated multiple instances in which co-circulating CXCR4 compared to CCR5-using variants are more neutralisation-resistant to autologous plasma.[42] The model further predicts that passage of some neutralisation-sensitive R5 variant in the presence of CXCR4-iAb should lead to the emergence of the CXCR4-using virus.

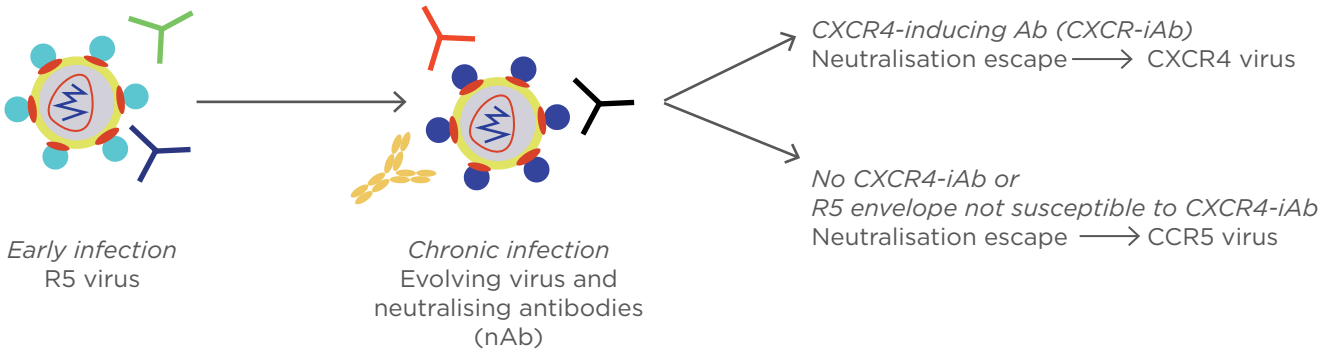


**Figure 2: Potential model for emergence of CXCR4-using strains.**

CCR5-using viruses (primarily R5) are present early after virus acquisition. Over time, the virus envelope glycoprotein changes in response to neutralising antibodies. CXCR4-using viruses emerge in some individuals that develop unique antibodies termed CXCR4-iAb. Individuals that lack CXCR4-iAb continue to have CCR5-using strains.

Indeed, this was observed with the emergence of a CXCR4-utilising variant after passaging a R5 variant in the presence of autologous contemporaneous plasma *in vitro*.[42] Furthermore, neutralisation-resistant CXCR4-using strains have been shown to emerge late in disease, suggesting that these types of strains emerge only after prolonged exposure to host humoral immune pressure.[55] Isolation and characterisation of these proposed CXCR4-iAb will provide definitive support for this proposed mechanistic model. It should be noted, however, that there is no direct evidence from human passive infusion clinical studies that a N160 or a N332 glycan bnAb induces co-receptor switching.

There is a body of other data also supporting the notion that co-receptor switching may occur as a consequence of neutralisation escape. For instance, HIV-1B, HIV-1C, and HIV-1D X4 compared to co-circulating R5 variants often have distinctive V3 loop motifs.[21,23] The X4 strains in these subtypes often have a 2–3 amino acid V3 loop insertion in the same general V3 loop region (Figure 3). Yet, the forces promoting V3 insertions remain unclear. The similarity in the V3 loop insertions among HIV-1B, HIV-C, and HIV-1D X4 variants suggests that these highly divergent viruses are independently converging to a similar solution to escape a common selection pressure, likely nAb. Indeed, nAb selective pressure has been associated with insertions observed in V1 through V4 envelope domains.[46-48,56] This data argues that humoral immune pressure selects for X4 variants with the observed V3 loop insertions because there is low likelihood that random mutations will lead to a shared genotypic and phenotypic characteristic. Thus, neutralisation escape is hypothesised as one mechanism among the diverse non-mutually exclusive postulated processes that potentially explains co-receptor switching.[22]

## CLINICAL IMPLICATIONS FOR THE INTERSECTION BETWEEN BROADLY NEUTRALISING ANTIBODIES THERAPY AND CO-RECEPTOR USAGE

The intersection between HIV co-receptor usage and the future use of bnAb as HIV therapy has a number of important clinical implications. First, the association between co-receptor usage and differential bnAb sensitivity to N160 and N332 loop directed-bnAb could be leveraged for determining the presence of neutralisation-insensitive viruses prior to treatment. Prior to implementing cART, infected individuals are examined for the presence of drug-resistant variants using sequence-based assays.[57] Similar pre-treatment tests are not available to test susceptibility for these future antibody-based therapies. Extensive sequence variation in the HIV-1 envelope has made it difficult to develop a genotype-based test for predicting decreased antibody neutralisation sensitivity.

| Viral Isolate | #Seq | Phenotype | v3 loop sequence | | |
|---|---|---|---|---|---|
| HIV-1B 1239 | | | | | |
| | 1 | X4 | CTRPNNNTRKSVRI | G I G | RGRAWSRTTDIIGDIRQAHC |
| | 2 | R5/X4 | CTRPNNNTRKGINI | ——G | PGRAWYRTTDIIGDIRQAHC |
| | 1 | R5 | CTRPNNNTRKGINI | ——G | PGKAWYRTTDIIGDIRQAHC |
| | 8 | R5 | CTRPNNNTRKGINI | ——G | PGRAWYRTTDIIGDIRQAHC |
| 4102 | | | | | |
| | 3 | X4 | CTRLNNNKRKRIRI | G H I | GPGRTI YATEGIRGDIRQAHC |
| | 2 | X4 | CTRLSNNKRKRIRI | G H I | GPGRTI YATEGIKGDIRQAHC |
| | 1 | R5/X4 | CTRPNNNTRKRISM | ——— | GPGRVYYTTGEIIGDIRRAYC |
| | 1 | R5 | CTRPNNNTRKSIPI | ——— | GPGKAFYATGDIIGDIRKAYC |
| | 1 | R5 | CTRPNNNTRKSITI | ——— | GPGKAFYATGDIIGDIRKAYC |
| | 11 | R5 | CTRLNNNTRKSIHI | ——— | GPGGAFYATGDIIGDIRQAYC |
| HIV-1C DM146 | | | | | |
| | 8 | R5/X4 | CTRPDNNTRRRVRM | G I G | PGQTFYTNDIIGDIRRAHC |
| | 5 | R5 | CTRPDNNTRRSVRM | ——G | PGQVFYTNDIIGDIRRAHC |
| | 2 | R5 | CTRPDNNTRRSVRM | ——G | PGQVFYTNDIIGDIRQAHC |
| | 1 | R5 | CTRPDNNTRRSVRM | ——G | PGQVFYTNDIIGDIRQYHC |
| | 1 | R5 | CTRPDNNTRRSVRM | ——G | PGQAFYTNDIIGDIRQAHC |
| | 1 | R5 | CTRPDNNTRR IVRM | ——G | PGQVFYTADIIGDIREASC |
| DM268 | | | | | |
| | 11 | X4 | CTRPSNNTRRRVRI | G I G | RGQAFDATQEIIGDIRQAHC |
| | 7 | R5 | CTRPSNNTRRRVSI | ——G | PGQYTDATGEIIGDIRKAHC |
| HIV-1D DM2 | | | | | |
| | 1 | X4 | CTRPNYNTRKAIHT | G P G | QGQAVYTAAKIVGNIRQAHC |
| | 6 | R5 | CSRPYNNTRQGTHI | ——G | PGQALFTTTRIVGDIRQAHC |
| | 3 | R5 | CLRPYNNTRQGTHI | ——G | PGQALFTTTRIIGDIRQAHC |
| | 1 | R5 | CTRPYNNTRQGTHI | ——G | PGQALFTTTRIVGDIRQAHC |
| | 1 | R5 | CSRPYNNTRQGTHI | ——G | PGQALFTTTRIVGDIRQAHC |
| | 2 | R5 | CSRPYNNTRQGTHI | ——G | PGQALFYTARIVGDIRQAHC |
| DM4 | | | | | |
| | 2 | X4 | CTRPYNSTRKGVHV | G H V | GPGRAFWTQNIVGNIRHAHC |
| | 6 | R5/X4 | CTRPYNSTRKGVHV | G H V | GPGRAFWTQNIVGNIRHAHC |
| | 3 | R5 | CTRPYNNTRTGVHV | ——— | GPGRAYWTQNIVGNIRHAHC |
| | 3 | R5 | CTRPYNNTRQGI HV | ——— | GPGRAYWTQNIVGNIRHAHC |

**Figure 3: Representative examples of co-circulating HIV-1B, HIV-1C, and HIV-1D R5, R5X4, and X4 strains.**

The alignment of the predicted amino acid sequence (denoted by single letter abbreviation) of phenotypically confirmed X4, R5, and R5/X4 envelope variable 3 (V3) loops. The subtype is listed and underlined on the left. Columns indicate the subject ID, number of envelopes with the same predicted V3 loop sequence, and the confirmed co-receptor phenotype. Bold red letters denote the predicted insertions. HIV-1C and HIV-1D sequences are from previous publications[21,23] while HIV-1B sequences are from a manuscript under consideration.

Individuals that contain variants with envelopes that lack the predicted glycan at position 160 or 332 are resistant to N160 and N332-directed bnAb, respectively. There are numerous HIV strains, however, that have decreased sensitivity to the N160 and N332 loop-directed bnAb even though they contain the required predicted glycosylated amino acids.[58] In the absence of

reliable sequence-based tests, bnAb clinical trials have often employed phenotypic assays. This entails the laborious process of examining neutralisation sensitivity of virus stocks incorporating HIV envelopes isolated from patient samples.[10,12] Patient viruses are generated either through outgrowth cultures or *in vitro* virus stock preparations using envelope amplification, cloning, and transfection. Furthermore, these phenotypic methods lack sensitivity because outgrowth cultures and *in vitro* virus generation mostly capture major variants but not minor yet clinically important strains present in the patients' viral quasispecies.[59] On the other hand, numerous sequence-based methods can accurately predict co-receptor usage.[60-62] It is possible that an infected individual's circulating virus envelope sequence can be interrogated with high resolution using next-generation sequencing, and subsequently the receptor usage can be predicted through previously defined sequence-based algorithms. The predicted presence of CXCR4-using virus would imply decreased sensitivity of the virus population to N160 and N332 bnAb, based on the association between co-receptor usage and bnAb susceptibility as highlighted above. This sequence-based test would limit the use of bnAb among patients that harbour relatively neutralisation-insensitive viruses, which would prevent both the virus from developing resistance to other drugs in a combination cocktail and unnecessary costs.

The second important clinical implication of the link between co-receptor usage and antibodies is the theoretical possibility that N160 and N332 bnAb-based therapies may promote the persistence or emergence of CXCR4-using strains. This outcome would be clinically significant because presence and emergence of CXCR4-utilising viruses is associated with worse and more rapid disease progression.[63,64] The potential for favouring the emergence of CXCR4-using virus with the use of N160 and N332-directed bnAb needs to be seriously considered prior to its widespread use because the therapeutic use of these bnAb may paradoxically worsen rather than improve disease outcomes. In humans and animals treated with N332-directed bnAb (10-1074 and PGT121), variants often escape neutralisation pressure by eliminating the glycan at 332 amino acid position, but this outcome is not universal.[3,4,7,10-12] It has been suggested that changes to the 332 glycan may yield a strain that is highly susceptible to other autologous antibodies present in chronically infected individuals.[65] Thus, in the presence of N332-directed bnAb, viruses may explore other sequence paths towards resistance, such as evolving toward CXCR4-usage. As opposed to *in vitro* passage, more pathogenic, phenotypically confirmed R5X4 or X4 strains have not been observed to emerge with N332 bnAb treatment. However, it is possible that CXCR4-using viruses evolve only after multiple rather than a small number of antibody infusions, which would be more consistent with clinical observations that these viruses emerge later in disease. Because of the association of X4 viruses with poorer prognosis and more rapid clinical progression, avoiding either the selection for a pre-existing or the emergence of X4 variant with bnAb treatment is clinically crucial. Viruses resistant to traditional ARV often have decreased replication capacity, and thus their emergence is not associated with worse disease outcome. In contrast to these ARV resistant strains, emergence of CXCR4-using viruses in the presence of specific bnAb would potentially lead to greater morbidity because co-receptor switching has been associated with faster disease progression.[63,64] Furthermore, emergence of CXCR4-using strains would eliminate the ability to treat patients with CCR5 receptor inhibitors. Indeed, the CCR5 inhibitors are considered an alternative second-line agent in cART regimens, and thus the loss of this ARV class will also limit options for ARV salvage regimens. The potential for selection or emergence of CXCR4-using virus with use of bnAb will require further examination as bnAb enter the clinical sphere.

## CONCLUSIONS

N160 and N332-directed bnAb are in clinical trials, and these novel therapeutics will likely enter clinical practice in the near future. The overlap of the HIV envelope domain targeted by these antibodies with the envelope regions involved in co-receptor usage provides the scientific rationale for believing that HIV variants that use different co-receptors to enter cells likely have varying susceptibility to these bnAb. Indeed,

newer studies show that X4 compared to R5 HIV are less sensitive to N160 and N332 bnAb, and neutralisation pressure, such as during bnAb-based treatment, may favour the persistence and emergence of CXCR4-using strains. The authors speculate that the linkage between co-receptor usage and susceptibility to variable loop-directed bnAb can be exploited to develop assays to predict pre-treatment neutralisation sensitivity, as well as give insight into the potential complications with future N160 and N332 bnAb.

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