

Contents

Message from the Editor	
<i>Marco H.K. HO</i>	P.3
Interview with Founder Honorary Secretary – Dr. Helen Chan	
<i>Helen H.L. CHAN and Marco H.K. HO</i>	P.4-5
Asthma	
Recent advances of immuno-regulatory and alarmin cytokines in allergic diseases	P.6-10
<i>Chun-kwok WONG</i>	
Ear Nose & Throat	
Association between rhinitis and eustachian tube dysfunction in adolescents	P.11
<i>Birgitta Y.H. WONG</i>	
Environment/Microbes	
Differentiating viral and non-viral respiratory illness in hospitalized children by evaluation of immune signatures in nasopharyngeal aspirate	P.12-15
<i>Renee W.Y. Chan</i>	
Food Allergy	
An update on the diagnostic strategy for fish allergy	P.16-17
<i>Agnes S.Y. LEUNG</i>	
Immunology/Drug Allergy	
The establishment of non-invasive nasal epithelial lining fluid collection method for the detection of localized IgA and mucosal immune response	P.18-20
<i>Renee W.Y. Chan</i>	
Meeting Highlights	
Annual General Meeting (AGM) 2023 and The 12th Hong Kong Allergy Convention (HKAC 2023)	P.21
Course in ADAPT: Advances in drug allergy & penicillin testing	P.22
Upcoming Events / Meetings	P.23

Council Members

President

Professor Gary W.K. WONG

Vice President

Dr. Philip H. LI

Honorary Secretary

Dr. Helen H.L. CHAN

Honorary Treasurer

Dr. Roland C.C. LEUNG

Immediate Past President

Dr. Marco H.K. HO

Editor-in-Chief

Dr. Marco H.K. HO

Associate Editors

Dr. Jason Y.K. CHAN

Dr. Allie LEE

Dr. Agnes S.Y. LEUNG

Council Members

Dr. Alson W.M. CHAN

Dr. Patrick C.Y. CHONG

Dr. Gilbert T. CHUA

Professor Ellis K.L. HON

Dr. Fanny W.S. KO

Dr. Christopher K.W. LAI

Dr. Agnes S.Y. LEUNG

Dr. Daniel K.K. NG

Co-opt Member

Dr. Christine Y.Y. WAI

Advisors

Dr. Jane C.K. CHAN

Professor Yu-lung LAU

Dr. Alfred Y.C. TAM

Dr. Tak-fu TSE

Dr. Robert Y.M. TSENG

Dr. John K.S. WOO

Dr. Donald Y.C. YU

Dr. Patrick M.P. YUEN

Editorial Board

Sub-editors

Asthma

Name

Specialty

Respiratory Medicine

Dr. Veronica L. CHAN

Respiratory Medicine

Dr. Alice S.S. HO

Respiratory Medicine

Dr. Lai-yun NG

Respiratory Medicine

Dr. Julie K.L. WANG

Respiratory Medicine

Ear Nose & Throat

Otorhinolaryngology

Dr. Jason Y.K. CHAN

Otorhinolaryngology

Dr. Birgitta Y.H. WONG

Otorhinolaryngology

Dr. David C.M. YEUNG

Environment/Microbes

Respiratory Medicine

Dr. Jane C.K. CHAN

Respiratory Medicine

Dr. Roland C.C. LEUNG

Eye Allergy

Ophthalmology

Dr. Allie LEE

Ophthalmology

Dr. K.W. KAM

Food Allergy

Paediatric Immunology, Allergy and Infectious Diseases

Dr. Gilbert T. CHUA

Paediatric Immunology, Allergy and Infectious Diseases

Dr. Marco H.K. HO

Paediatrics

Dr. Agnes S.Y. LEUNG

Paediatric Respiratory Medicine

Dr. Alfred Y.C. TAM

General Allergy

Paediatric Immunology, Allergy and Infectious Diseases

Dr. Alson W.M. CHAN

Immunology/Drug Allergy

Immunology

Dr. Elaine Y.L. AU

Immunology

Mr. Andy K.C. KAN

Skin Allergy

Paediatrics

Dr. David C.K. LUK

Dermatology and Venereology

Dr. Christina S.M. WONG

Allied Health Professionals

Registered Dietitian

Ms. June K.C. CHAN

Registered Nutritionist

Dr. Sonal HATTANGDI-HARIDAS

Registered Pharmacist

Mr. Andrew W.T. LI

Registered Dietitian

Ms. Sabrina W.S. MOK

Registered Pharmacist

Ms. Chara Y.W. YIP

Message from the Editor

Dr. Marco H.K. HO

*MBBS(HK), MD(HK), MRCP(UK), FRCPCH, FRCPE, FRCP, FHKCPaed, FHKAM(Paed)
Specialist in Paediatric Immunology, Allergy and Infectious Diseases
Immediate Past President, Hong Kong Institute of Allergy*



The world is increasingly irrational. Many astounding horrors and ridicules are unfolding to us day by day. Scientific rationalism in an irrational world is critically important which would bring us clarity, balance and peace of mind. Science, unlike politics, is not an ideology or belief system; it is a process. HKIA is a catalyst of the process for better education, research and service in the interests of allergy sufferers. In this fall issue, there are two important Firsts. We have an interview about one of HKIA's founders Dr. Helen Chan on birth of HKIA, its mission and how she views the health system landscape and humanity. First of this kind in our Newsletter. Many insightful and visionary ideas from Dr. Chan! Another First is that 3 articles in this Issue were original works produced wholly or in part through HKIA's grant support. Professor Chun-kwok Wong gave an excellent overview about his original studies on immuno-regulatory and alarming cytokines in allergic diseases which may pave way to novel therapeutic intervention targets. Dr. Renee W.Y. Chan back-to-back shared two articles on her original works with wheezing and viral studies; - differentiating viral and non-viral respiratory illness in hospitalized children by evaluation of immune signatures in nasopharyngeal aspirate; and localized IgA and mucosal immune response to virus.

Dr. Agnes S.Y. Leung and her team have literally put Hong Kong Seafood Research on the world map. This time, she narrated in the real world how to incorporate diagnosis of fish allergy in couple with dietary advancement therapy, ie selective eating instead of en bloc avoidance. Tedious work but good for patient's quality of life and health. Last but not least, Dr. Birgitta Y. H. Wong brought our attention to a recent paper analysing the association between rhinitis and eustachian tube dysfunction in adolescents. An apparent known fact but with emerging new understanding and possible interventional procedures.

There has been packed with important events in this fall season too, there are again many "Firsts". It gives me great pleasure to attend the "first physical Allergy Convention since pandemic" - 12th HKAC which was successfully held on 7 - 8 October 2023 at the Hong Kong Convention and Exhibition Centre. Many of us have braved through the Typhoon Koinu which gave a T8 storm signal on Sunday 8 October and enthusiastic participants stayed the very end. The Observatory hoisted the Increasing Gale or Storm Signal No. 9 on Sunday evening, the first time in Allergy Convention's History. We are grateful again to have devoted support from the American College of Allergy, Asthma & Immunology (ACAAI) and British Society for Allergy & Clinical Immunology (BSACI). We were very privileged to have Hon Andrew Leung Kwan-yuen, GBM, GBS, JP President of Legislative Council of Hong Kong who graciously officiated the opening of Allergy Convention and Chris Corrigan, Professor of Asthma, Allergy & Respiratory Science at King's College London lecture to us at the inaugural (first) Tak Hong Lee Memorial Lecture. We have also just finished our AGM at Hong Kong Club held on 7 November presided by Professor Gary Wong and new councils were elected. Under the able leadership of Dr. Philip Li, the first Course in ADAPT: Advances in Drug Allergy & Penicillin Testing was conducted on 23 – 24 September and 4 – 5 November 2023 respectively. First time, Hong Kong Institute of Allergy had co-organized with the Faculty of Medicine of the University of Hong Kong and the University of Western Australia to cater the unmet demand in drug allergy diagnosis and delabeling unnecessary outdated drug labels. A photo album highlights these memorable events.

I hope you have a productive season, until next time, take care.

Dr. Marco H.K. Ho
Editor, HKIA e-newsletter
The Hong Kong Institute of Allergy

Interview with Founder Honorary Secretary – Dr. Helen Chan

Never Forget our Original Intention in Establishing the Institute

It is my pleasure to have a zoom Interview with Dr. Helen on 15 September 2023 on zoom. Dr. Helen Chan is the Founding Secretary of the Institute. Together with the late Dr. Avery Chan, Dr. Ka-ho Chan, Dr. Jane Chan, Dr. Christopher Lai, Dr. John Leung, Professor William Wei, the late Dr. William Yip, Dr. Donald Yu, Dr. Hip-cho Yu and Dr. Patrick Yuen, Dr. Chan set up the Institute in 1996. Dr. John Leung was the one who gave the name of “Hong Kong Institute of Allergy” to the newly formed organisation. Since then, Dr. Chan serves as the Honorary Secretary of the Institute until now. The President’s Medal is the highest honour that the Hong Kong Institute of Allergy (HKIA) can bestow. It recognises the distinction of an individual not only for clinical and/or scientific achievements but also for contributions to the Institute and to the growth of the discipline of allergy in the HK community. The Medal is awarded every two years at our international HKIA Allergy Convention. Dr. Helen Hei Ling Chan was the awardee of 2018 President’s Medal. She is also a previous awardee of the prestigious International Distinguished Fellowship of the ACAAI.



Annual General Meeting 2014
when Professor Tak Lee was first on board
(31 Oct 2014)

Question 1: What are your views on the Past, the Present and the Future of Allergy. What are the connections with the group of specialists?

In the early 1990s, Allergy was considered a Cinderella specialty with little scientific understanding of mechanisms and allergists were akin to “quacks.” Only a minority of doctors accepted allergy testing and immunotherapy was often viewed as witchcraft. There were no formalized training programmes for allergy in HK. As a result, allergic patients could not benefit from a correct diagnosis, allergen avoidance and effective holistic management.

In light of the need of allergy awareness among medical professionals and the growing prevalence of allergic disorders in Hong Kong, one of the main missions of the HKIA is to introduce and promote allergy as a medical discipline and to foster a professional forum for fellowship and scientific exchange for the benefits of allergic patients in Hong Kong. We start by educating and training doctors, then nurses, and then other paramedical professionals and patients.

Starting from 1998, the American College of Allergy, Asthma and Immunology (ACAAI) was invited to send speakers to Hong Kong to give lectures to and to educate the doctors and healthcare professionals. We are still doing this practice every 2 years at the Hong Kong Allergy Conventions with the hope that finally “Allergy” will be recognised as a specialty one day.

Today, the Council of the Institute is still working hard to educate more doctors on allergy, and with the wish that they will join this specialty for the benefits of patients. I am thrilled of seeing many young exuberant allergists and trainees joining the allergy field and I certainly hope we can grow bigger and stronger.

Question 2: Have you got any bitter experience along the work with the Institute?

Dr. Avery Chan was my classmate and helped setting up the Institute with us. Unfortunately he died with cancer when he was still young. Another miserable memory is the loss of Professor Tak Lee. When Tak came back to Hong Kong in 2014, Dr. Christopher Lai and myself persuaded him to join the Institute and to lead the Council for promoting the discipline. It is also very sad that he died in August 2022. I feel very heartbreakingly that we lost these 2 important colleagues.

One good thing is that I nominated Tak to receive the “International Distinguish Fellow Award” of the ACAAI in 2017. His work has been well recognized internationally. I hope his legacy will prevail.

Question 3: What is your view on the development of the healthcare landscape?

Healthcare industry is definitely expanding as people are living longer. We hope that we can use less resources but everyone lives a healthier life. I am a huge believer of “Preventive Healthcare”. It is much better to do healthy things and to eat properly to prevent diseases instead of taking drugs after you get sick. Aligning with many old documentaries and



Dinner with Patron of Year 2015, Dr. Wing-man Ko
(10 July 2015)



Representing of HKIA to attend the World Allergy Congress with Dr. Gilbert Chua (14 - 17 Oct 2015)

emerging evidences, I intuitively envision human beings should live up to 120 years old. The reason why we cannot be so nowadays is because people are not doing the right things nor eating the right food. I believe it is realistic that many of us can become centenarian for our generation. The next generation may easily be able to live up to 120 years old if they consistently do the right things. Aging healthily and not making ourselves a burden neither to the family nor to the healthcare system in Hong Kong is possible. The key mindset is taking good care of one's own health. In such spirit, I am often disappointedly seeing many people delaying seeking treatment from doctors when they get sick.

Hong Kong should be proud of her excellent healthcare system and health indexes. There are nonetheless from time to time us face resource limitation, workforce complacency and constraints. I can hardly agree with any knee jerk response of oversimplifying to "we-need more-doctors" as the gold standard solution. Sometimes less is more. The crux is to have

optimum number of healthcare professionals who are motivated to give their best care to patients. My humble view boils down to firstly, offer a nice remuneration package, and secondly, instigate the culture that healthcare is a noble job. With that we can attract the capable people with a right mindset.

Question 4: Do you have a quote or a reminder to our future allergists?

Last but not least, going back to HKIA, we should always remain faithful to our original mission which was (and still is) to promote allergy as a discipline and to foster a professional forum for fellowship and scientific exchange for the benefits of patients in HK. Ultimately we are here to alleviate sufferings of allergic patients and to have better prevention of allergic diseases. I have been witnessing the positive changes over the last 3 decades and I am sure more new blood will carry on our baton and sustain our Institute's vibrancy and contemporaries.



Council Members attending the Annual Dinner of the Federation of Medical Societies of Hong Kong (31 Dec 2015)



Dr. Helen Chan celebrating her birthday with her sister, Ms. Agnes Chan Mei-ling (on the left)



Dr. Helen Chan celebrating the day she became a mother-in-law at her son, Ryan's (3rd from left) wedding to Vinci (2nd from left)



A relaxing daily life: Dr. Helen Chan with her family



Dr. Helen Chan, her husband, Dr. Tak-fu Tse (on the left), and son Ryan (on the right), joyfully welcoming his little girl to their family.

Original work supported by HKIA Research Grant

Recent advances of immuno-regulatory and alarmin cytokines in allergic diseases

Professor Chun-kwok WONG

BSc (HK), MPhil (HK), PhD (HK), FRSB (UK)

Professor, Department of Chemical Pathology

Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong



Cytokines and chemokines in allergic diseases

Cytokines and chemokines are a rapidly expanding research field in laboratory medicine, inflammation and allergic disease. Inflammatory diseases are generally associated with dysregulation of cytokines and chemokines such as the imbalance of T helper (Th) type 1 and 2 cytokines, over-activation of Th17 cells, aberrant regulatory T/B cells and regulatory cytokines, and cytokine/chemokine storm. This leads to the inflammatory reaction, including infiltration of immune cells (e.g. T cells, macrophages, dendritic cells, eosinophils, basophils, type 2 innate lymphoid cells) into the inflammatory sites, increased intercellular interaction, tissue/cell damage, airway remodeling, mucus secretion in allergic disease. Therefore, the evaluation of the immunopathological roles and dysregulation of cytokines and chemokines in inflammatory diseases will not only further foster our understanding of the immunopathological mechanisms but also furnish a biochemical basis for the development of disease activity markers and novel therapeutic cytokine-targeting agents in allergic diseases. The objectives of these three projects, funded by the Hong Kong Institute of Allergy (HKIA) funded projects (2016-2018) were in an attempt to evaluate the immunological roles of novel regulatory and alarmin cytokines and their underlying immunological and molecular mechanisms in allergic inflammation of allergic asthma and atopic dermatitis.

IL-37 in Allergic Asthma

In the first HKIA-funded project “Immuno-regulatory Roles of the Novel Anti-inflammatory Cytokine Interleukin-37 in Allergic Asthma”, the primary objective is to elucidate the *in vitro* and *in vivo* immunomodulatory role of the novel anti-inflammatory cytokine IL-37, a member of the IL-1 cytokine family, in allergic asthma. It has been shown that respiratory bacterial and viral infection can provoke allergic inflammation in allergic asthma.^{1,2} The regulatory cytokine IL-37b, the largest and best characterized variant of IL-37, is an immunosuppressor that exerts anti-inflammatory activity in inflammatory diseases.³ In this study, IL-37b showed significant suppression of the inflammatory reaction in culture of human eosinophils and airway cells upon stimulation by the toll-like receptor (TLR)2 ligand, the bacterial cell wall component peptidoglycan (PGN). IL-37b can also reduce the activation of intracellular inflammation-related signaling molecules I κ B α , Akt and extracellular signal-regulated kinases (ERK)1/2, and suppress the transcription of inflammation-related

genes such as BOLA2B, CAMP, DPM3, ELOB, C4ORF48, S100A9, TFF3, NPIP15 and PYCARD. In ovalbumin (OVA)-induced allergic asthmatic mice, intravenous administration of IL-37b can alleviate airway inflammation, as shown by significant improvement of lung function, decreasing allergy-related OVA-specific IgE concentration, cytokines and chemokines including Th2-related IL-4, IL-5 and IL-10, IL-17, chemokine CCL11 and CCL5 in plasma, bronchoalveolar lavage fluid (BALF) and lung tissue. IL-37b also retarded the development of asthma by suppressing eosinophil count in BALF and up-regulating the percentage of CD25hiFoxp3+ regulatory T cells in spleen and lung tissue. Similar findings were further validated using humanized allergic asthmatic non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, which mimic the human immune system, to confirm the therapeutic potential of IL-37b in human allergic asthma.⁴ The above is illustrated in Figure 1.

In this HKIA-supported project, we have gained promising insights into the anti-inflammatory activity and underlying intracellular and molecular mechanisms of IL-37b of eosinophils-mediated allergic inflammation in allergic asthma. Given the pivotal role of eosinophils in many other allergic diseases such as atopic dermatitis, IL-37b may also be a potential therapeutic agent for various eosinophilic disorders. This study laid a solid foundation leading for the subsequent Research Grants Council General Research Fund (2018/19) funded project, “Regulation of eosinophil/basophil-innate lymphoid type 2 cells axis by fundamental innate immunity inhibitor IL-37 in atopic dermatitis.⁵⁻⁷

High-Mobility Group Box 1 in Atopic Dermatitis

The second HKIA-funded project, “High-Mobility Group Box 1-Mediated Activation of Eosinophils Interacting with Dermal Fibroblasts: a Crucial Link between Tissue Damage and Allergic Inflammation in Atopic Dermatitis” aims to elucidate the cellular mechanisms mediating the activation of human eosinophils and dermal fibroblasts by human high-mobility group box 1 (HMGB1), a skin-lesion-related prototypical damage-associated molecular pattern (DAMP) molecule and DNA-binding inflammatory cytokine, in atopic dermatitis (AD). AD is characterized by eczematous skin lesions with inflammatory infiltrates composed predominantly of eosinophils, basophils, Th and memory T cells.⁸ The pathogenesis of AD involves repeated abnormal innate and adaptive immune responses to environmental pathogens when the skin barrier is disrupted.⁸

Aeroallergens, such as house dust mites, pollen and contact allergens can induce allergic inflammation in AD through eosinophil activation and production of reactive oxidative species (ROS).⁸ Moreover, bacterial *Staphylococcus aureus* (*S. aureus*) is another extrinsic causative factor in the pathogenesis of AD, because it colonizes almost all skin lesions skin in AD.⁹

HMGB1, a nuclear DNA-binding cytokine, similar to that of alarmin IL-33, is one of the key DAMPs released by necrotic or apoptotic cells that activate the innate immune system.^{10,11} HMGB1 is also actively secreted by monocytes/macrophages in response to cytokines (e.g., interferon- γ), bacterial lipopolysaccharide (LPS)¹¹ or chemical contact allergens.¹² It is a prototypical DAMP molecule that is released during skin lesions in AD. Our previous studies have shown that pathogen-associated molecular pattern-mediated intracellular signal transductions can modulate the activation of eosinophils/basophils upon interaction with bronchial epithelial cells/dermal fibroblasts.^{13,14} Using in vitro and animal studies, this study has highlighted the crucial role of eosinophils and HMGB1 in the cellular mechanisms of allergic inflammation in AD and its underlying intracellular mechanisms. Using ex vivo co-culture system and AD mice model, results revealed a novel HMGB1/receptor for advanced glycation endproducts (RAGE)-stimulated pathway that induces the activation of eosinophils through interaction with dermal fibroblasts. As illustrated in Figure 2, the pruritogenic cytokine IL-31 released from allergen activated Th2 cells, causing scratching behavior, which in turn causes tissue damage and releases the DAMP HMGB1. This subsequently activates eosinophils, which interact with dermal fibroblasts via distinct intracellular signaling pathways, including p38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)- κ B to release chemokines such as CXCL8 and CCL4 which can mediate the infiltration of immune effector cells into the inflammatory site to amplify the allergic inflammation in AD. The results therefore depict a mechanism by which the alarmin HMGB1 would play an important role in the pathogenesis of AD. In addition, our results concerning the vicious effects of HMGB1 in AD also shed light on the idea that implementing therapeutic interventions to block the HMGB1 pathway may help to alleviate AD symptoms.

IL-38 and Innate Lymphoid Type 2 Cells in Atopic dermatitis

The third HKIA funded project “The Immunological Link between Pattern Recognition Receptors and the Activation of Innate Lymphoid Type 2 Cells in Allergic Inflammation”, aims to elucidate the in vitro infection-mediated activation of innate lymphoid type 2 cells (ILC2s) and the underlying intracellular mechanisms in allergic inflammation. Innate lymphoid cells (ILCs) are a new family of innate immune effector cells with morphological similarity to B and T lymphocytes without rearranged antigen receptors, which are important early regulators of immune responses at mucosal surfaces.^{15,16} ILCs are instructed by cytokines and epithelial-derived lipid mediators, stromal and myeloid cells for regulating the type of Th cells and intensity of the immune response including innate immunity-mediated inflammation and lymphoid tissue formation by producing cytokines and

other mediators, and through intercellular interactions.¹⁵⁻¹⁷ There are three functionally different ILC subsets: group 1 (ILC1s), group 2 (ILC2s) and group 3 ILCs (ILC3s), which are innate equivalents of Th1, Th2 and Th17, respectively. Group 1 ILCs include natural killer cells and other innate cells that produce IFN- γ and are dependent on the Th1 transcription factor T-bet for their development and function; group 2 ILC comprise ILCs that produce type 2 cytokines (IL-4, IL-5, IL-9 and IL-13) and require transcription factor GATA3 and ROR α ; and group 3 ILC subtypes that produce IL-17 and/or IL-22 and utilize ROR γ t for their differentiation.¹⁸

To evaluate the immunopathological role of innate lymphoid type 2 (ILC2) cells in allergic inflammation, we performed in vitro and in vivo experiments to elucidate the immunoregulatory activities of the regulatory IL-1 family cytokine IL-38 on ILC2 cells. In co-culture, upon stimulation with the viral RLR ligand Poly(I:C)/LyoVec or infection-related tumor necrosis factor (TNF)- α for the expression of cytokines/chemokines/adhesion molecules, the immunoregulatory cytokine IL-38 significantly inhibited the induced inflammatory IL-6, IL-1 β , CCL5 and CXCL10 production and the anti-viral interferon- β , and intercellular adhesion molecule-1 (ICAM-1) expression in co-culture. Mass cytometry and RNA-sequencing analysis revealed that IL-38 could antagonize the activation of intracellular STAT1,3, p38 MAPK and ERK 1/2 and NF- κ B pathways and upregulate the expression of the host defense-related gene POU2AF1 together with the anti-allergic response gene RGS13.¹⁹

Intraperitoneal injection of IL-38 into house dust mite (HDM)-allergic asthmatic mice could ameliorate airway hyper-reactivity through reducing accumulation of eosinophils in lung and inhibiting the expression of Th2-related cytokine IL-4, IL-5 and IL-13 in bronchoalveolar lavage fluid (BALF) and lung homogenates. Histological examination indicated the alleviation of lung inflammation by reducing cell infiltration and goblet cell hyperplasia, together with reduced ILC2, Th2 and Th17 cells but increased proportions of regulatory T cells in lung, spleen and lymph nodes. IL-38 administration suppressed airway hyperresponsiveness and asthma-related IL-4 and IL-5 in humanized asthmatic NOD/SCID mice, together with significantly decreased CCR3+ eosinophils in BALF and lung, and lower percentages of human CD4+CRTH2+Th2 in lung and mediastinal lymph nodes.¹⁹ The above is illustrated in Figure 3.

Taken together, our results demonstrated the immunological role of innate lymphoid type 2 cells in allergic inflammation, which may be the anti-inflammatory target of the immunoregulatory cytokine IL-38 in allergic disease. In this HKIA-funded project, using mass cytometry and RNA sequencing analysis in co-culture, we have identified the crucial intracellular mechanisms regulating viral RLR ligand poly (I:C)/LyoVec-mediated allergic inflammation. Together with asthmatic murine experiments, results can advance our knowledge of the immunopathological mechanisms by which the activation of innate lymphoid type 2 cells can trigger allergic inflammation. This published result will establish a biochemical basis for the development of therapeutic interventions, targeting ILC2 cells in allergic diseases.¹⁹

References

1. Schröder NW, Crother TR, Naiki Y, Chen S, Wong MH, Yilmaz A, Slepchenko A, Schulte D, Alsabeh R, Doherty TM, Peterson E, Nel AE, Arditi M. Innate immune responses during respiratory tract infection with a bacterial pathogen induce allergic airway sensitization. *J Allergy Clin Immunol.* 2008; 122:595-602. [\[Crossref\]](#) [\[Pubmed\]](#)
2. Ritchie AI, Farne HA, Singanayagam A, Jackson DJ, Mallia P, Johnston SL. Pathogenesis of Viral Infection in Exacerbations of Airway Disease. *Ann Am Thorac Soc.* 2015; 12 Suppl 2: S115-32. [\[Crossref\]](#) [\[Pubmed\]](#)
3. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol.* 2010; 11: 1014-1022. [\[Crossref\]](#) [\[Pubmed\]](#)
4. Zhu J, Dong J, Ji L, Jiang P, Leung TF, Liu D, Ng LG, Tsang MS, Jiao D, Lam CW, Wong CK. Anti-Allergic Inflammatory Activity of Interleukin-37 Is Mediated by Novel Signaling Cascades in Human Eosinophils. *Front Immunol.* 2018; 9:1445. [\[Crossref\]](#) [\[Pubmed\]](#)
5. Hou T, Sun X, Zhu J, Hon KL, Jiang P, Chu IM, Tsang MS, Lam CW, Zeng H, Wong CK. IL-37 Ameliorating Allergic Inflammation in Atopic Dermatitis Through Regulating Microbiota and AMPK-mTOR Signaling Pathway-Modulated Autophagy Mechanism. *Front Immunol.* 2020; 11:752 [\[Crossref\]](#) [\[Pubmed\]](#)
6. Hou T, Tsang MS, Chu IM, Kan LL, Hon KL, Leung TF, Lam CW, Wong CK. Skewed inflammation is associated with aberrant interleukin-37 signaling pathway in atopic dermatitis. *Allergy.* 2021;76:2102-2114. [\[Crossref\]](#) [\[Pubmed\]](#)
7. Hou T, Tsang MS, Kan LL, Li P, Chu IM, Lam CW, Wong CK. IL-37 Targets TSLP-Primed Basophils to Alleviate Atopic Dermatitis. *Int J Mol Sci.* 2021;22:7393. [\[Crossref\]](#) [\[Pubmed\]](#)
8. Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunol Rev.* 2011;242:233-246. [\[Crossref\]](#) [\[Pubmed\]](#)
9. Huang JT, Abrams M, Tlougan B, Rademaker A, Paller AS. Treatment of *Staphylococcus aureus* colonization in atopic dermatitis decreases disease severity. *Pediatrics.* 2009;123:e808-814. [\[Crossref\]](#) [\[Pubmed\]](#)
10. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu Rev Immunol.* 2011;29:139-62. [\[Crossref\]](#) [\[Pubmed\]](#)
11. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, Karisola P, Reunala T, Wolff H, Lauferma A, Alenius H. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol.* 2012;132:1392-1400. [\[Crossref\]](#) [\[Pubmed\]](#)
12. Galbiati V, Papale A, Galli CL, Marinovich M, Corsini E. Role of ROS and HMGB1 in Contact Allergen-Induced IL-18 Production in Human Keratinocytes. *J Invest Dermatol.* 2014; 134:2719-2727. [\[Crossref\]](#) [\[Pubmed\]](#)
13. Wong Wong CK, Dong J, Lam CW. Molecular mechanisms regulating the synergism between IL-32 α and NOD for the activation of eosinophils. *J Leukoc Biol.* 2014;95:631-642. [\[Crossref\]](#) [\[Pubmed\]](#)
14. Jiao D, Wong CK, Qiu HN, Dong J, Cai Z, Chu M, Hon KL, Tsang MS, Lam CW. NOD2 and TLR2 ligands trigger the activation of basophils and eosinophils by interacting with dermal fibroblasts in atopic dermatitis-like skin inflammation. *Cell Mol Immunol.* 2016; 13:535-50. [\[Crossref\]](#) [\[Pubmed\]](#)
15. Lim AI, Verrier T, Vosshenrich CA, Di Santo JP. Developmental options and functional plasticity of innate lymphoid cells. *Curr Opin Immunol.* 2017; 44:61-68. [\[Crossref\]](#) [\[Pubmed\]](#)
16. Roediger B, Kyle R, Le Gros G, Weninger W. Dermal group 2 innate lymphoid cells in atopic dermatitis and allergy. *Curr Opin Immunol.* 2014;31:108-114. [\[Crossref\]](#) [\[Pubmed\]](#)
17. Vercelli D. From Amish farm dust to bacterial lysates: The long and winding road to protection from allergic disease. *Semin Immunol.* 2023;68:101779. [\[Crossref\]](#) [\[Pubmed\]](#)
18. Mjösberg J, Spits H. Human innate lymphoid cells. *J Allergy Clin Immunol.* 2016;138:1265-1276. [\[Crossref\]](#) [\[Pubmed\]](#)
19. Sun X, Hou T, Cheung E, Iu TN, Tam VW, Chu IM, Tsang MS, Chan PK, Lam CW, Wong CK. Anti-inflammatory mechanisms of the novel cytokine interleukin-38 in allergic asthma. *Cell Mol Immunol.* 2020; 17:631-646. [\[Crossref\]](#) [\[Pubmed\]](#)

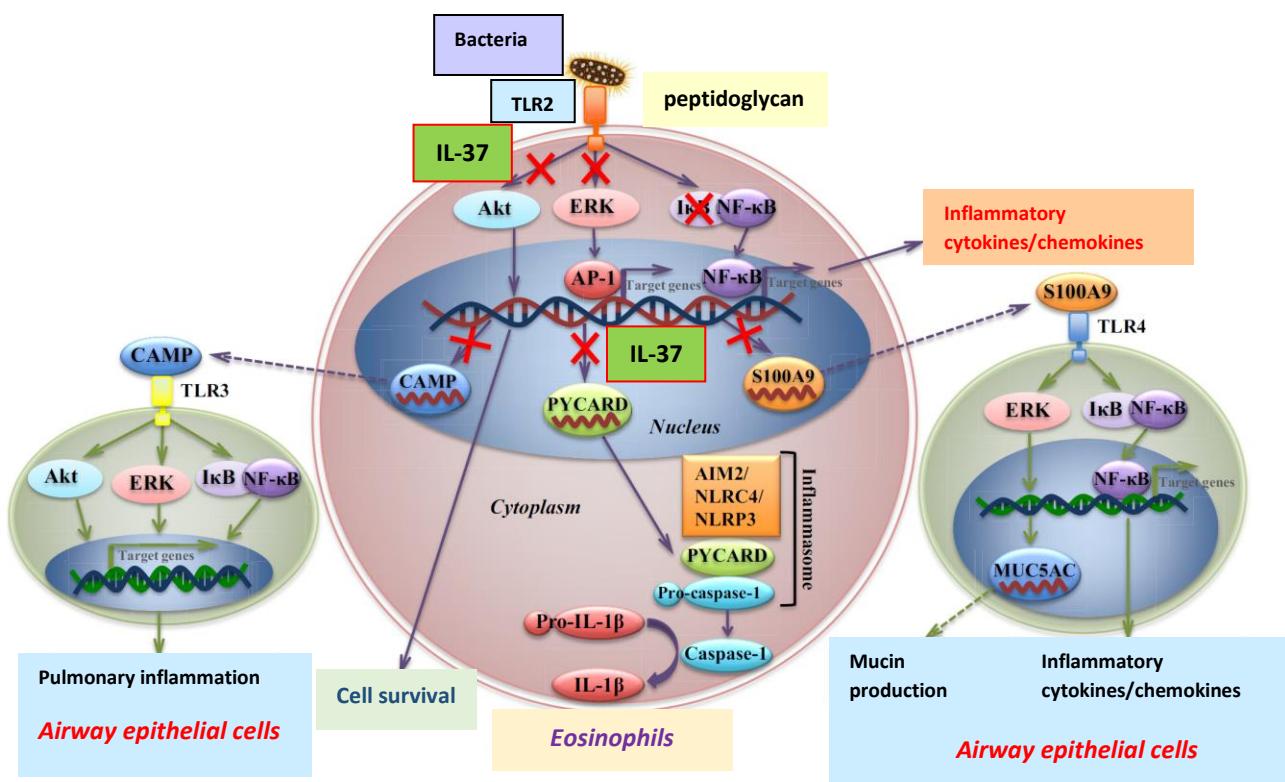


Figure 1. IL-37-mediated intracellular signaling cascades in eosinophils for the suppression of allergic airway inflammation.⁴

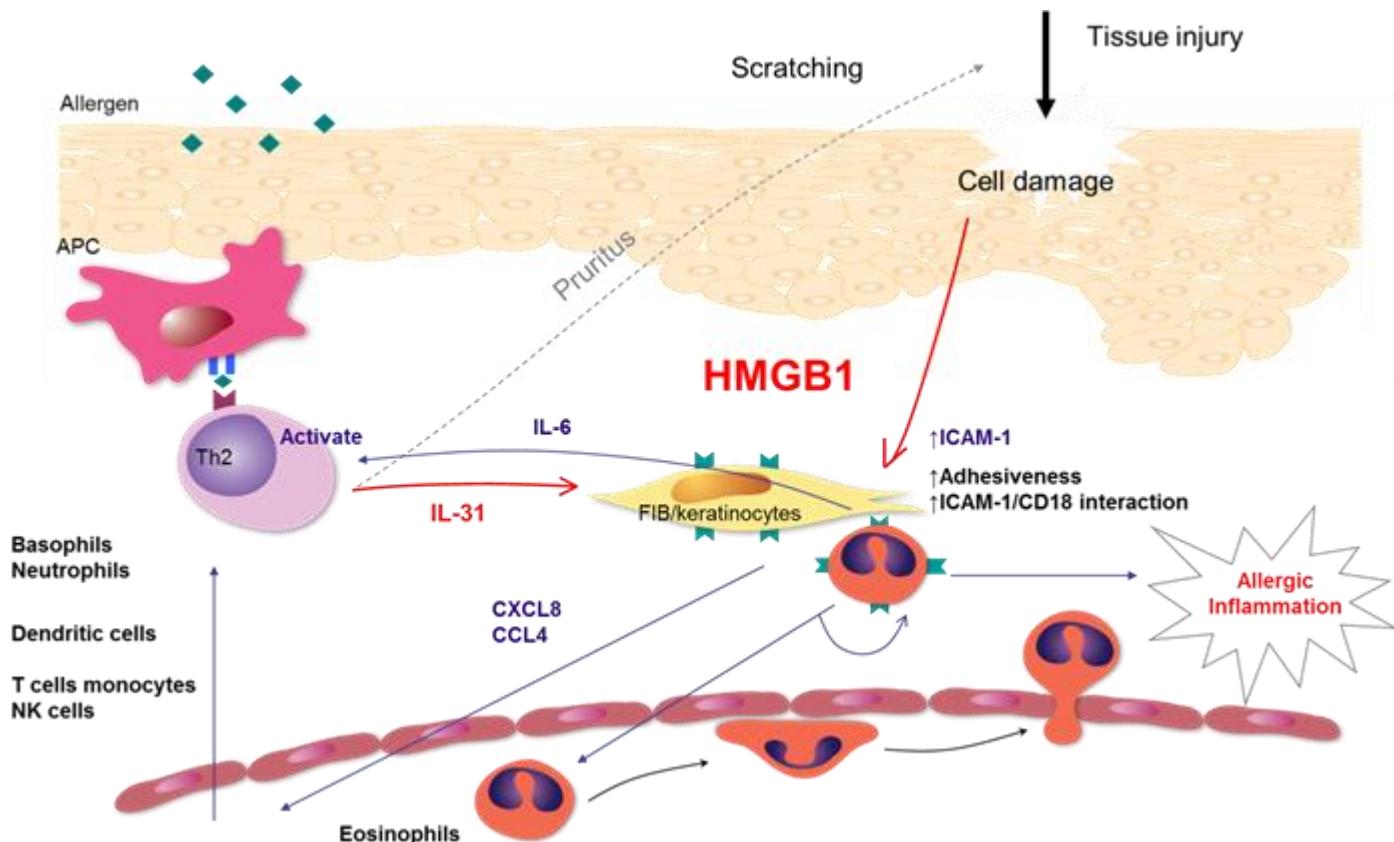


Figure 2. A novel HMGB1/RAGE-stimulated pathway that induces the activation of eosinophils by interacting with dermal fibroblasts.

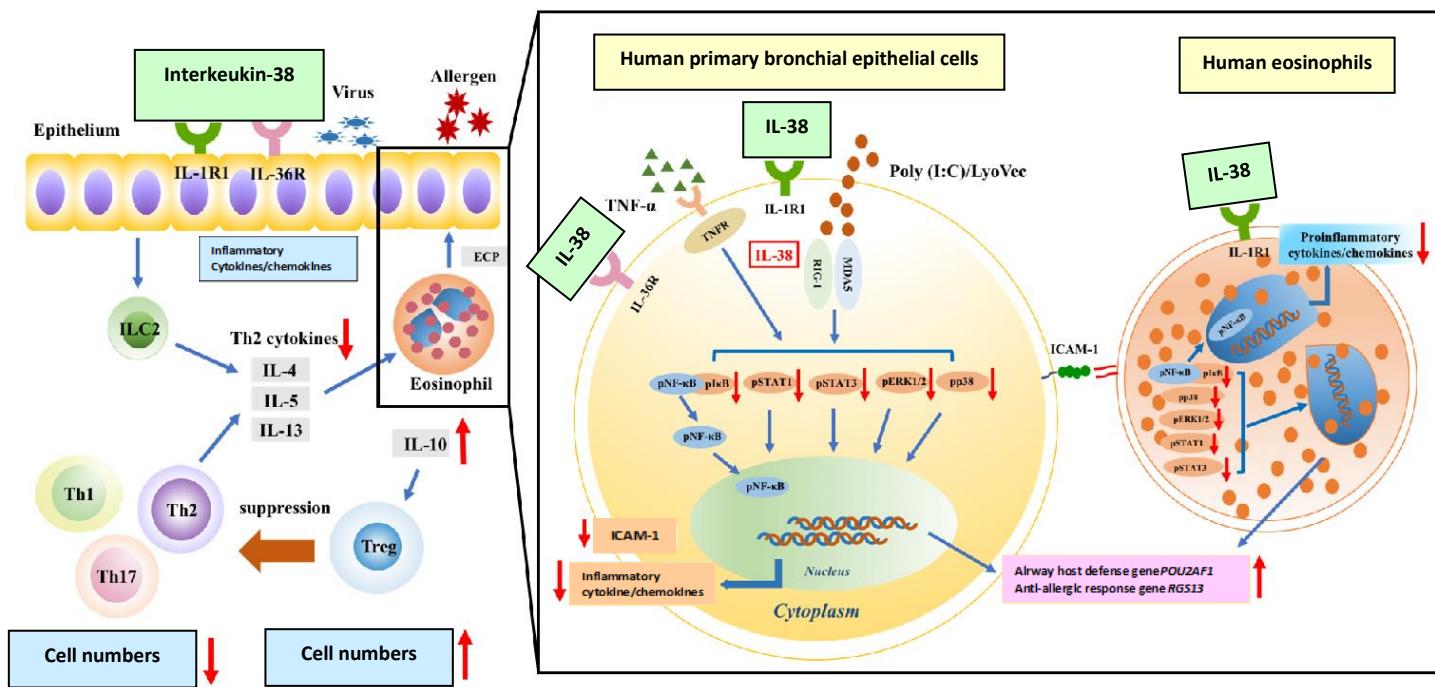


Figure 3. Anti-inflammatory activities of IL-38 in allergic airway inflammation. In murine allergic asthma, IL-38 can inhibit the accumulation of eosinophils, ILC2s and Th2 cells and the release of CCL11, eosinophilic cationic protein (ECP) and the Th2- cytokines IL-4, IL-5 and IL-13. In addition, IL-38 can promote Tregs, which are regulated by IL-10 to maintain immune homeostasis. In our in vitro study, we focused on the interaction between eosinophils and bronchial epithelial cells. Activation of co-cultured human primary epithelial cells and eosinophils by the viral mimic dsRNA RLR ligand poly (I: C)/LyoVec or pro-inflammatory TNF- α could be suppressed by IL-38, and this suppression was mediated by the downregulation of the p38, STAT1, STAT3, ERK1/2 and NF- κ B pathways and upregulation of the expression of the airway host defence gene POU2AF1 and the anti-allergic response gene RGS13 in bronchial epithelial cells, leading to significantly reduced expression of ICAM-1 and pro-inflammatory cytokines and chemokines. In addition, IL-38 was able to reduce the phosphorylation of p38, STAT1, STAT3, ERK1/2 and I κ B α in eosinophils of the co-culture system, thereby ameliorating allergic airway inflammation.¹⁹

Association between rhinitis and eustachian tube dysfunction in adolescents

Dr. Birgitta Y. H. WONG

MBBS (HK), MRCSED, FRCSED (ORL), FHKCRL, FHKAM (Otorhinolaryngology)

Specialist in Otorhinolaryngology

Chief of Service, Consultant & Honorary Clinical Associate Professor

Department of ENT, Queen Mary Hospital, The University of Hong Kong, Hong Kong



Patients with rhinitis often demonstrate various otologic symptoms. Among them, extensive studies had been on Eustachian tube dysfunction (ETD). Underlying mechanisms include mechanical obstruction caused by inflammation from allergies, infection and dysfunction of the tensor veli palatine muscles.¹ As the Eustachian tube is shorter and more horizontal in young children, we may expect the dysfunction will improve after childhood. Recently there is a paper published on the association between rhinitis and Eustachian tube dysfunction in a nationally-representative population of US adolescents and particularly further focusing on non-allergic rhinitis and allergic rhinitis adolescents.²

The paper included adolescents with age between 12 and 19 from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) cycle who completed the allergy and audiology assessments. The allergy assessment included self-reported questionnaire, serum total and allergen-specific immunoglobulin E. Audiology assessment consisted of tympanometry. Three outcome representing ETD were evaluated independently including positive response of having 3 or more ear infection, with a myringotomy and tube insertion for middle ear effusion and abnormal tympanometry defined as type B or C tympanogram in either ear. Otolaryngologists placing myringotomy tubes likely follow the Clinical Practice Guideline for children experiencing otitis media with effusion for 3 months or longer with associated hearing impairment, or for children with recurrent acute otitis media and middle ear effusion on examination i.e. 3 episodes in 6 months or 4 episodes in 1 year.²

For the results, a total of 1955 adolescents completed the allergy and audiology assessment. There were 160 patients with non-allergic rhinitis, 333 with allergic rhinitis and 1462 without rhinitis. Over one-third (38.8%) of adolescents reported history of 3 or more ear infections and 11.4% reported history of myringotomy tubes insertion. Abnormal tympanometry was found in 14%. Adolescents with rhinitis were significantly more likely to report ear infection and tubes insertion compared to those without rhinitis ($P<0.001$). However, association between rhinitis and abnormal tympanometry was not statistically significant for type B and type C tympanograms. Interesting, on further evaluation of results, the association between rhinitis and ETD was strongest for those with non-allergic rhinitis than allergic rhinitis. The non-allergic rhinitis adolescent group had twice the odds of ear infections and three times the odds of myringotomy tube insertion. The

author suggested that a specific inflammatory mechanism maybe involved in ETD in this population and warrants further studies.² Allergic rhinitis is an IgE-mediated inflammatory response while the pathophysiology of non-allergic rhinitis is independent of IgE. Non-allergic rhinitis has many subtypes including vasomotor rhinitis, infectious rhinitis, gustatory rhinitis, alcohol-induced rhinitis, hormonal and drug-induced rhinitis, atrophic rhinitis and nonallergic rhinitis with eosinophilia syndrome (NARES) with different underlying cellular inflammation and neurogenic factors.^{3,4} The author commented that this may explain why traditional treatment for allergic rhinitis such as intranasal steroid spray and antihistamine are ineffective for Eustachian tube dysfunction. Cochrane review also found that intranasal antihistamines and decongestants did not benefit children with OME.⁵

Recent years, Eustachian tube balloon dilatation has been used for patient with medically refractory Eustachian tube dysfunction. A pediatric cohort study had shown improvement in tympanometry and mucosal inflammation after balloon dilation⁶ though the procedure is not currently popular among pediatric age group yet. Further studies on the relationship and underlying inflammatory mechanisms between different types of rhinitis and Eustachian tube dysfunction is needed and the role of possible surgical management.²

References

- Fireman P. Otitis media and Eustachian tube dysfunction connection to allergic rhinitis. *J. Allergy Clin Immunol* 1997; 99(2); S787-97. [\[Pubmed\]](#)
- Adams DR et al. Are rhinitis and Eustachian tube dysfunction associated in United States adolescents? *Int J Pediatr Otorhinolaryngol* 2023 Apr; 167:111511. [\[Crossref\]](#) [\[Pubmed\]](#)
- Dykewicz MS et al. Rhinitis 2020: a practice parameter update. *J Allergy Clin Immunol* 2020; 146(4);721-767. [\[Pubmed\]](#)
- Baroody FM et al. Nonallergic rhinitis: mechanism of action. *Immunol Allergy Clin North Am.* 2016; 36(2);279-287. [\[Pubmed\]](#)
- Griffin G et al. Antihistamines and/or decongestants for otitis media with effusion (OME) in children. *Cochrane Database Syst. Rev.* 2011 Sep 7:2011 (9):CD003423. [\[Pubmed\]](#)
- Toivonen J et al. Balloon dilation for obstructive Eustachian tube dysfunction in children. *Otol Neurotol.* 2021;42(4):566-572. [\[Pubmed\]](#)

Original work supported by HKIA Research Grant

Differentiating viral and non-viral respiratory illness in hospitalized children by evaluation of immune signatures in nasopharyngeal aspirate

Dr. Renee W.Y. Chan

BSc (Hons), MPhil, PhD, Diploma (Epidemiology)
Associate Professor, Department of Paediatrics
The Chinese University of Hong Kong, Hong Kong



Respiratory infection is the most common reason for hospitalisation in children and most of the respiratory tract infections are of viral origin. Viral wheeze is common in infants and young children and most of them outgrow the problem, while some would have persistent wheeze with different intensities. Therefore, we aim to differentiate viral and non-viral wheeze in children in the in-patient setting by measuring the host response genes and protein expression in the nasopharyngeal aspirate to compare the sensitivity and specificity of the infection type with the PCR microbial detection assay.

Subject recruitment

Nasopharyngeal aspirate (NPA) was collected from paediatric patients aged 0-17 years with symptoms of respiratory illness and admitted to the paediatric ward at the Prince of Wales Hospital (PWH) in Sha Tin, Hong Kong between August 2018 and July 2019 by nurses in a negative pressure room. Samples collected from patients receiving corticosteroids or other immunosuppressive drugs within four weeks before NPA collection, patients with compromised immunity, chromosomal aberrations, structural lung diseases, gastroesophageal reflux, recurrent aspiration, and patients admitted beyond seven days since disease onset were excluded from the study. As part of our clinical management, we tested the samples for the presence of nine respiratory viruses, including adenovirus, human metapneumovirus, influenza A and B, parainfluenza 1, 2, and 3, respiratory syncytial virus, and enterovirus/rhinovirus.

Sample and Data Processing

Samples were separated into their cell pellet and supernatant components by centrifugation. We treated the cell pellets with cell lysis buffer, while the supernatant was aliquoted into small volumes and stored at -80°C until RNA extraction and measurements. Quantitative polymerase chain reaction (qPCR) was performed for β -actin, chemokine (C-C motif) (CCL5), CCL8, chemokine (C-X-C motif) ligand (CXCL)10, CXCL11, interferon induced protein with tetratricopeptide repeats (IFIT)2 and 2'-5'-oligoadenylate synthetase like (OASL) using primers from Invitrogen and SYBR Premix

Ex Taq (TaKaRa, Cat. #RR820). Levels of secreted proteins CXCL10 and CXCL11 were determined by enzyme-linked immunosorbent assay (ELISA). Collected data was analysed by GraphPad Prism Version 6.01 software. Receiver operator characteristic (ROC) curves and their associated area under the curve (AUC) statistics were calculated for each measured gene and protein by Prism. The Youden Index (J) was used to determine a threshold. Assuming sensitivity and specificity hold equal diagnostic importance, $J = \text{Sensitivity} + \text{Specificity} - 1$, J provides an optimal threshold. The Mann-Whitney test was used whenever appropriate. Two-sided p -values <0.05 were considered statistically significant.

Results

From August 2018 to August 2019, 230 paediatric patients were recruited from the paediatric isolation ward of Prince of Wales Hospital. 55% (127/230) of them were male and the overall median age was 2.44 years old (range from 29 days to 16.11 years old) with no significant difference in age distribution between the two genders. 16.1% (37/230) of the recruited subjects had wheeze during admission, and there were no differences in gender and age distribution between the wheeze and non-wheeze groups (Table 1).

Virus detection

A total of 230 nasopharyngeal aspirates (NPA) were collected freshly from each subject. A panel of nine respiratory viruses including adenovirus (ADV), human metapneumovirus (hMPV), influenza (IV) A and B, parainfluenza (PIV) 1, 2, and 3, respiratory syncytial virus (RSV), and enterovirus/rhinovirus (EV/RV) were included in the test. A genotyping test was carried out for EV/RV positive specimen by a nested PCR of the viral protein(VP)4/VP2 region followed by Sanger sequencing and alignment.

The virus detection rate between the wheeze and no wheeze groups were not significant (Table 2). However, a higher percentage of patients with wheeze had EV/RV or RSV when compared with those without wheeze ($p = 0.004$ and $p = 0.039$, Chi-square test). Moreover, within those with EV/RV detected, RV-C contributed

significantly more to cases with wheeze than those without ($p = 0.009$). In contrast, patients with wheeze were less likely to have IVAs (H1 and H3) detected ($p = 0.004$) than those without wheeze. Patients with wheeze and rhinovirus infection were less likely to have rhinovirus of species A (RV-A) ($p = 0.011$).

Pre-selected host transcript and protein signature are upregulated in the NPA of virus infected paediatric patients

To ensure the sample quality, samples with less than 200 detected copies of β -actin gene were excluded, the overall sample number for the downstream analysis was 114. When comparing levels of the genes and proteins measured¹, samples that were confirmed to contain a virus by the clinical laboratories' panel test demonstrated a significant increase in all tested gene and protein expression (Figure 1).

Predictive value of the presence of virus using host signatures

In order to determine the predictive ability of these host transcript and protein expression, receiver operating characteristic (ROC) curves were made for each host signature and the area under curve (AUC) were calculated. Overall, each of the host signature tested for its predictivity of viral infection was greater than 0.72 (Table 3).

Using the ROC curves, thresholds for all the tested host signatures were derived using the Youden Index calculation (Table 4). These thresholds provided the basis for predicting a viral infection. When the host transcript or protein level was above the threshold, viral infection was predicted, and if the host signature level was below the threshold, no viral infection was predicted.

Using these established thresholds, the positive predictive value (PPV) was above 0.92 except for IFIT2 (PPV=0.76) while the specificity was above 0.92 in the case of CCL8, CXCL10 and CXCL11 gene. However, in all these host signatures, the negative predictive value (NPV) was below 0.5.

Discussion

Distinctive peripheral blood transcriptome signature has shown promise in diagnostic discrimination between viral and bacterial infection.^{1,2} Future research should also consider if an in-depth transcriptome study can be done systematically for the

identification of a better local host signatures for the prediction of viral and bacterial infection. This might be superior to the blood cells or plasma samples as the nasal mucosa is the site of entry for the first line of defence.

The traditional PCR-based test cannot differentiate if a virus is active in the biospecimen and between active and subclinical or latent infection. An alternative strategy that can address these problems involves a pan-viral, host-signature-based test that will detect the presence of any viral species. The development of a reliable pan-viral test will likely lead to a more targeted disease management.

The current study provided evidence that a pan-viral test to predict viral infection is feasible. The high PPV of the tests using Youden Index thresholds indicates that such tests can be useful in determining the presence of a viral infection in a clinical setting. However, the low NPV of these tests would mean a viral-negative prediction is less certain and would require further testing. Thus, the Youden Index is only ideal in scenarios where sensitivity and specificity are equally important. There may be instances where false negatives cannot be tolerated in a clinical setting, and the Youden Index threshold is no longer applicable. In this study, using the Youden Index created tests with exceptional PPV but low NPV. The main benefit of this is that the test would do so correctly, and the physician can be confident that the test is correct. However, the low NPV would mean that if the test predicted the absence of a virus, further testing would be required to confirm this, which may defeat the original purpose of a pan-viral detection tool.

We would like to thank the Hong Kong Institute of Allergy Research Grant 2018 to support this project.

References

- Landry ML, Foxman EF. Antiviral Response in the Nasopharynx Identifies Patients With Respiratory Virus Infection. *J Infect Dis.* 2018;217(6):897-905. [\[Crossref\]](#) [\[Pubmed\]](#)
- Herberg JA, Kaforou M, Wright VJ, Shailes H, Eleftherohorinou H, Hoggart CJ, et al. Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children. *JAMA.* 2016;316(8):835-45. [\[Crossref\]](#) [\[Pubmed\]](#)

	n = 230			
Age, years old (median ± range)	2.44 ± 16.03			
Gender distribution	Male (n=127, 55%)		Female (n=103, 45%)	
Age, years old (median ± range)	2.58 ± 15.93		2.43 ± 15.95	
	Wheeze 22, 17%	Non-wheeze 105, 83%	Wheeze 15, 15%	Non-wheeze 88, 85%
Age, years old (median ± range)	2.19 ± 8.94	2.74 ± 15.93	2.83 ± 10.53	2.40 ± 15.95

Table 1. Demographics of the recruited subjects, and the subgrouping of wheezer and non-wheezer.

	Wheeze (n=37)	No wheeze (n=193)	p value
Virus detected (n, %)	27, 73%	120, 62%	0.210
Gender, Male (n, %)	17, 64%	67, 56%	0.292
Age, years (median ± range)	2.41 ± 10.91	2.44 ± 16.03	0.562
EV/RV			
RV-A	2, 15%	13, 65%	0.011
RV-B	1, 8%	0, 0%	0.394
RV-C	8, 62%	3, 15%	0.009
<i>undefined</i>	2, 15%	4, 20%	>0.999
RSV	7, 26%	13, 11%	0.039
PIVs	2, 7%	13, 11%	0.595
IVAs (H1 and H3)	1, 4%	37, 31%	0.004
IVB	0, 0%	7, 6%	0.198
ADV	1, 4%	15, 13%	0.185
hMPV	1, 4%	9, 8%	0.479
Co-detection of more than 1 agent	2, 7%	6, 5%	0.618

Table 2. Demographics of the wheezer and non-wheezer and the distribution of respiratory virus being detected.

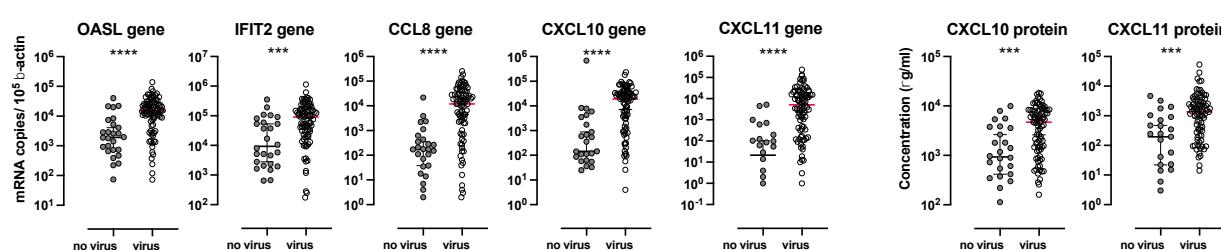


Figure 1. Comparing gene expression and protein levels between NPA samples 114 paediatric patients with and without a viral infection. Copies of mRNA were normalized to the number of β -actin mRNA copies in the same sample. Center line and error bar represent the median and 95% CI. Statistical significance was determined using Mann-Whitney tests, *** $p < 0.005$ and **** $p < 0.0001$.

Host signature	Area Under Curve (AUC)		95% CI		P-value	
	All (n=114)	Wheezy (n=22)	All	Wheezy	All	Wheezy
OASL gene	0.78	0.61	0.68 to 0.88	0.33 to 0.90	<0.0001	0.4174
IFIT2 gene	0.73	0.61	0.62 to 0.84	0.34 to 1.00	0.0004	0.4174
CCL8 gene	0.81	0.89	0.73 to 0.89	0.74 to 1.00	<0.0001	0.0064
CXCL10 gene	0.82	0.95	0.72 to 0.91	0.86 to 1.00	<0.0001	0.0015
CXCL11 gene	0.88	0.99	0.81 to 0.95	0.96 to 1.00	<0.0001	0.0005
CXCL10 protein	0.75	0.86	0.64 to 0.85	0.70 to 1.00	0.0002	0.0099
CXCL11 protein	0.74	0.66	0.64 to 0.85	0.38 to 0.96	0.0002	0.2532

Table 3. Area under curve (AUC) statistics of the ROC curves generated for each host signature using the data of all paediatric subjects recruited with respiratory symptoms and above the sample QC (n=114) and the wheezy subset (n=22).

Host signature	Threshold	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Sensitivity	Specificity	Accuracy
Gene Copies per 10⁵ b-actin						
OASL	8,698	0.94	0.47	0.71	0.85	0.74
IFIT2	8,605	0.76	0.34	0.71	0.50	0.66
CCL8	2,421	0.97	0.47	0.69	0.92	0.74
CXCL10	8,741	0.98	0.42	0.61	0.96	0.69
CXCL11	1,035	0.97	0.47	0.69	0.92	0.74
Protein pg/mL						
CXCL10	3,881	0.92	0.37	0.56	0.85	0.63
CXCL11	1,059	0.92	0.37	0.56	0.85	0.63

Table 4. The thresholds (maximal Youden Index) for each host signature, and the corresponding sensitivity, specificity and accuracy generated from the data of all paediatric subjects recruited with respiratory symptoms and above the sample QC (n=114).

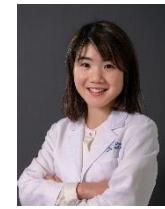
An update on the diagnostic strategy for fish allergy

Dr. Agnes S.Y. LEUNG

MB ChB (CUHK), MRCPCH (UK), FHKCPaed, FHKAM (Paediatrics)

Assistant Professor, Department of Paediatrics

Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong



Fish is one of the major food allergens, particularly affecting regions where fish consumption is high. Our team began researching fish allergies after observing that many young children in Hong Kong experience severe allergic reactions to grass carp at a young age. We noted that the most common culprit fish species causing allergic reactions were grass carp, salmon, and grouper, with freshwater fish being the first to cause allergic reactions in 75% of subjects.¹

Our initial proposal, supported by the research grant from the Hong Kong Institute of Allergy, focused on evaluating the varying levels of allergenicity between freshwater fish (grass carp) and saltwater fish. Preliminary findings found that among the six fish species commonly consumed in Hong Kong (salmon, golden threadfin bream, grass carp, pompano, mandarin fish and tilapia, the protein levels of parvalbumin were lower in salmon and pompano, but higher in golden threadfin bream, grass carp, mandarin fish and tilapia.

The difference in allergenicity among fish species prompted our investigation into grass carp's allergenicity. We identify grass carp as the major allergen in Hong Kong, with a major IgE-binding protein, parvalbumin, being more allergenic than common carp, salmon, and cod parvalbumins.² The strong allergenicity of *Cten i 1*, a new major allergenic parvalbumin isoform from grass carp, contributes to the high IgE reactivity of grass carp. In a collaborative effort with a Japanese group, we further identified fish allergens in salmon and grass carp and evaluated the sensitization patterns of fish-allergic subjects from Hong Kong and Japan.³ Results showed common allergens like enolase, GAPDH, and parvalbumin, as well as salmon-specific allergens collagen and aldolase. Parvalbumin was the major allergen, with a 74.7% sensitization rate. However, Japanese subjects showed a more diverse allergen sensitization pattern. Cooking methods, such as baking and frying, modified the allergen composition of salmon and influenced allergic manifestations.

We further conducted oral food challenges for twenty-two subjects with a history of immediate allergic reactions within 2 hours of fish consumption. 71% of subjects were challenge-positive to grass carp or salmon, while 29% were challenge-negative for both. Of those challenged positive, 14 subjects were positive to grass carp only, one subject was positive to salmon only, and only five subjects were positive to both grass carp and salmon. The cumulative median dose of carp to which those reacted in the challenge was 10 g, much lower than that of salmon (53 g). During this process,

we also established a recipe for double-blinded placebo-controlled food challenges (DBPCFC) to diagnose grass carp allergy. This recipe has adequate blinding, was acceptable and was able to elicit allergic reactions in the active sample but not placebo in DBPCFCs conducted in subjects across different age ranges.

With these findings, we recently further examined the clinical characteristics, immunological profile, and tolerance pattern of fish, and shellfish in fish-allergic individuals.⁴ 249 individuals from six allergy clinics in Hong Kong experienced fish-allergic reactions between 2016 and 2021. This collaborative effort found that fish-allergic participants had a gradient of IgE sensitization to fish corresponding to their β -parvalbumin levels, the so-called "fish ladder of allergenicity". 40% of fish-allergic individuals reported tolerance to one or more types of fish, more commonly to fish with lower β -parvalbumin levels, like tuna and salmon, than β -parvalbumin-rich fish, such as tilapia, catfish, and carp. This suggests that individuals with lower IgE titers may have selective tolerance to β -parvalbumin-poor fish, so it is safer to choose fish species with lower levels for oral food challenges. In this study, we found that 41% of individuals with fish and shellfish co-sensitization reported tolerance to crustaceans or mollusks, while half avoided shellfish, with 33% lacking sensitization. Patients with fish allergies do not need to avoid shellfish as false positive sensitization is common due to cross-reactivity with dust mites. Most importantly, findings from this study confirm the earlier age of fish allergy onset in the Chinese population, and we hypothesized that early exposure to β -parvalbumin-rich fish is linked to the early onset of fish allergy, while exposing at-risk children to fish with lower levels of β -parvalbumin may delay it. Further experimental study is needed to validate this theory.

Overall, our recent findings affirm that fish-allergic patients can have selective tolerance to specific fish species, and most of the time, they can also consume shellfish without problems, although thorough and careful evaluation and counseling are required from experienced allergists. Besides parvalbumin, there are also other fish components of interest. Further research is required to understand the intricacies involved in diagnosing fish allergies.

Acknowledgment: We thank the support of the Hong Kong Institute of Allergy for our research. We thank collaborators at QE (Dr. Chan WH, Polly Ho, Yau YS), PMH/YCH (Dr. Mike Kwan, QU Lee, Joshua Wong, Ivan Lam), QMH (Dr. Marco Ho, Jaime SRD), UCH (Dr. James Cheng), KWH (Dr. Chan WS), PYNEH (Dr. M Li),

AHNH (Dr. Vivien Mak, Grace Chiang) and TMH (Dr.

Esther Wong) for their support. We would like to express our gratitude to all patients and their families for participating in the study and for their unwavering support.

References

1. Leung ASY LN, Wai CY, Xu KJY, Lam MCY, Wong GWK, Leung TF. Characteristics of fish-allergic patients in Hong Kong. *Allergy* Aug 2020. [\[Crossref\]](#) [\[Pubmed\]](#)
2. Leung NYH, Leung ASY, Xu KJY, et al. Molecular and Immunological Characterization of Grass Carp (*Ctenopharyngodon idella*) Parvalbumin Cten i 1: A Major Fish Allergen in Hong Kong. *Pediatr Allergy Immunol*. Apr 22 2020;doi:10.1111/pai.13259 [\[Crossref\]](#) [\[Pubmed\]](#)
3. Wai CYY, Leung NYH, Leung ASY, et al. Differential patterns of fish sensitization in Asian populations: Implication for precision diagnosis. *Allergol Int*. Jul 2023;72(3):458-465. doi:10.1016/j.alit.2023.03.003 [\[Crossref\]](#) [\[Pubmed\]](#)
4. Leung ASY, WAi CYY, Leung NYH, Ngai NA, Chua GT, et a. Real-world sensitization and tolerance pattern to seafood in fish-allergic individuals. *JACI-in practice*. Oct 2023. doi.org/10.1016/j.jaip.2023.09.038 [\[Crossref\]](#)

Original work supported by HKIA Research Grant

The establishment of non-invasive nasal epithelial lining fluid collection method for the detection of localized IgA and mucosal immune response

Dr. Renee W.Y. Chan

BSc (Hons), MPhil, PhD, Diploma (Epidemiology)
Associate Professor, Department of Paediatrics
The Chinese University of Hong Kong, Hong Kong



In respiratory system, the upper airway epithelium is in proximity to the environment. Nasal epithelium functions as a physical barrier and exerts innate immune response, such as the secretion of mediators, proteases and anti-microbial peptides, to act against the stimuli and maintain the equilibrium state of the microenvironment. The epithelium also constantly secretes interleukin-2 (IL-2), IL-5, IL-6, IL-10 and TGF- β for the growth and differentiation of B-cell to Immunoglobulin A (IgA) producing plasma cells. In addition, the epithelium also produces secretory component, a glycoprotein that stabilizes the secretory IgA and transports the polymeric IgA and IgM.¹

IgA is the most produced antibody in the human body. It binds to and neutralized pathogens to prevent infection at mucosal sites and also contributes to the initiation of inflammation. The IgA also initiates the inflammation by the formation of immune complexes that trigger the Fc alpha Receptor I (Fc α R).² At the mucosa, IgA is produced by local plasma cells in the form of dimer. The polymeric immunoglobulin receptor (pIgR) on the basolateral side of the epithelial cells can leverage the removal of antigen or microbe bound IgA across the epithelium through a vesicular transport mechanism. In the circulation, IgA is produced by bone marrow plasma cells in the form of monomer at concentrations of 1-3mg/ml of serum. Patient with a serum deficiency of IgA have a high incidence of respiratory infections.³ No known reports of individuals deficient in secretory IgA but with normal serum values and it is not known if this deficiency is transient or permanent.⁴

A dependable, non-invasive self-administered sampling method that can be used across different age groups with repeatable is ideal for longitudinal studies. Recently, Reboli *et al.* introduced a novel collection method using Leukosorb medium, an absorbent made of fibrous matrix designed to isolate leukocytes from whole blood. This method collects nasal fluid and isolates different biomarkers from nasal epithelial lining fluid, providing an innovative sampling method.⁵ A similar method was used to evaluate the immune response in experimental rhinovirus infection in patients with allergic asthma.⁶ Therefore, this project aimed to establish a non-invasive

method of collecting the nasal epithelial lining fluid (NELF) from subjects for the analysis of various parameters, including respiratory viral RNA and local mucosal immune responses.

Optimization of the dimensions of nasal strip for all ages

To ensure the nasal strip can be widely used, it must fit all populations, including different age groups with varying nose sizes. The dimensions for adults were adopted from Reboli's publication, with 4mmW×40mmL, including a 12mmL holding region. A shortened version of 4mmW and 27mmL, with a 12mmL holding region, was designed for paediatrics. In addition, a modified L-shaped version with the same collection dimension for adult and paediatric subjects was adopted and used in this study.

Viral RNA and mucosal IgA stability in NELF collected by nasal strips

To determine the stability of viral RNA over time, nasal strip pairs were collected from six Covid-19 patients and stored for 24-to-72 hours at room temperature. The viral RNA of nucleoprotein protein of SARS-CoV-2 was determined by qRT-PCR. The results showed that the viral RNA remained detectable, with no significant difference in CT value even after 72 hours.⁷ To test the stability of immunoglobins, nasal strip pairs were collected from four subjects and stored for 5, 7, and 14 days at room temperature. NELF was eluted by 300 μ L PBS from nasal strips, and total IgA was determined by the Total IgA ELISA kit (abcam). There was no significant difference in total IgA between Day 0 and Days 5, 7, and 14 by Student *t*-test ($p=0.95$, 0.37, and 0.45, respectively).

Measurement of total IgA in NELF in a cross-sectional school-based study

After the verification of the nasal strip method, we conducted a school-based study in a local primary school to check for its use in a community setting with healthy children. Thirty-four subjects were recruited with 47% (16/34) of them were female. The mean age of the female and male students was 9.8 ± 2.1 years old and 9.8 ± 2.3 years old. Their NELF samples were collected and

subjected to total IgA measurement. The concentration of the total IgA was \log_{10} transformed the normality of dataset passed the D'Agostino & Pearson test. The mean and standard error of mean of the total IgA

concentration was $413.16 \pm 62 \mu\text{g/mL}$ and $439.05 \pm 96 \mu\text{g/mL}$ in female and male subjects. No significant differences in the mean of the total IgA concentration between the two genders was found by Student *t* test ($p=0.60$). Age posed no significant effect in the concentration of total IgA measured within this cohort (age range: 6 to 15 years old) in both genders.

Measurement of Spike protein specific IgA, IgG, and neutralising antibody in NELF in a longitudinal prospective SARS-CoV-2 study

In a mucosal immunity study of Covid-19 paediatrics patients, longitudinal NELF were collected at admission and every seven days during in-patient period and during follow-up consultation after being discharged.⁸ Thirty-four paediatric patients at the median age of 12.5 years old (range 6-17) were recruited with 32% male. A total of 188 NELF were collected longitudinally, and 88 paired plasma samples were collected as a comparison. All subjects were tested negative to other respiratory pathogens in a PCR multiplex panel during admission.

The SARS-CoV-2 S1-specific IgA was detected in 54% of the NELF and 43% of the plasma samples of the paediatric patients within the first four days of disease diagnosis. A minority of NELF samples were tested positive with S1-specific IgG after twelve days of disease diagnosis. The result indicated that the local and systemic antibodies had a different kinetics during an acute infection.

Moreover, the mucosal antibody detection would shed light to disease severity association. When we compared the nasal S1-specific IgA level on day 0-to-4 after diagnosis, the asymptomatic paediatric patients had a significantly higher level of S1-specific IgA than the symptomatic paediatric patients ($p<0.01$), together with a trend of a higher percentage of NELF with positive S1-specific IgA at the same time point ($p=0.05$, Fisher's Exact test). Therefore, the nasal strip could open up a new dimension for the study of local immune responses.

Measurement of IgE in NELF in patients with allergic symptoms

Apart from infectious diseases, the nasal strip method can also serve in the allergy clinic. Thirty six patients (5-41 years old) were recruited in the local allergy outpatient clinic and IgE in their NELF was also evaluated for the correlation with allergic diseases in a cross-sectional manner. Two allergists while atopic graded severity score of allergic rhinitis, asthma, and eczema and food allergy status were indicated by skin prick test. Total IgE and cytokines in NELF were measured by ELISA. Serum IgE levels were retrieved from clinical records. Correlation was tested using Spearman's test. Mucosal and serum IgE were found to be correlated ($r=0.60$,

$p=0.02$) while a negative correlation between age and mucosal IgE was observed ($r=-0.43$, $p=0.01$). Mucosal IgE had a positive correlation with eczema severity ($r=0.54$, $p=0.001$) but not asthma severity.

Potential of nasal epithelial lining fluid collected by nasal strip

Total IgA measurements in NELF of thirty-four primary school students served as a proof-of-concept on the possibilities of utilizing nasal strip to provide mucosal measurement in the community settings while the measurements of SARS-CoV-2 viral gene and spike protein 1 specific IgA and IgG in patients with acute Covid-19 demonstrated the feasibility of this self-administrated non-invasive collection method in virological and immunological aspects during acute respiratory infection. The nasal IgE measurement pioneered the research area of local mucosal immunity in relation to the allergic disease.

We would like to thank the Hong Kong Institute of Allergy Research Grant 2020 to support this project.

References

1. Salvi S, Holgate ST. Could the airway epithelium play an important role in mucosal immunoglobulin A production? Clin Exp Allergy. 1999;29(12):1597-605. [\[Crossref\]](#) [\[Pubmed\]](#)
2. Hansen IS, Baeten DLP, den Dunnen J. The inflammatory function of human IgA. Cell Mol Life Sci. 2019;76(6):1041-55. [\[Crossref\]](#) [\[Pubmed\]](#)
3. Ammann AJ, Hong R. Selective IgA deficiency: presentation of 30 cases and a review of the literature. Medicine (Baltimore). 1971;50(3):223-36. [\[Pubmed\]](#)
4. Rockey JH, Hanson LA, Heremans JF, Kunkel HG. Beta-2a Aglobulinemia in Two Healthy Men. J Lab Clin Med. 1964;63:205-12. [\[Pubmed\]](#)
5. Rebuli ME, Speen AM, Clapp PW, Jaspers I. Novel applications for a noninvasive sampling method of the nasal mucosa. Am J Physiol Lung Cell Mol Physiol. 2017;312(2):L288-L96. [\[Crossref\]](#) [\[Pubmed\]](#)
6. Hansel TT, Tunstall T, Trujillo-Torralbo MB, Shamji B, Del-Rosario A, Dhariwal J, et al. A Comprehensive Evaluation of Nasal and Bronchial Cytokines and Chemokines Following Experimental Rhinovirus Infection in Allergic Asthma: Increased Interferons (IFN-gamma and IFN-lambda) and Type 2 Inflammation (IL-5 and IL-13). EBioMedicine. 2017;19:128-38. [\[Crossref\]](#) [\[Pubmed\]](#)
7. Chan RWY, Chan KC, Chan KYY, Lui GCY, Tsun JGS, Wong RYK, et al. SARS-CoV-2 detection by nasal strips: A superior tool for surveillance of paediatric population. J Infect. 2021;82(4):84-123. [\[Crossref\]](#) [\[Pubmed\]](#)

8. Chan RWY, Chan KCC, Lui GCY, Tsun JGS, Chan KYY, Yip JSK, et al. Mucosal Antibody Response to SARS-CoV-2 in Paediatric and Adult Patients: A Longitudinal Study. *Pathogens*. 2022;11(4).
[\[Crossref\]](#)[\[Pubmed\]](#)

Annual General Meeting 2023

7 November 2023

This year's AGM was held physically on 7 November 2023 at The Hong Kong Club. Council members and many members attended the meeting and had an enjoyable evening!



The 12th Hong Kong Allergy Convention
7-8 October 2023
Hong Kong Convention and Exhibition Centre

Organizer

HONG KONG 香港過敏科醫學會
INSTITUTE OF ALLERGY

Advances in Allergy Care 2023

The 12th Hong Kong Allergy Convention (HKAC 2023)

7 - 8 October 2023

The 12th Hong Kong Allergy Convention was successfully held on 7 - 8 October 2023 at the Hong Kong Convention and Exhibition Centre. With the theme of "**Advances in Allergy Care 2023**", the programme highlighted the novel discoveries in mechanisms and development of cutting-edge treatment and preventative paradigms for allergic diseases. A group of prominent speakers in the field of Allergy, Asthma and Clinical Immunology delivered state-of-the-art lectures in exciting areas of fundamental and applied science as well as teaching on new approaches to personalized practice.

The Organizing Committee would also like to extend their sincere thanks to all the speakers, the supporting organizations and sponsors for their ever unfailing support.

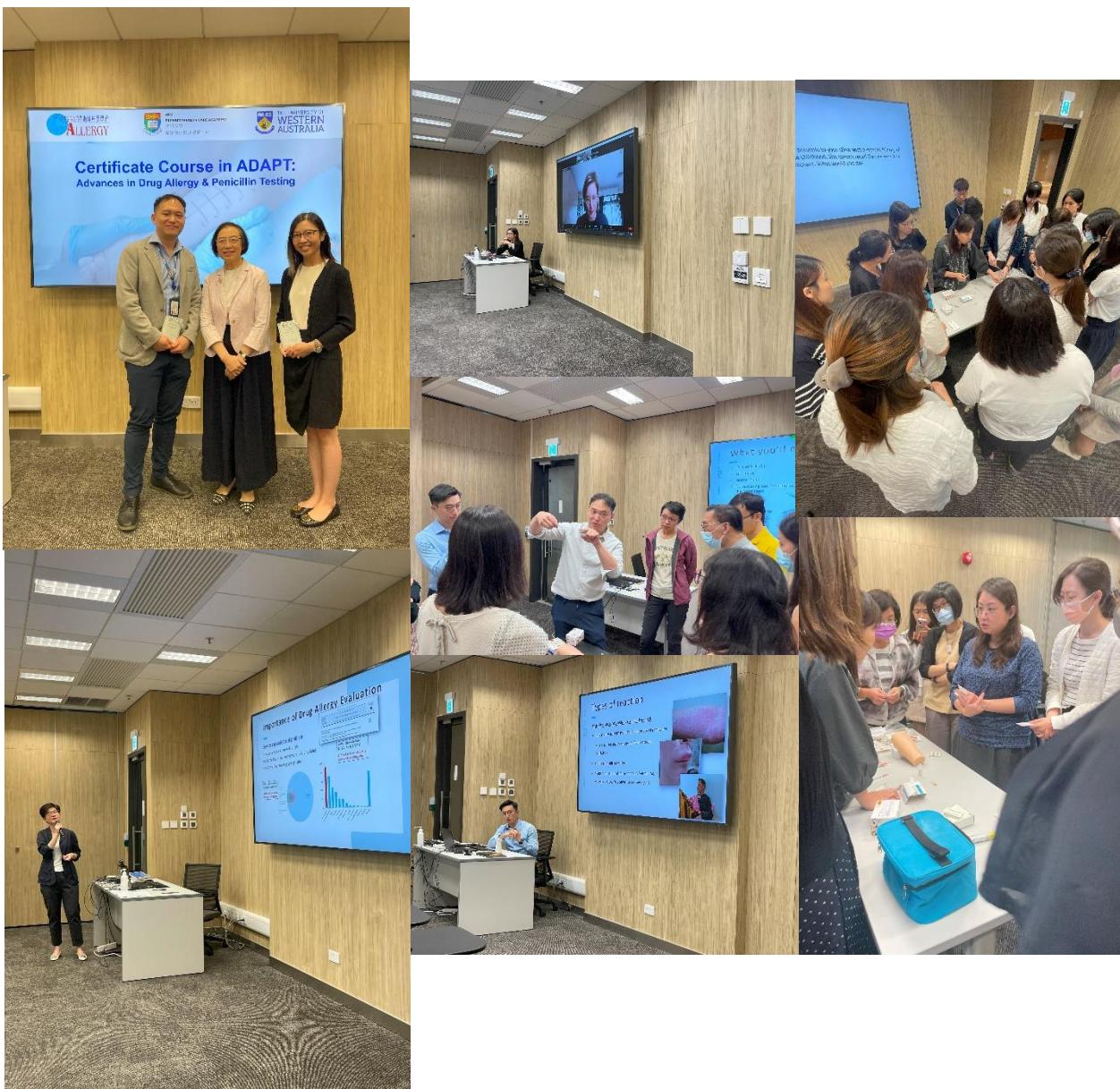


Course in ADAPT: Advances in Drug Allergy & Penicillin Testing

23 – 24 September and 4 – 5 November 2023

Hong Kong Institute of Allergy had co-organized with the Faculty of Medicine of the University of Hong Kong and the University of Western Australia on a course on drug allergy entitled: ADAPT – Advances in Drug Allergy & Penicillin Testing. Two identical courses were run on 23 – 24 September and 4 – 5 November 2023 at the HKU campus.

This course was the first of HKU's Primary Health Care Academy Series and it was presented by an international faculty of renowned drug allergy specialists from both Hong Kong and Australia. This course was specifically targeted towards both practising doctors and nurses, especially for front-line colleagues working in both out-patient and/or hospital settings. Both physician (ADAPT-P) and nursing (ADAPT-N) streams, taught by physicians and nurses experienced in drug allergy were conducted. There were about 30 participants for the first course held in September 2023.



Overseas Meetings

EAACI Congress 2024 (European Academy of Allergy and Clinical Immunology Congress 2024)
31 May - 3 June 2024 / Valencia, Spain (<https://eaaci.org/events/congress/eaaci-congress-2024/>)

ERS 2024 (European Respiratory Society Congress 2024)
7 - 11 September 2024 / Vienna, Austria (<https://www.ersnet.org/congress-and-events/congress/>)

CHEST 2024 (The American College of Chest Physicians Annual Meeting 2024)
6 - 9 October 2024 / Boston, MA (<https://www.chestnet.org/Learning-and-Events/Events/CHEST-Annual-Meeting>)

ACAAI 2024 (American College of Allergy Asthma and Immunology Annual Scientific Meeting 2024)
24 - 28 October 2024 / Boston, MA (<https://annualmeeting.acai.org/>)

Local Meeting

HKTS / CHEST Annual Scientific Meeting 2024 (ASM 2024)
24 March 2024, Hong Kong

28th Congress of the Asian Pacific Society of Respirology (APSR 2024)
7 - 10 November 2024, Hong Kong