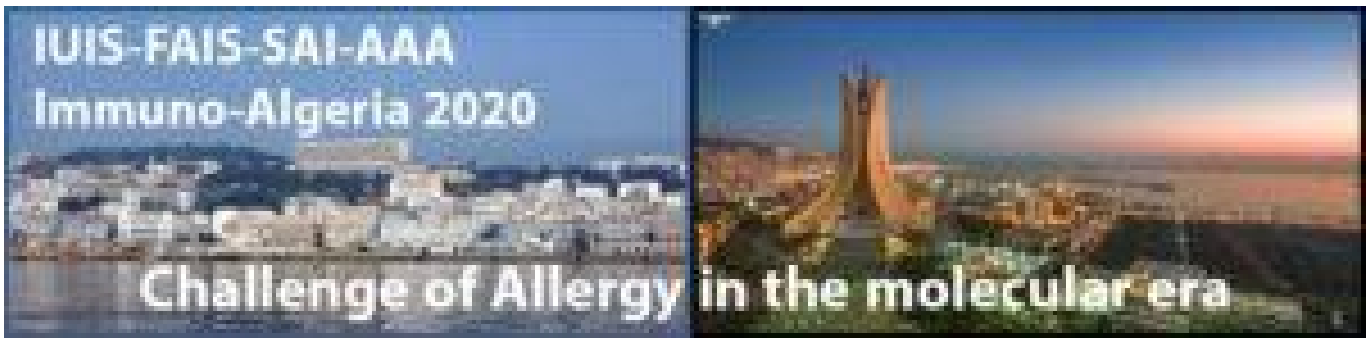
Immuno-Algeria: Non-invasive allergy biomarkers & next-gen immunotherapies



IUIS-FAIS-SAI-AAA Immuno-Algeria course took place remotely between 11th May -12th June. The theme of the course was *"Challenge of Allergy in the Molecular Era*". To ensure that all attendees had the immunological knowledge required for advanced content that was going to be discussed during the meeting, weekly immunology refresher lectures were provided during the month of May. This was followed by a 2 week long meeting focused on allergy content. This week we higlight lectures by Professors Carsten Schmidt-Weber and Claude Lambert that discussed non-invasive methods to diagnose and monitor allergy response, and a lecture by Professor Rudolph Valenta on "Next generation of allergen-specific immunotherapies: molecular approaches".

After a brief historical introduction, Prof Schmidt (Germany) started his lecture by reporting on the need for markers of endotyping (underlying pathobiological mechanism), disease prediction and therapy monitoring in the field of allergic

diseases.

Endotyping is essential for medical practice, as it enables in-depth characterisation of pathobiological mechanisms associated with clinical symptoms . For example, transcriptomic analysis of skin biopsies from people with different skin diseases (e.g. eczema, psoriasis) allows the identification of molecules that are differentially expressed depending on the disease. Combining transcriptomic analysis with clinical data/phenotypes could inform the identification of potential biomarkers of a certain clinical presentation. Prof Schmidt gave an example of endotyping between eczema and psoriasis which identified High iNOS and low CCL27 as a potential classifier for psoriasis which was superior to clinical presentation alone.

For disease prediction, Prof Schmidt gave the example of airway allergy, where there is a need for non-invasive markers to improve diagnosis at an early stage (early intervention chance for cure) and in chronic disease (endotyping targeted therapy). This has led to the creation of the ADAPT Consortium to combine biomarkers and clinical findings into a single risk score (ADAPT Algorithmprevention).

Another area of allergy where non-invasive markers are needed is during early life. In this part of his lecture, Pr Schmidt highlighted the difficulties associated with the molecular characterisation of different asthma phenotypes in childhood (in comparison with adulthood). Additionally, challenges in the identification of predictors for persistent asthma and transient wheeze, respectively. Regarding airways secretions, he demonstrated that analysis of molecules secreted by epithelial cells in nasal samples seems to be more reliable than markers from infiltrating cells. He gave the example of experimental biomarkers in nasal secretions for diagnosis of allergic rhinitis, which performs locally much better than systemically. Thus demonstrating that local analysis dynamics are in general more powerful than systemic assessment. He also

gave an overview of the studied nasal biomarkers from different sources (epithelial cells, infiltrating cells and both) during asthma.

Finally, he ended his talk by describing numerous non-invasive/ minimally-invasive biomarker candidates (from serum, microbiopsies, exhalates, secretions, excretions) which have been suggested and tested for primary prevention, differential diagnosis, endotyping and secondary prevention of allergic and non-allergic diseases.

Pr Claude Lambert (University of Saint Etienne, France) presented an interesting lecture on **T cell analysis in hypersensitivity**. He began his talks with a brief introduction of type I and IV hypersensitivity mechanisms, the role of the cytokine imbalance in the pathogenesis of the delayed type of hypersensitivity and by extension the role of T cell subsets in these reactions.

Prof Lambert discussed how flow cytometry could be used to monitor T cells in allergy diseases, specifically :

- T cell maturity: naive, central memory, effector memory and terminally differentiated effector cells
- Tissue homing (chemokine receptors)
- Functional polarisation and activity following non-specific (PHA, PMA/ionomycin) or a specific (antigen e.g. allergen peptides) stimulation.

He then emphasised the importance of quality assurance when performing flow cytometry assays as it may negatively affect analysis. He also gave a hands-on (online) practical example of flow cytometry analysis using a specialised flow cytometric software.

His second lecture focused on the use of flow cytometry to investigate immediate hypersensitivity. He began the lecture by using clinical cases of urticaria and quick edema to explain the mechanisms of type I hypersensitivity. He

specifically focused on the structure, activation and degranulation of the two main cells involved in this type of reaction: basophils and mast cells. In this summary, we shall focus on the use of the basophil activation test (BAT), a functional *ex vivo* test to investigate type I hypersensitivity. Basophils were first identified as **circulating IgE+ cells**, and determination of activated basophils by flow cytometry was first described with CD63. Now alternative basophil markers have been identified such as **CD203c (basophil-specific ectoenzyme E-NPP3)**, basophil lineage specific marker, which in combination with IgE identified activated basophils. Other described basophil markers include: **CD123+HLADR-**, **CRTH2+CD3-**, **CCR3+CD45+**, CCR3+CD3-, among others. He explained the basic principle of the test (*sampling, allergen stimulation, labelling, flow cytometric analysis using identification and activation markers of basophils*) and the possible technical issues when performing it (*controls, gating strategy, interpretation etc.*). BAT is a multifaceted and promising tool for the allergologist, who can use it to :

- Diagnose patients with food, insect venom, and drug allergies.
- Monitor patients on allergen immunotherapy,
- Monitor Anti-IgE treatment
- Study the natural resolution of allergy.

However, the proper performance of the BAT test requires special attention, which to many could be challenging due the technical expertise required.

Professor **Rudolph Valenta** began the talk by highlighting the importance of molecular diagnosis in providing specific and personalised allergenic immunotherapy (AIT) for patients. Molecular allergy diagnosis has revolutionised the identification of causal allergens in multi-sensitized patients, which eventually leads to a better targeted vaccination. Specific diagnosis also leads to targeted

treatment and favourable patient outcomes. He also highlighted the importance of molecular allergy in monitoring the efficiency of treatments in patients. One way to monitor immunotherapy is to measure specific IgE levels using microarrays. Current vaccination in allergic patients is accompanied with a decrease in specific allergen IgE levels and an increase in IgG1 and IgG4 levels.

The future perspectives of molecular diagnosis aim to:

- Diagnosis individuals earlier in life and facilitate prophylactic treatment
- Predict the development of allergy by detecting IgE in sensitised but asymptomatic children
- Predict genetic susceptibility for allergen-specific IgE sensitisation in not yet sensitized children

The elucidation of the molecular structures of the allergens and the determination of IgE and T cell epitopes recognized by allergic patients facilitate the design of vaccines that target different immune mechanisms. Some of the novel strategies being investigated are synthetic peptides targeting allergen-specific T cells, recombinant hypoallergenic allergen derivatives, and recombinant peptide carrier-based vaccines. These new strategies are under clinical trials and aim to decrease the number of injections of allergens in patients. Beside therapeutic vaccination, the trend now is to develop prophylactic vaccination: a personalized therapy, administered after the early identification of sensitization profiles in children, and will hopefully be available a decade from now.

References:

Valenta et al., 2018. Chapter Five — Molecular Aspects of Allergens and Allergy. Advances in Immunology

Summary by Sawsan Feki & Mezghiche Ikram