Final report

Short-term research fellowship Mouhamed Mounir Chentouh

Type of fellowship, duration and location

I have been at the Luxembourg Institute of Health (Luxembourg) from April 1st 2019 to Jun 30th 2019, with a Short Term Research fellowship in the Department Infection and Immunity (Director: Prof. Markus Ollert). The title of my internship was "ASSESSMENT OF THE ALLERGENICITY OF A NEW LEGUME FLOUR".

Dr Christiane Hilger, group leader of the 'Molecular and Translational Allergology' team, has been my supervisor, while Stéphanie Kler, a scientific researcher, helped me to carry out my laboratory experiments.

Research problem and objectives

New food products are entering the market without assessment of their allergenicity. They may generate new sensitizations, but they may also constitute a risk factor for specific allergic patients that react to allergen sources related to the new product. IgE-binding to highly identical allergens from different sources (IgE-cross-reactivity) can translate into clinical cross-reactivity. The pathogenesis-related (PR) protein family 10, the non-specific lipid transfer proteins (nsLTP) and profilins are well known examples of panallergens in pollen and plant foods. Legumes are important crop plants which have a high nutritional value. However they also account for a high incidence and severity of allergic reactions. Peanuts are the most prevalent trigger of allergic symptoms, followed by peas, lentils, soybeans, chickpeas, lupin, mung beans and red grams.

The present project aimed at evaluating the allergenicity of cowpea, a new legume flour. Cowpea, also commonly known as southern pea or black-eyed pea, is of high nutritional value as it is an important source of crude protein, being rich in glutamic acid, aspartic acid and lysine.

In particular, patients with an allergy to legumes might have an increased risk when ingesting cowpeas or cowpea flour because of potential cross-reactive molecules common to different legumes. The newly identified allergens will improve diagnosis of patients at risk as they can be used as components in novel diagnostic approaches such as molecular or component-resolved diagnosis (CRD).

Work program and results

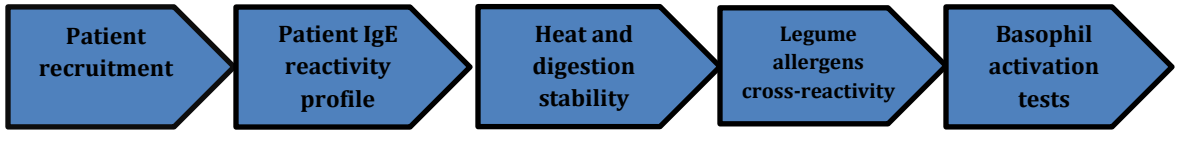

Fig 1: work program during the fellowship

The detailed information of each step of workflow (Fig. 1) is as below:

Patient recruitment

Legume and/or peanut allergic patients (n= 29) were recruited by Dr. Françoise Codreanu-Morel, Immuno-Allergology Unit at Centre Hospitalier de Luxembourg (CHL) (National Ethics Committee approval 20180705). Study participants were 2-12 years of age, male or female, with an allergy to legumes. Inclusion criteria include a positive skin prick test to at least one legume and/or cowpea and sIgE to one or several legumes.

Patient IgE reactivity profile

The majority of the patient sera showed IgE-binding to cowpea, pea and peanut protein extract in immunoblot. Patient sera were more reactive to pea and peanut proteins than to cowpea.

IgE-immunoreactivity of the patient sera to cowpea proteins could be due to cross-reactivity between legume vicilins, since vicilins are the main storage proteins in the seeds, they are the most abundant protein in the legume proteome and they are also known to be responsible for high IgE-cross-reactivity because of their important amino-acid sequence similarity.

2D SDS-PAGE and IgE immunoblotting of cowpea proteins followed by mass spectrometry revealed that indeed immunoreactivity of the patient sera to cowpea proteins is essentially due to vicilin fragments.

Assessment of legume IgE-cross-reactivity

In order to investigate IgE-cross-reactivity between legumes, as well as the role of cowpea vicilin, an enriched fraction of vicilin was prepared by means of successive sodium chloride precipitations from cowpea protein extract. IgE-cross-reactivity was assessed by means of IgE-ELISA inhibition assays using pea, peanut and cowpea crude protein extracts as well as the vicilin fraction prepared from cowpea.

ELISA inhibition assays demonstrated that cowpea extract was able to inhibit IgE-binding to pea and peanut to a great extent. In particular, vicilin was able to induce an average inhibition of 36% (ranging from 3 to 82%) of IgE binding to peanut extract in 46% of the patients and an average inhibition of 35% (ranging from 5 to 85%) of the IgE binding to pea extract in 89% of the patients.

Basophil activation tests

Basophil activation tests were performed using lentil, lupin, mungbean, soja, peanut, pea and cowpea protein extract, with allergen concentrations ranging from 10^{-4} ug/ml to 1000ug/ml.

Two distinct parameters were used for analysis of the results, CD63+ was used for the determination of the reactivity profile, while AC50 (allergen concentration that was able to activate 50 % of the basophils that were activated in the stimulation control (Anti FcЄRI)) was used for the determination of the sensitivity of the patient toward each extract.

All patients reacted positively to cowpea proteins in the BAT assay, however, they were more reactive and more sensitive to pea, peanut and lentil, (AC50 threshold < 10ug/ml) than to lupin,

mungbean, soja and cowpea. Those legumes were also able to activate basophils of the patients, but activation required a higher concentration of allergen (AC50 threshold > 20ug/ml).

Heat and digestion stability

The digestion stability of cowpea, pea and peanut proteins was investigated by means of enzymatic digestion with pepsin and trypsin followed by SDS-PAGE and immunoblot using a polyclonal anti-Ara h 1 antibody cross-reacting with pea and cowpea vicilin.

Simulated digestion experiments showed that pea and peanut proteins were resistant to gastric digestion, but degraded already after 1 minute of intestinal digestion, while cowpea proteins were highly resistant to both gastric and intestinal digestion.

Heat stability was assessed by cooking the grains in water for 1 hour at 90°C, extracting both soluble and insoluble fractions and analyzing the proteins by SDS-PAGE and immunoblot. Results showed that both cowpea, pea and peanut proteins were still immunoreactive even after 1 hour of cooking.

Conclusion

We have identified vicilin as a new allergen in cowpea. ELISA inhibition assays showed that it is highly cross-reactive with pea and peanut allergens. In-vitro and in-vivo assays confirm a potential allergenicity of cowpea in patients allergic to peanut and/or other legumes within the Fabaceae family.

The results of my fellowship will be presented as a poster at the EAACI-ISMA meeting in Amsterdam (November 2019) and we are planning to include our data into a manuscript that will be submitted next year.

Acknowledgement and personal reflection

I would like to express my special thanks of gratitude to Professor Markus Ollert and Dr Christiane Hilger for their support in applying for this fellowship, as well as the EAACI for giving me this generous award. I also extend my gratitude particularly to the Dr Christiane Hilger for hosting me at the Luxembourg Institute of Health and for her academic and material investment into my project, my thanks goes also to Dr Françoise Morel for her excellent collaboration and devotion and to all my colleagues and friends of the Infection and Immunity Department who freely shared their knowledge and skills and greatly supported this project.

Finally, I would like to say that nothing would have been possible without the fellowship award. This fellowship allowed me to achieve a great part of the work plan of my thesis, and gave me the opportunity to live a great and a productive research experience at the Luxembourg Institute of Health that will contribute to my professional as well as personal development, and will help me to carry out other future collaboration in Europe.