How immunoblotting and mass spectrometry can help to diagnose mustard allergy.

J. Courtois[1], C. Bertholet[2], E. Cavalier[2], N. Gillard[3], S. Tollenaere[4], B. Quinting[4], R. Gadisseur[2]

1: CRIG Liège, Belgium, j.courtois@crig.be, 2: CHU Liège, Belgium, 3: CER Group Marloie, Belgium, 4: HELMo Liège, Belgium

Introduction

We describe a clinical case study of severe allergy to a variety of mustard, *Sinapis alba*, in an adult patient without any previous food nor respiratory allergy history.

Objectives

The diagnosis of allergy to mustard is based on anamnesis, skin prick test and specific IgE (sIgE) measurement to total mustard extract. Actually, the *in vitro* diagnostic tools cannot help the physician to define the precise mustard allergens involved in the allergic reaction and are unable to support evaluation of potential cross-reactions. Indeed, no molecular allergen component is commercially available for mustard. We aimed to adapt a 2D immunoblot method to mustard. Afterwards, mass spectrometry (LC-MS/MS) was used to identify precisely the allergens bound to sIgE.

Methods

We analyzed the serum of a 37 y.o. man presenting a grade 2 reaction (facial quincke edema with respiratory distress) after eating food containing the mustard species *Sinapis alba*. He had positive sIgE results for mustard extract (0.62 KUA/L) and a positive realistic SPT to foods containing mustard. We extracted total proteins of *Sinapis alba* seed. The different proteins were separated based on their isoelectric point and their molecular weight. The patient serum was analyzed by 2D Western blot in order to evaluate its sIgE reactivity against the different protein spots. Finally, the protein spots recognized by the patient sIgE were precisely identified by LC-MS/MS.

Results

The patient sIgE sensitization profile showed three specific protein spots. The first protein spot was observed at 18 kDa and pH 5 to 6. A second protein spot was localized around 14 kDa and pH 5. Finally, the third protein spot was situated around 15 kDa and pH 7. The LC-MS/MS analysis of these 3 spots pointed out 2 allergens already described in mustard allergy: sin a 1 (2S-albumin) and sin a 2 (11S-globulin).

Conclusion

In this study, a 2D immunoblot provided a specific sensitization profile for a patient presenting a grade 2 allergy to mustard with low sIgE to total mustard extract and without any other history of allergy. The protein spots recognized by the sIgE concerned two main allergens identified by LC-MS/MS as sin a 1 and sin a 2. Those allergens are classified in the storage protein family which is associated to severe reactions to food and could be highly cross-reactive. We pointed out specific mustard allergens that could be associated to severe reactions such as facial quincke edema with respiratory distress.