11S globulin identified as a new bean allergen

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Legumes are one of the most frequent causes of food allergy, especially in children[1]. In Mediterranean areas and India, lentils and chickpeas are considered the most allergenic legumes. However, beans, also widely consumed in these populations, are less allergenic and usually present cross-reactivity with other legumes as lentils, chickpeas and peas. Numerous studies have demonstrated a high degree of cross reactivity between legumes[2].

The common bean, *Phaseolus vulgaris*, belongs to the Fabaceae family. Although there are not many studies focused on this legume, some proteins have been identified as allergens. Among them, the major seed storage protein is a vicilin *Phaseolin (Pha v)*, which belongs to the 7S globulin family with a molecular weight of 47.5 kDa[3]. This protein has also been described in red and white kidney bean. Moreover, an IgE-binding protein of 32 kDa is also identified in green beans as *Pha v Chitinase*, closely related to the major avocado allergen[4]. Besides, a 31 kDa major allergen of the red kidney bean (*Pha l*) was purified and identified as phytohemagglutinin with cross-reactivity to peanut and black gram[5]. On the other hand, Profilin (*Pha v 5*), Bet v 1-like (*Pha v 6*) and lipid transfer protein (LTP-*Pha v 3*) have also been described in the common bean with molecular weights of 14.4, 17 and 10 kDa, respectively, with a high degree of cross reactivity with others vegetables[6,7].

We present a 13 year-old boy without previous atopy history or food allergy who is attended in our consultation after suffering two anaphylactic reactions after eating pinto beans. He describes these episodes as generalized hives, labial angioedema, sneezing, nasal stuffiness and dyspnea 30-45 minutes after eating pinto beans. The reaction was completely resolved with intramuscular methylprednisolone and dexchlorpheniramine in less than 3 hours. Previously, he had tolerated all legumes and after the episode he
continued eating the remainder (peanuts, lentils, chickpeas, green peas and green beans) and tree nuts without incidents. However, he did not eat pinto or white beans again. Furthermore, he refers rhinoconjunctivitis symptoms and morning cough in autumn and spring months.

We performed skin prick tests to commercial extracts (Leti Pharma, Madrid, Spain) of soy, peanut, lentil, chickpea, green pea, white bean, green pea and broad bean with negative result for all of them. Nevertheless, skin prick-prick tests to fresh pinto and white beans were positive, eliciting an average diameter of wheal of 7mm and 5mm, respectively. Besides, skin prick test to pollen extracts were positive to *Platanus, Olea, Phleum and Lollium*. We also performed a blood test showing a total serum IgE (ImmunoCAP®, Thermo Fisher Scientific, Uppsala, Sweden) of 210 kUA/l and specific IgE antibodies were negative to peach, Pru p 3 (LTP), Pru p 4 (profilin), green peas, white beans, pinto beans, green beans, chickpeas and lentils (all of them with IgE<0.06 kUA/l).

In order to complete the immunological study, we prepared extracts with cooked white, pinto, red and black beans. Briefly, 100 g of each type of bean beaten with its cooking water, were dissolved in phosphate-buffered saline (PBS) and was kept overnight at 4°C under constant magnetic stirring. Then, the extract was filtered using a double-gauze filtration system. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, under reducing conditions, displayed protein bands ranging between 130 and 10 kDa [Figure 1a]. In addition, a Western blot with beans extract was performed and after the incubation with the patient’s serum, that revealed an IgE-binding protein about 15 kDa in white, pinto and red beans extracts; that was not the case of black beans extract which did not show any band. Meanwhile, no IgE-binding bands appeared in the negative control [Figure 1b].

A protein band with an apparent molecular weight of 15 kDa recognized by the patient’s serum was extracted from the gel and identified by mass spectrometry, as previously described[8], as well as by searching a nonredundant protein sequence database (NCBI) using the Mascot program (http://www.matrixscience.com) in the Proteomic Service of Complutense University of Madrid, which is a member of the ProteoRed Network. Research conducted with protein databases identified 15 kDa IgE-binding band as a legumin or 11S globulin.
We would like to highlight some relevant findings from this clinical case. The patient had an exclusive allergy to beans, both immunologically and clinically. In Mediterranean countries, the most frequent cause of legume allergy is lentil, and cross-reactivity to chickpea or pea is frequent. Common beans are usually well tolerated and some patients have allergy to green beans in the context of LTP allergy.

The relation between allergy to pollens and legumes has already been addressed in some studies. In a Spanish study, it was demonstrated through inhibition ELISA, cross-reactivity between peas and white beans and four pollens: *Lolium perenne, Olea europea, Artemisia vulgaris y Betula alba*[2]. In our case, the patient's allergy to pollens cannot be due to cross-reactivity to beans since there are no seed storage proteins in pollens.

7S globulins or vicillins are the major allergens of legumes (i.e. lentils, chickpea or pea) and cross-reactivity is frequent among them[9]. The only IgE binding band recognized by the patient's serum was a 11S globulin or legumin. 11S globulins have been reported as major allergens in several seeds, nuts and legumes and associated with severe reactions[10]. Although cross-reactivity among different 11S globulins is not well known, has been reported (i.e. between mustard and nuts) in some cases, but not in others. It might be explained depending on the epitopes involved in sensitization. In view of the results, our patient should have been sensitized to the specie-specific epitopes of bean 11S globulin.

In conclusion, we present a case of 13-year-old boy with selective severe allergy reaction to beans in which a 15 kDa protein was reported. The allergen was identified as 11S globulin or legumin with no cross-reactivity with other food allergens (neither seeds, nuts nor legumes). To our knowledge, this is the first time an 11S globulin has been identified in beans. More studies are needed to fully characterize this allergen family.

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Conflicts of interests:

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Nothing to declare.
References


Figure.


b) IgE-immunoblot of bean extract with patient’s and negative control (no atopic) serum. W: white, B: black, R: red, P: pinto. MW: molecular weight, kDa: kilodalton.