

Identification of the Soybean Hull Allergens Responsible for the Barcelona Asthma Outbreaks

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Key Words

Asthma outbreaks · Allergens, main · Soybean hull allergens

Abstract

Background: Soybean hulls were identified as the etiologic agent responsible for the asthma outbreaks that occurred in Barcelona and Cartagena, Spain. **Objective:**

To identify the main soybean hull allergens using the sera of 18 asthmatic epidemic patients from Barcelona and to compare the results to those previously reported.

Methods: Specific IgE to a soybean hull allergen extract was determined by radioimmunoassay and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10–20% Tris-tricine gradient gel followed by Western blot. **Results:** Specific IgE, which was detected in 15 of the 18 (83.3%) sera, indicated two nonoverlapping band patterns on the SDS-PAGE/Western blot: (1) pattern 1, present in 11 sera, consisted of three bands with estimated molecular weights (MWs) of 8, 7.5 and 7 kD, which are the MWs described for the allergens Gly m 2, Gly m 1A and Gly m 1B, respectively, and (2) pattern 2, present in 3 sera, consisted of a band with an estimated MW of 8.2–8.3 kD and four additional bands in a MW range of 25–36 kD. The remaining positive sera indicated very faint bands. **Conclusions:** This study confirms that soybean hulls contain three main al-

lergens with MWs of 8, 7.5 and 7 kD, and indicates additional higher MW allergens, which selectively bind specific IgE of the sera that do not react with the three low MW components. This dichotomous and nonoverlapping pattern of allergen recognition has not been previously described.

Introduction

Asthma outbreaks that took place in Barcelona and Cartagena, Spain, were associated with the unloading of soybeans in the city harbors [1, 2]. An identification and partial characterization study of the allergens involved demonstrated two glycoproteins with a molecular weight (MW) less than 14.4 kD, both of which bound specific IgE present in the plasma of 2 asthmatic epidemic patients (AEP) from Barcelona [3]. Studies performed with AEP sera from Cartagena identified a main soybean hull allergen (SHA), Gly m 1, which contains two isoallergens, Gly m 1A and Gly m 1B, with MWs of 7.5 and 7 kD [4, 5]. A second SHA, Gly m 2, with a MW of 8 kD was further purified and associated with the Barcelona asthma outbreaks [6].

The purposes of this study are to identify the main SHAs using sera of AEP from Barcelona and to compare the results to those previously reported.

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Fig. 1. SDS-PAGE/Western blot of the soybean hull extract, incubated with the 18 AEP sera. The lane numbers correspond to the serum numbers on table 1.

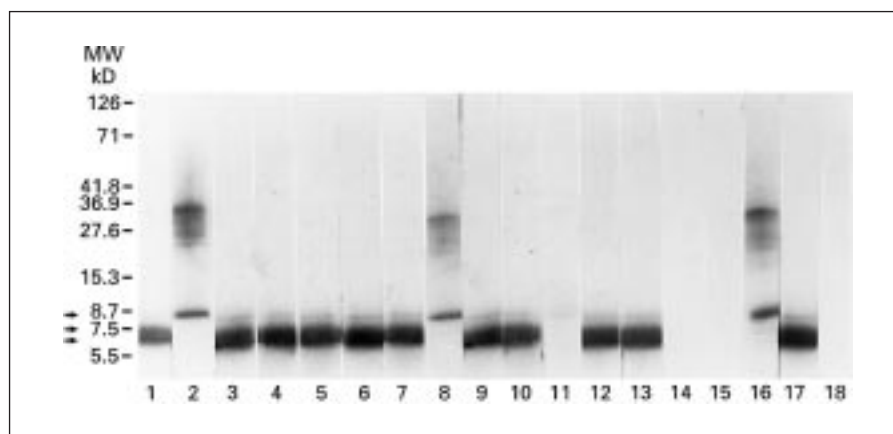


Table 1. Specific IgE (%TCB) to SHA extract

Serum No.	TCB %
1	5.2
2	6.4
3	12.9
4	10.1
5	9.9
6	10.5
7	6.2
8	4.7
9	31.5
10	12.2
11	1.9
12	5.6
13	8.7
14	0.2
15	0.1
16	2.8
17	3.2
18	0.3

Note that 37,400 counts were added to the radioimmunoassay.

Specific IgE Determination

Specific IgE determination to an SHA extract was performed by radioimmunoassay as previously described [6]. The results were expressed as the percentage of total counts bound (% TCB) and were considered positive if the values were higher than the 400% of the value found for the control pool.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis/Western Blot

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/Western blot of the SHA extract was performed as previously described [6], using 10–20% polyacrylamide Tris-tricine gradient gels [7]. The determination of MWs was performed according to the method of Weber and Osborn [8].

Results

The 400% of the specific IgE value found for the control pool was 0.6% TCB. Thus, values higher than 0.6 were considered positive. Fifteen of the 18 (83.3%) sera contained specific IgE to SHAs (table 1). These sera also had a positive Western blot.

SDS-PAGE/Western blot indicated the presence of two nonoverlapping band patterns (fig. 1): (1) 11 of the 15 (73.3%) positive sera (No. 1, 3, 4, 5, 6, 7, 9, 10, 12, 13 and 17) strongly reacted with two bands with MWs of 7.5 and 7 kD, and weakly, specially serum No. 1, with a band with a MW of 8 kD, and (2) 3 of the 15 (20%) positive sera (No. 2, 8 and 16) reacted to two strongly radiostained bands with MWs of 8.2–8.3 and 36 kD and to additional bands with estimated MWs of 25, 27 and 34 kD. The remaining positive serum (No. 11), which presented low levels of specific IgE, indicated two extremely faint bands with MWs of 8.2–8.3 and 36 kD.

Materials and Methods

Human Sera

Sera were obtained from 18 AEP seen in emergency rooms during the 26 asthma outbreaks that occurred in Barcelona, Spain, between 1981 and 1987. All subjects gave voluntary consent. A serum pool was made by mixing an equal volume of 5 individual sera from non-allergic subjects and was used as a control. All samples were stored at –20°C until analysis.

Discussion

In this work, 18 sera of AEP from Barcelona were used to identify the SHAs responsible for soybean-induced asthma. Specific IgE, which was detected in 15 (83.3%) sera, indicates two different and nonoverlapping band patterns on the SDS-PAGE/Western blot using a 10–20 Tris-tricine gel. Pattern 1 consists of three allergens with MWs of 8, 7.5 and 7 kD, which are the MWs reported for the allergens Gly m 2, Gly m 1A and Gly m 1B, respectively [5, 6]. Pattern 2 consists of an allergen with an estimated MW of 8.2–8.3 kD and four additional allergens with a higher MW. Rodrigo et al. [3], using two plasmas of AEP from Barcelona and a 12.5% Tris-glycine nongradient gel, described two allergenic zones, one represented by two allergens with a MW lower than 14.4 kD and another zone with allergens in the MW range of 21.5–42 kD. Analogous results were found by Gonzalez et al. [4] using 7 sera of subjects affected by the outbreaks in Cartagena and a 8–25% Tris-glycine gradient gel. Differences in the methodology employed are probably

responsible for the discrepancies in resolution and in MW assignation to the different IgE binding components. The Tris-tricine system gel employed in this study discriminates a MW range from 2 to 120 kD and it seems to be more appropriate than Tris-glycine gel systems to characterize the SHAs.

The meaning, if any, of the two nonoverlapping band patterns described is unknown. The sera employed in this study were taken at different times and the two patterns could merely represent different types of exposure to SHA. If the patterns of allergen recognition were associated with differential characteristics, such as severity of presentation of the disease, immunoblots using the methodology employed in this study could be a practical tool for the diagnosis of soybean-induced asthma. This observation should be investigated.

In conclusion, this study confirms the existence of three main SHA with MWs of 8, 7.5 and 7 kD and indicates the existence of additional higher MW allergens, which are also involved in soybean-induced asthma.

References

- 1 Sunyer J, Anto JM, Rodrigo MJ, Morell F: Case-control study of serum immunoglobulin-E antibodies reactive with soybean in epidemic asthma. *Lancet* 1989;1:179–182.
- 2 Navarro C, Marquez M, Hernando L, Galvan F, Zapatero L, Caravaca F: Epidemic asthma in Cartagena, Spain, and its association with soybean sensitivity. *Epidemiology* 1993;4:76–78.
- 3 Rodrigo MJ, Morell F, Helm RM, Swanson MC, Greife A, Anto JM, Sunyer J, Reed CE: Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemics. *J Allergy Clin Immunol* 1990;85:778–784.
- 4 Gonzalez R, Zapatero L, Caravaca F, Carreira J: Identification of soybean proteins responsible for respiratory allergies. *Int Arch Allergy Appl Immunol* 1991;95:53–57.
- 5 Gonzalez R, Polo F, Zapatero L, Caravaca F, Carreira J: Purification and characterization of major inhalant allergens from soybean hulls. *Clin Exp Allergy* 1992;22:748–755.
- 6 Codina R, Lockey RF, Fernandez-Caldas E, Rama R: Characterization of a soybean hull allergen responsible for the Barcelona asthma outbreaks. II. Purification and sequencing of the Gly m2 allergen. *Clin Exp Allergy* 1997; 27:424–430.
- 7 Schagger H, von Jagow G: Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;166: 368–397.
- 8 Weber K, Osborn K: The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem* 1969;244:4406–4412.

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