

Soybean aeroallergen around the port of New Orleans: a potential cause of asthma¹

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Abstract

There have been reported epidemics of severe asthma in Barcelona, Spain, linked to a 10 kDa low molecular mass (LMM) allergen from soybean hulls that became airborne during unloading of ships. As a preliminary probe of the potential for dispersion of this allergen in USA cities, four automated air samplers were placed around a grain elevator in New Orleans and operated continuously from May to October 1990. The allergen was extracted from the filters and immunochemically assayed for soybean aeroallergen. On 31 separate days, the airborne allergen concentration in at least one of the samples was over 10 000 U/m³, similar to those observed in Barcelona on some epidemic days. Areas North and East of the elevator were most affected. Serologic studies showed that of 50 asthmatics from New Orleans who were participants in an unrelated clinical study 4 or 8% demonstrated elevated titers of IgE antibody to LMM soybean allergen. Only 1 of 475 control sera (half of which were also asthmatic) obtained elsewhere in the US was positive for LMM soybean IgE antibody. Based on the findings in this study, there is a great possibility that on some days there is enough soybean allergen in the air and a sufficient frequency of soybean aeroallergen RAST positive asthmatics in New Orleans to warrant further investigation of the contribution of soybean aeroallergen to asthma around the port of New Orleans.

Keywords: Aeroallergen; Soybean asthma; Immunochemical Assay; Air sampling

1. Introduction

Soybean dust produced during the unloading of grain freighters in the harbor of Barcelona, Spain, has caused epidemics of asthma among residents of the city (Anto et al., 1989; Sunyer et al., 1989). Twenty-six outbreaks of asthma have been identified in Barcelona since 1981.

Epidemic days of acute asthma resulted in 1155 emergency room admissions and asthma exacerbations on epidemic days were unusually severe resulting in 20 fatalities and multiple intensive care unit admissions. Detailed investigation of these epidemics revealed that the outbreaks of asthma in Barcelona were caused by soybean dust generated by unloading soybeans in the Barcelona harbor where wind conditions carried the soybean dust over the city. Further immunochemical investigation identified the source of the aeroallergen of soybean dust to be a low molecular mass (LMM) glycoprotein concentrated in the soybean hull (Swanson et al., 1991; Rodrigo et al., 1990).

Abbreviations: kDa, kilo-Dalton; LMM, Low molecular mass; RAST, Radioallergosorbent test.

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The United States is a major producer and exporter of soybeans. In fact, 9 of the 13 asthma epidemic days identified in Barcelona correlated with unloading soybeans from ships which originated in the United States (Anto et al., 1989). It was postulated that asthma from soybean dusts may occur in the United States. Because New Orleans is a major port for exporting soybeans, we measured the concentration of the LMM soybean aeroallergen in the air near a grain elevator in New Orleans and screened 50 individual sera from asthmatics in New Orleans for IgE antibody to this soybean allergen.

2. Materials and methods

2.1. Air sampling

Four automated air samplers equipped with two week polytetrafluoroethylene filter cassettes (Quantec-Air, Inc., Rochester, MN) were placed North, South, East and West of a grain elevator in New Orleans (Fig. 1). The automated cassette changed the filter every day automatically for two-week periods. With a flow rate of one liter per second, the daily 23 h collections totaled 83 m³ per sample.

2.2. Preparation of LMM soybean allergen reference standard

The reference soybean allergen was extracted by mixing the hulls with glass beads and loading them into a 40 × 2.6 cc glass column with 200 cc of 200 mM NH₄HCO₃. The LMM fraction which contains almost all the allergen (Swanson et al., 1991), was prepared by placing 100 ml of soybean hull extract in a dialysis casing that permits diffusion of molecules smaller than 10 000 molecular weight which was then placed inside a second casing that permits diffusion of molecules smaller than 3500 molecular weight containing 300 ml distilled water. After exhaustive dialysis, the fluid between the bags was concentrated to the original volume of the whole extract. This preparation containing the LMM fraction of soybean allergen was assigned an arbitrary concentration of one million U/ml. Measurements of airborne LMM soybean allergen was expressed in units per cubic meter (U/m³). It was divided into aliquots and stored in saturated sucrose at –20°C.

2.3. LMM soybean allergen measurement

The polytetrafluoroethylene air filter from each day was extracted in phosphate buffered saline (0.1 M, pH 7.4) containing 0.2% bovine serum albumin and stored in 50% glycerine at –20°C until assayed. The

LMM Soybean allergen was adsorbed to microtiter wells of Immulon II microtiter Removawell™ strips (Dynatech, Alexandria, VA). Each well contained 100 µl of 200 mM carbonate buffer (pH 9.2) with LMM allergen (1:1000 v/v) and incubated at room temperature overnight. The plates were washed three times with 0.1 M phosphate buffer containing 0.1% tween 20.

The inhibition assay was conducted by adding 50 µl of either the air sample extract, buffer control or serial dilutions of the reference standard. Then, 50 µl of 13 pooled sera containing a high titer antibody specific for LMM soybean allergen was added to each well. The high titer IgE serum pool was obtained from 13 individuals who experienced epidemic asthma in Barcelona. After a second overnight incubation at room temperature, the wells were washed and 100 µl (20 ng) of affinity purified ¹²⁵I labeled rabbit anti-IgE was added and again incubated overnight. After a final washing, the wells were counted in a RIAstar gamma counter and the allergen concentration was calculated by log-logit regression analysis.

2.4. Patient serology

Previously collected and stored serum samples from 50 asthmatics from New Orleans and 475 control patients were assayed for IgE antibody to LMM soybean allergen. The 50 asthmatics from New Orleans were participants in an unrelated clinical study and all had documented dust mite allergic asthma. The control sera included 45 asthmatics from Tampa, Florida, 57 subjects with ragweed allergic rhinitis or allergic asthma from Minnesota, Iowa or Wisconsin, 135 fiberglass factory workers from Ohio, 33 workers in enzyme manufacturing and 212 individuals who lived in tightly constructed homes and had a high frequency of asthma and other respiratory disease. Approximately half of these 475 individuals had asthma.

2.5. LMM soybean RAST

The radioimmunoassay for LMM soybean IgE antibody was performed by adding 100 µl of test serum to each well. Control wells contained a normal non-allergic serum and a reference positive serum. After a second overnight incubation, the wells were washed and 100 µl of an affinity purified ¹²⁵I labeled rabbit anti-IgE was added. The wells were washed then counted in a RIAstar gamma counter and the antibody concentration expressed as a percent of the normal control serum binding. Duplicate serum samples which demonstrated binding greater than 400% above the normal control serum was interpreted as positive for soybean IgE antibody.

3. Results and discussion

The four air samplers which were operated around a grain port in New Orleans continuously from May to October, 1990, were placed approximately 1–2 km around the grain facility in cardinal positions. On 31 separate days, the airborne concentration of at least one of the samples was over 10 000 U/m³, similar to those observed in Barcelona on some epidemic days. LMM soybean allergen in the atmosphere was measured predominantly in areas North and East of the port (Fig. 2).

Air sampling conducted in an identical manner at the Rochester, Minnesota airport demonstrated airborne concentrations of LMM soybean aeroallergen up to 1500 U/m³ during soybean harvest time. Air samples collected in the spring demonstrated no detectable soybean allergen. A personal breathing zone air sample taken from a farmer during harvest operations demonstrated exposure to the LMM soybean allergen of 3500 U/m³ when he was combining soybeans. He was in an enclosed cab during the combining.

Serologic studies showed that 4 of 50 (8%) dust mite sensitive asthmatics in New Orleans contained elevated titers of IgE antibody to LMM soybean allergen. Only 1 of 475 (0.2%) control asthmatic and non-asthmatic sera was positive for LMM soybean IgE antibody.

LMM soybean allergen was used for all assays since this glycopeptide contains most of the soybean allergenic activity. This major allergen is present predominantly in the telae and pods of the soybean plant. It was measured in all parts of the soybean plant throughout its maturation (Swanson et al., 1991).

The results of the air sampling studies showed that significant concentrations of LMM soybean allergen

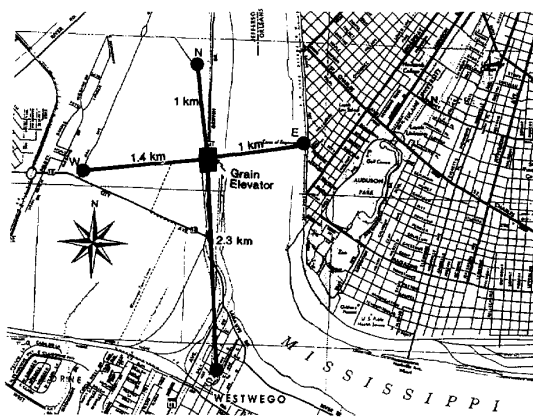


Fig. 1. A map depiction of air sampler placements around a grain elevator along the Mississippi River in New Orleans. Sampler sites to the North, South and West of the elevator were on the West side of the river and the site East of the elevator was on the East bank of the river.

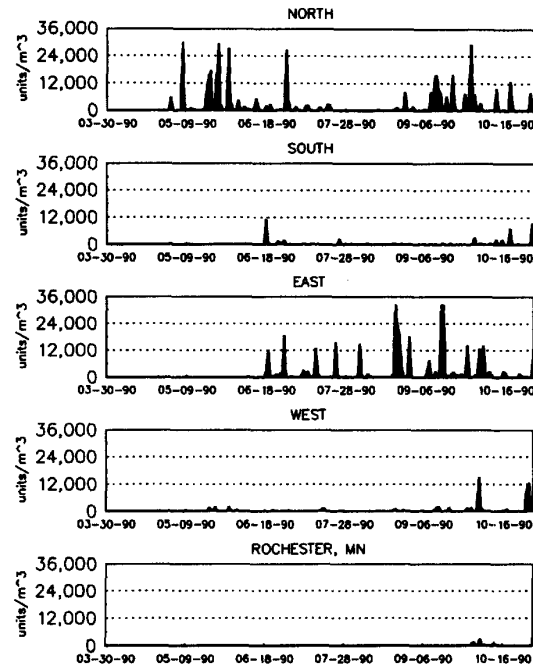


Fig. 2. Time plots of airborne concentrations of low molecular mass soybean allergen from May through October, 1990 in New Orleans. The bottom plot is from air samples taken in an identical manner from the Rochester, MN airport during the same time period.

became airborne around the port of New Orleans from May to October. Measurable levels were also found in Rochester, Minnesota during the fall harvest. Since the serum pool, the LMM soybean allergen standard and the assay technique were all the same as those used in the studies of the Barcelona epidemic, the New Orleans soybean concentrations of 10 000 U/m³ are comparable to the mean soybean aeroallergen concentration of 7100 U/m³ measured in Barcelona on epidemic days. The Barcelona studies suggest that these allergen concentrations correlate to soybean dust concentrations of 10–25 µg/m³ (Anto et al., 1989; Aceves et al., 1991).

Study of dust mite sensitive asthmatics in New Orleans revealed that 8% (4 of 50) of subjects had elevated soybean IgE antibody compared with only 0.2% (1 of 475) controls. Investigation of the asthma outbreaks in Barcelona showed that 64 of 86 (74.4%) patients with epidemic asthma demonstrated elevated titers of IgE antibody to commercial soybean extract compared with 4 of 86 (4.6%) of nonepidemic asthmatics (Sunyer et al., 1989). These differences in prevalence of sensitization to soybean may have been even more striking if the RAST using LMN soybean allergen had been used in Spain. Further serologic study of asthma epidemics in Barcelona revealed that 10 of 10 patients with epidemic asthma, 1 of 10 patients with nonepidemic asthma, 1 of 10 patients with asthma who did not live in Barcelona

and 0 of 10 patients without asthma demonstrated IgE antibody to a soybean allergen extract.

The Barcelona asthma epidemics affected 688 individuals or approximately 1 in 2000 residents. Therefore, the observation that 8% of mite sensitive asthmatics in New Orleans generate IgE antibody to LMM soybean allergen suggests that soybean aeroallergen may be a potential cause of asthma in New Orleans. Additional and detailed epidemiologic studies are necessary to further investigate the true prevalence of LMM soybean allergy in the general or asthmatic population in New Orleans. In fact, atopy or skin reactivity was one risk factor for epidemic asthma in Barcelona (Sunyer et al., 1992).

Other investigators have considered the possibility that soybean aeroallergens may be an unrecognized cause of asthma exacerbation following the description of asthma outbreaks caused by soybean dust in Barcelona. Hernando reported similar asthma epidemics likely caused by airborne soybean dust in Cartagena, Spain (Hernando et al., 1989) as did Alvarez-Dardet and colleagues in Valencia, Spain (Alvarez-Dardet et al., 1989). Epidemic outbreaks of asthma have been observed in New Orleans (Weill et al., 1964; Salvaggio et al., 1970). However, grain dust or soybean dust was never proven to be a cause of these outbreaks and a subsequent epidemiologic survey showed no clear asthma epidemic days in New Orleans (Goldstein and Cuzick, 1983).

Ingestion of food containing soybean is a recognized cause of anaphylaxis (Mortimer, 1961). Asthma caused by soybean dust has previously been recognized as a cause of asthma in farmers, mill workers, and soybean handlers (Pepys, 1986). The identification of asthma epidemics in Barcelona and suspicion of asthma in other cities caused by soybean dust raises the possibility that asthma exacerbations triggered by inhalation of soybean aeroallergens may affect people in sectors of many communities. Recently in Barcelona it was shown that the installation of filters on soybean storage silos prevented airborne dispersion of allergenic soybean dust and eliminated the outbreaks of asthma caused by inhalation of the dust (Anto et al., 1993).

We conclude that LMM soybean aeroallergen is in the air around a grain elevator in New Orleans. Concentrations of this aeroallergen, especially in areas North and East of the port were very high. Of interest, was the observed frequency of elevated IgE titers spe-

cific for LMM soybean allergen (8%) in a group of 50 New Orleans asthmatics. Additional detailed epidemiologic study and air sampling are needed to determine whether or not LMM soybean allergen is an important aeroallergen in asthma and whether changes in dust control techniques are indicated at soybean processing facilities in New Orleans.

References

- Aceves, M., Grimalt, J.O., Sunyer, J., Anto, J.M. and Reed, C.E. (1991) Identification of soybean dust as an epidemic asthma agent in urban areas by molecular marker and RAST analysis of aerosols. *J. Allergy Clin. Immunol.* 88, 124–134.
- Alvarez-Dardet, C., Belda, J., Pena, M. and Nolasco, A. (1989) Outbreak of asthma associated with soybean dust. *N. Engl. J. Med.* 321, 1127–1128.
- Anto, J.M., Sunyer, J., Rodriguez-Roisin, R., Suarez-Cervera, M. and Vazquez, L. (1989) Toxicoepidemiological Committee. Community outbreaks of asthma associated with inhalation of soybean dust. *N. Engl. J. Med.* 320, 1097–1102.
- Anto, J.M., Sunyer, J., Reed, C.E. et al. (1993) Preventing asthma epidemics due to soybeans by dust-control measures. *N. Engl. J. Med.* 1760–1763.
- Goldstein, I.F. and Cuzick, J. (1983) Daily patterns of asthma in New York City and New Orleans: An epidemiologic investigation. *Environ. Res.* 30, 211–223.
- Hernando, L., Navarro, C., Marquez, M., Zapatero, L. and Galvan, F. (1989) Asthma epidemics and soybean in Cartagena (Spain). *Lancet* 1, 502.
- Mortimer, E. (1961) Anaphylaxis following ingestion of soybean. *J. Pediatr.* 51, 139–151.
- Pepys, J. (1986) Occupational allergic lung diseases caused by organic agents. *J. Allergy Clin. Immunol.* 5, 1058–1062.
- Rodrigo, M.J., Morell, F., Helm, R.M. et al. (1990) Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemic. *J. Allergy Clin. Immunol.* 85, 778–784.
- Salvaggio, J., Hasselblad, V., Seabury, J. and Heiderscheit, L.T. (1970) New Orleans asthma. II. Relationship of climatologic and seasonal factors to outbreaks. *J. Allergy* 45, 257–265.
- Sunyer, J., Rodrigo, M.J., Anto, J.M. and Morell, F. (1989) Clinical and Toxicoepidemiological Committee. Case-control study of serum immunoglobulin-E antibodies reactive with soybean in epidemic asthma. *Lancet* 1, 179–182.
- Sunyer, J., Anto, J.M., Sabria, J. et al. (1992) Risk factors of soybean epidemic asthma. The role of smoking and atopy. *Am. Rev. Respir. Dis.* 145, 1098–1102.
- Swanson, M.C., Li, J.C., Wentz-Murtha, P., et al. (1991) Source of the aeroallergen of soybean dust: A low molecular mass glycopeptide from the soybean tela. *J. Allergy Clin. Immunol.* 87, 783–788.
- Weill, H., Ziskind, M.M., Dickerson, R.C. and Derbes, V.J. (1964) Epidemic asthma in New Orleans. *J. Am. Med. Assoc.* 190, 75–78.