MEDICAL INTELLIGENCE

KUNITZ SOYBEAN TRYPSIN INHIBITOR

A Specific Allergen in Food Anaphylaxis

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ALTHOUGH foods are common causes of anaphylaxis,[1] the responsible allergens, with the exception of the one from codfish muscle,[2,3] remain uncharacterized. Soybean products are recognized causes[1] and are commonly encountered, often in an unrecognizable form,[4] in processed and restaurant foods. In some cases, IgE antibody has been documented by radioallergosorbent methods and skin testing with crude soybean extracts, rather than purified allergens, as test materials.[5,6]

Proteinase inhibitors from plants are widely used in biochemical research and are highly characterized molecules.[7] The best known is the Kunitz soybean trypsin inhibitor (SBTI).[8-10]

This report documents a specific IgE-antibody response to this polypeptide in a patient with anaphylactic reactions after ingestion of soybean products.

CASE REPORT

A woman had angioedema and urticaria after eating certain legumes, including peas, lentils, peanuts, and kidney, lima, and navy beans. The most violent reactions occurred after eating foods containing soybean products. A native of Chile, she had eaten soybeans uneventfully as a dietary staple at least once a week until she emigrated to Canada in 1974. Her first allergic reaction occurred in 1972, when she had laryngeal edema and systemic symptoms after eating an unidentified kind of bean. After an identical episode one week later, she avoided all beans until 1975 when, as a postpartum patient in a hospital, she ate a hamburger that had been cooked in soya oil. Ten minutes later she had an anaphylactic reaction (facial edema, dyspnea, dysphagia, and malaise) that required emergency treatment with oxygen and intravenous medications. In 1976, within minutes of tasting her infant's soya formula, she had a similar reaction that culminated in syncope. She has been unable to eat restaurant or processed foods with equanimity since then. In 1978, at the age of 31 years, she began to work in a laboratory in which SBTI is frequently used. This study was prompted by the potential hazard of such work to someone with known soybean-induced anaphylaxis.

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METHODS

Skin tests were performed with commercial extracts (Hollister-Stier, Toronto; 2000 protein nitrogen units per milliliter), commercial SBTI (Type 1-S, Sigma Chemical Company, St. Louis, Mo.) prepared by the method of Rackis and Anderson,[9] and purified SBTI.

Commercial SBTI was purified by gel filtration on Bio-Gel P 60 (Bio-Rad Laboratories, Richmond, Calif.) in phosphate-buffered saline at a pH of 7.4. Fractions were monitored for protein content at 280 nm, for trypsin inhibitory activity by hydrolysis of tosyl-L-arginine methyl ester,[11] and for ability to inhibit IgE binding by radioallergosorbent methods.

The homogeneity of SBTI was verified by cellulose acetate electrophoresis and, after dithiothreitol reduction, by electrophoresis in sodium dodecyl sulfate-polyacrylamide disc gels (10 per cent gels).[12] Slices of a duplicate cellulose acetate electropherogram were eluted with buffer, and the eluates tested for skin reactivity and inhibition of IgE binding. Purified SBTI was measured spectrophotometrically.[10]

A specific radioallergosorbent assay was performed conventionally.[3,13] For the coupling phase, final allergen concentrations were 0.5 μg of SBTI per milliliter or 15 μg of protein per milliliter for whole-soybean extract. Results were expressed as nanograms of [^{125}I]anti-IgE bound (means of duplicate assays) after subtraction of blank values (0.17 to 0.19 ng bound) obtained with buffer alone. Each assay contained 4 ng (0.025 μCi) of ^{125}I-labeled rabbit anti-IgE (Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden). Inhibition studies were performed with the skin-test extracts and with lima-bean trypsin inhibitor (Worthington Biochemical Corporation, Freehold, N.J.), ovomucoid trypsin inhibitor (Type II-0, Sigma Chemical Company, St. Louis, Mo.), peanut extract (1:10 wt/vol extract of an acetone powder), bovine-lung trypsin inhibitor (Trasylol, Boehringer, Montreal), and ragweed allergen Ra5, provided by Dr. L. G. Goodfriend.

Serum samples from other patients with soybean allergy were provided by Dr. Charles E. Reed of Rochester, Minn. Other serum samples were from unselected hospital patients, patients with

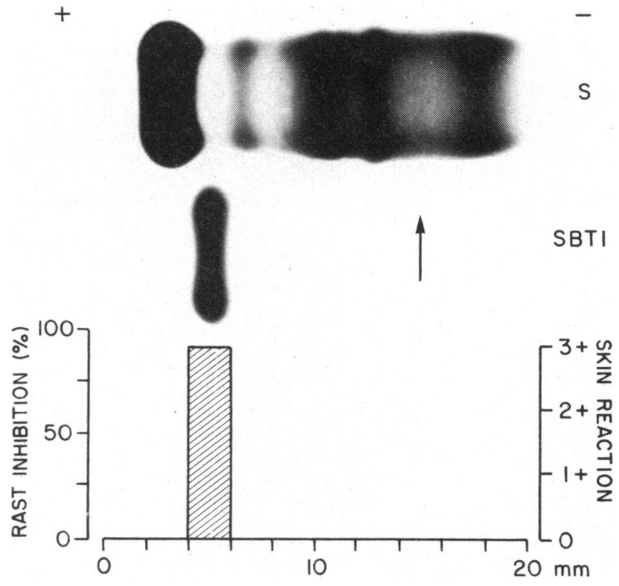

Figure 1. Cellulose Acetate Electropherogram of Purified SBTI.

Correspondence between homogeneous protein (SBTI), skin reactivity, and inhibition of SBTI-specific IgE binding determined by radioallergosorbent testing (RAST) is shown. Human serum (S) is included as an electrophoretic-mobility reference. Arrow indicates point of application.

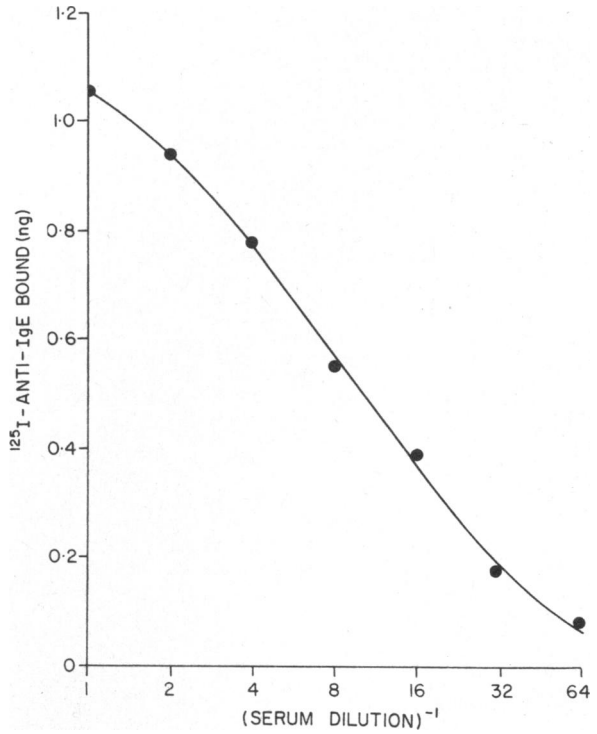

Figure 2. Results of SBTI-Specific Radioallergosorbent Assay.

Concentration-dependent binding of IgE from patient's serum to SBTI-coupled paper disks is reflected in subsequent binding of ^{125}I-labeled anti-IgE.

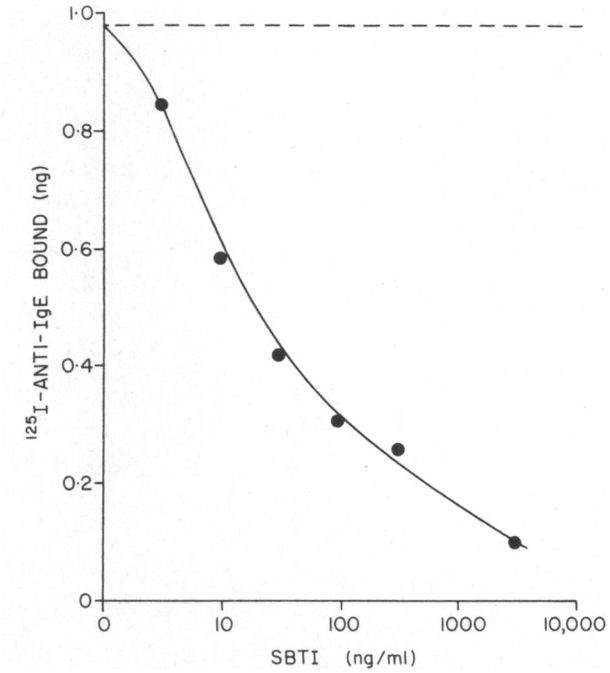

Figure 3. Inhibition of IgE Binding by SBTI.

Serum (final dilution, 0.5) was incubated with purified SBTI in the final concentrations shown for the IgE-binding phase of the radioallergosorbent assay. Broken horizontal line indicates control value (serum alone).

chronic urticaria and angioedema, and patients with elevated total IgE levels (1000 to 4000 IU per milliliter).

Passive transfer was performed on a normal skin site 48 hours after injection of patient's serum.

Results

In addition to immediate skin reactions to lima beans, kidney beans, navy beans, peanuts, peas, and other extracts, the patient had a 4+ reaction to soybean and to commercial SBTI (10 μg per milliliter). By gel filtration on Bio-Gel P 60, more than 97 per cent of this material eluted in a single peak that contained the trypsin inhibitory and skin-test activity (4+ reaction with peak material at 1.5 μg of protein per milliliter). Skin-test reactivity was restricted to this protein, which was homogeneous by cellulose acetate electrophoresis (Fig. 1) and gave a single band with an apparent molecular weight of 20,000 to 21,000 daltons by electrophoresis in polyacrylamide disc gels. It elicited a 4+ reaction in normal skin previously sensitized with patient's serum.

With SBTI as the ligand, binding of IgE from this serum was detectable by radioallergosorbent testing at a dilution of 1:64 (Fig. 2). The maximum amount bound with undiluted serum was 1.1 to 1.3 ng of [^{125}I]anti-IgE. Addition of SBTI to serum during the IgE-binding phase produced inhibition at concentrations as low as 3 ng per milliliter (Fig. 3). Inhibition of IgE binding on radioallergosorbent testing coincided with SBTI and skin-test reactivity studied in gel-filtration fractions and with electrophoresis (Fig. 1). Binding was unaffected by any of the other agents tested, indicating its specificity for SBTI. With whole-soybean extract as the ligand, IgE binding by the patient's serum (0.73 ng of anti-IgE bound) was completely inhibited by SBTI, indicating that SBTI was the sole allergen in soybean for this patient.

Serum samples from two other patients with angioedema and urticaria after eating soybeans were negative by SBTI-specific radioallergosorbent methods. One of these patients had a positive reaction in the whole-soybean test (0.88 ng of anti-IgE bound), which could be inhibited by the whole extract but not by SBTI. The other patient's reaction was negative by both assays, as were serum samples from two patients with a different syndrome — asthma after inhalation of soybean dust.

Specific binding was undetectable in serum from 10 nonallergic patients and 11 patients with high total IgE levels. Very low binding (mean, 0.045 ng of anti-IgE bound) was observed in 65 serum samples from patients with chronic urticaria and angioedema. The specificity of this low binding was not examined.

Discussion

The positive skin reactions, passive transfer with serum, and IgE antibodies demonstrable by radioimmunosorbent testing indicate that SBTI is a specific allergen in human beings and the sole soybean al-

lergen for this patient, in whom its identification has pinpointed an important occupational hazard. Other soybean allergens exist, as illustrated by the positive reaction to whole soybean but negative SBTI-specific reaction in a patient with a similar history. The negative reactions in both assays with serum samples from other subjects who were allergic to soybeans may reflect lack of representation of the pertinent allergen or allergens on the whole-soybean solid-phase matrix, an allergen resulting from gastrointestinal enzyme action on ingested soybean proteins, or a non-IgE mechanism, as suggested in the patients with asthma after inhalation of soybean dust.

Proteinase inhibitors such as SBTI may be of more than passing interest as food allergens. In general, they are of low molecular weight and relatively resistant to acid pH and proteolytic degradation[8,14] — properties that may be conducive to their rapid absorption and interaction with the immune system in relatively intact form.

Isolation and characterization of allergens and determination of their primary structure is a necessary but laborious prelude to study of the immunologic behavior of the native molecules and their fragments.[15] For many proteinase inhibitors, such as SBTI,[16] this structural information is on record.[7] Although further studies are required to determine the overall incidence of IgE reactivity to SBTI, even rare examples of IgE antibody to molecules of this type may help to clarify the molecular basis of allergenicity.

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REDUCTION IN 1,25-DIHYDROXYVITAMIN D IN CHILDREN WITH INCREASED LEAD ABSORPTION

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STUDIES in laboratory animals have demonstrated that a diet low in calcium increases lead retention and that there are associated biochemical and morphologic manifestations of enhanced lead toxicity.[1,2] This increased toxicity has been attributed to an increase in the gastrointestinal absorption of lead, although the precise roles of dietary calcium and vitamin D in modifying lead absorption have not been defined. Recent experimental observations suggest that calcium and lead compete for similar binding sites on mucosal proteins in the intestine.[3] Nonetheless, lead is bound mainly to a high-molecular-weight mucosal protein, whereas calcium is bound primarily to a lower-molecular-weight protein (calcium-binding protein).[3] Vitamin D and 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), the hormonal form of the parent vitamin, enhance lead acetate absorption in the distal small intestine of the rat, whereas vitamin D-dependent calcium absorption occurs in the proximal duodenum.[4,5] It is likely that vitamin D affects lead absorption in a manner somewhat different from the manner in which it affects calcium absorption.

Children with high blood levels of lead (>60 μg per deciliter) have reduced dietary intakes of calcium and vitamin D.[6-10] Impairment of appetite and thereby of nutrition are subtle clinical manifestations of lead toxicity[6]; low concentrations of 25-hydroxyvitamin D (25-OHD) in serum reflect the decreased intake of vitamin D, although the cause of the relatively low mean value of serum calcium has remained largely unexplained.[6-10] If accumulation of lead ions in renal cortical cells impairs $1,25\text{-}(OH)_2D$ biosynthesis, the

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