

Soybean trypsin inhibitor is an occupational inhalant allergen

To the Editor

Soybean is considered a common cause of food allergy. Kunitz soybean trypsin inhibitor (STI) has been identified as a potent allergen capable of inducing food anaphylaxis.¹ In addition, allergens from soybean flour and hull have been incriminated in occupational asthma²⁻⁵ and epidemic asthma outbreaks.⁶ Soybean flour is frequently added to dough to improve baking products and to bleach dough carotinoids.³⁻⁵ Baur et al^{3,4} reported that 20% to 32% of bakers with asthma or rhinitis had elevated soybean flour-specific IgE antibodies. In a recent study of 14 bakers with work-related respiratory symptoms who were sensitized to soybean flour, the results of serologic tests suggested that STI (21 kDa, pI 4.5) and lipoxidase were major allergens of soybean.⁴ Although specific IgE antibodies to STI were detected in that study,⁴ bronchial reactivity to STI has not been previously demonstrated.

We report on 2 bakers (one 35 and the other 37 years old) with work-related asthma symptoms who were occupationally exposed to soybean flour. The diagnosis of occupational asthma in these patients was confirmed by immunologic tests and specific inhalation challenges to wheat and soybean flour. We further investigated the role of STI as an inhalant allergen.

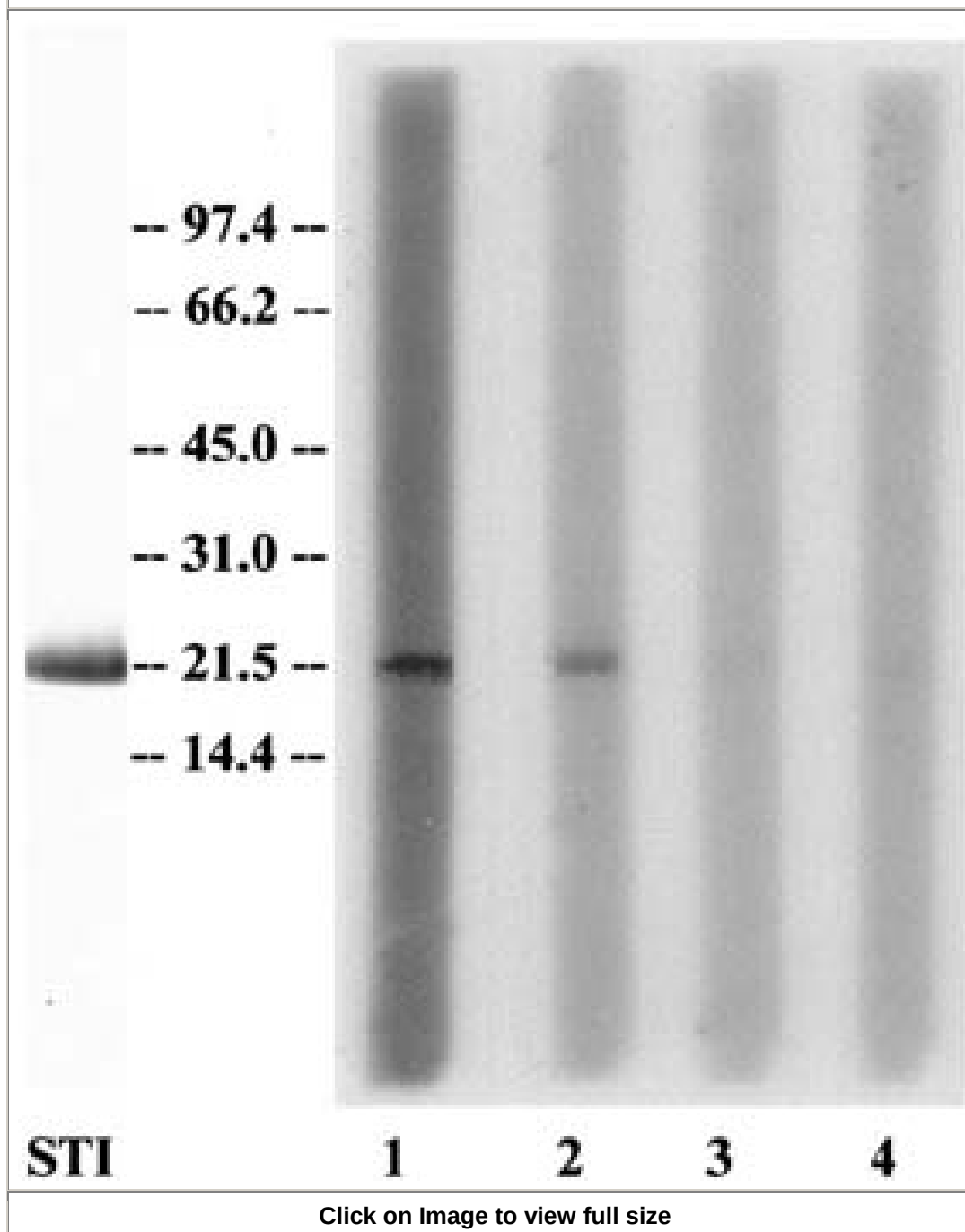
STI (T-9003) from Sigma Chemical (St Louis, Mo) was prepared at a concentration of 10 mg/mL in PBS and passed through a membrane with a 0.22- μ m pore size for sterilization. Both patients showed a positive skin test result to soybean flour and wheat flour 10% w/v (ALK-Abelló, Madrid, Spain) and to STI 10 mg/mL. The titers of specific IgE antibodies to soybean through use of the CAP technique (Pharmacia, Uppsala, Sweden) were <0.35 and 5.73 kU/L, respectively (results $\geq$ 0.35 kU/L were regarded as positive).

Specific inhalation tests with an aqueous extract of purified STI were performed through use of the tidal breathing method⁵ to assess the clinical relevance of sensitization to this allergen. The provocative concentration of purified STI causing a 20% fall in FEV₁ (PC₂₀ allergen) was calculated. Methacholine inhalation tests revealed bronchial hyperresponsiveness (PC₂₀, 0.31 and 0.25 mg/mL, respectively). Specific inhalation challenge to STI elicited isolated early asthmatic responses in both patients (PC₂₀ allergen, 6.87 and 0.025 mg/mL, respectively).

Polyacrylamide gel electrophoresis in the presence of dodecyl sulfate was performed with a 10% to 20% acrylamide gradient in a high molarity Tris buffer system under nonreducing

conditions⁷; 4 µg of STI was applied per lane. After running, the separated proteins were electrophoretically transferred to nitrocellulose membranes (Amersham, Buckinghamshire, United Kingdom). Unreacted membrane sites were blocked with PBS containing 1% BSA and 0.05% polysorbate 20 (Tween 20) for 30 minutes. After 4 washes with PBS-Tween 20 (0.05%), blots were incubated with human serum diluted 1:5 in PBS containing 1% BSA and 0.05% Tween 20. After washing, antihuman-IgE mouse mAb labeled with ¹²⁵I was added and incubated overnight at room temperature. IgE binding proteins were visualized by autoradiography on x-rays films (AX, Konica, Mahwah, NJ). Immunoblots showed IgE binding to purified STI with serum from the 2 bakers sensitized to STI but not with serum from 2 bakers with asthma who were nonsensitized to this allergen (Fig 1).

Fig. 1. *Left:* Coomassie blue–stained SDS-PAGE analysis of purified STI. *Right:* IgE binding to STI with sera from patients 1 (lane 1) and 2 (lane 2) and from 2 nonsensitized subjects with baker's asthma (lanes 3 and 4). *Center:* Molecular weight markers (kDa).



The positive cutaneous and IgE responses and the specific bronchial reactivity to purified STI in these 2 patients point out that this allergen might give rise to allergic respiratory symptoms in sensitized bakers. Defining a new allergen in baker's asthma has implications from a pathogenic and clinical standpoint. If an individual is shown to have a specific response to a particular allergen, avoidance of this component rather than total elimination of all exposures might be possible.

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