

## Identification of specific IgE to mesquite wood smoke in individuals with mesquite pollen allergy

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*To the Editor:*

Pollen from mesquite (genus *Prosopis*) is a potent allergen responsible for causing immediate hypersensitivity reactions in susceptible people in the western and southwestern United States.<sup>1</sup> A study by Novey et al<sup>1</sup> showed that according to skin prick testing (SPT) results, mesquite sensitization was the most prevalent pollen sensitization in a group of 100 atopic patients in California. The clinical relevance of these sensitivities was confirmed with nasal, conjunctival, and bronchial challenges.<sup>1</sup>

Over the past several years, the burning of mesquite wood for broiling and barbecuing has become popular because of the distinctive flavor that the smoke imparts to various foods.<sup>2</sup> Respiratory effects reported with exposure to mesquite smoke in mesquite broiler cooks have been attributed to hydrocarbons or other irritants.<sup>2</sup> However, it is known that allergens can survive the process of combustion and remain immunologically active in smoke.<sup>3</sup>

Three patients presented to our clinic with complaints of allergy symptoms after exposure to smoke from mesquite wood. We sought to identify immunologically active allergens in mesquite smoke that could explain the symptoms in these patients.

Of the 3 patients presenting to our clinic, 2 had complained of oral allergy symptoms after eating foods cooked over mesquite wood; the other had complained of rhinitis after being exposed to mesquite smoke used for recreational barbecuing. All patients had positive SPT results with mesquite pollen extract (1:20 w/v; Hollister-Stier, Spokane, Wash).

Serum was obtained from each patient and stored at 0°C. In addition, serum was obtained from 8 mesquite pollen SPT–positive patients without known symptoms to mesquite smoke and stored at 0°C.

Commercial mesquite pollen extract was dialyzed against distilled water overnight at 4°C in 3500-d dialysis tubing. The resulting extract was lyophilized and stored at 4°C.

Commercially available mesquite wood chips used for barbecuing were obtained and pulverized into small pieces. Protein extraction was performed in 0.125 mol/L NH<sub>4</sub> HCO<sub>3</sub> at 4°C overnight. The wood extract was centrifuged and the supernatant passed through 0.45-μm pore–sized filters. The filtrate was dialyzed against distilled water overnight at 4°C in 3500-d dialysis tubing. The resulting extract was lyophilized and stored at 4°C.

Additional mesquite woodchips were burned in an exhaust hood. Smoke was vacuum-collected in a distilled water trap over approximately 8 hours. The resultant extract was prepared in a manner similar to that used with the wood extract, as described above, including filtration, dialysis, and lyophilization, and stored at 0° C.

Extract protein concentrations were determined by a modification of the technique of Lowry et al.<sup>4</sup> Pollen, wood, and smoke extracts contained 443, 325, and 925 µg of protein per milligram of extract, respectively.

SDS-PAGE of the pollen, wood, and smoke extracts was accomplished as described previously.<sup>5</sup> In short, the mesquite pollen, wood, and smoke extracts were electrophoresed on BMA- PAGER (BioWhittaker, Rockland, Me) 10% to 20 % gradient gels along with low-molecular-weight controls (Pharmacia, Piscataway, NJ), which provided 6 calibration points (phosphorylase B, 94 kd; albumin, 67 kd; ovalbumin, 43 kd; carbonic anhydrase, 30 kd; trypsin inhibitor, 20.1 kd; α-lactalbumin, 14.4 kd).

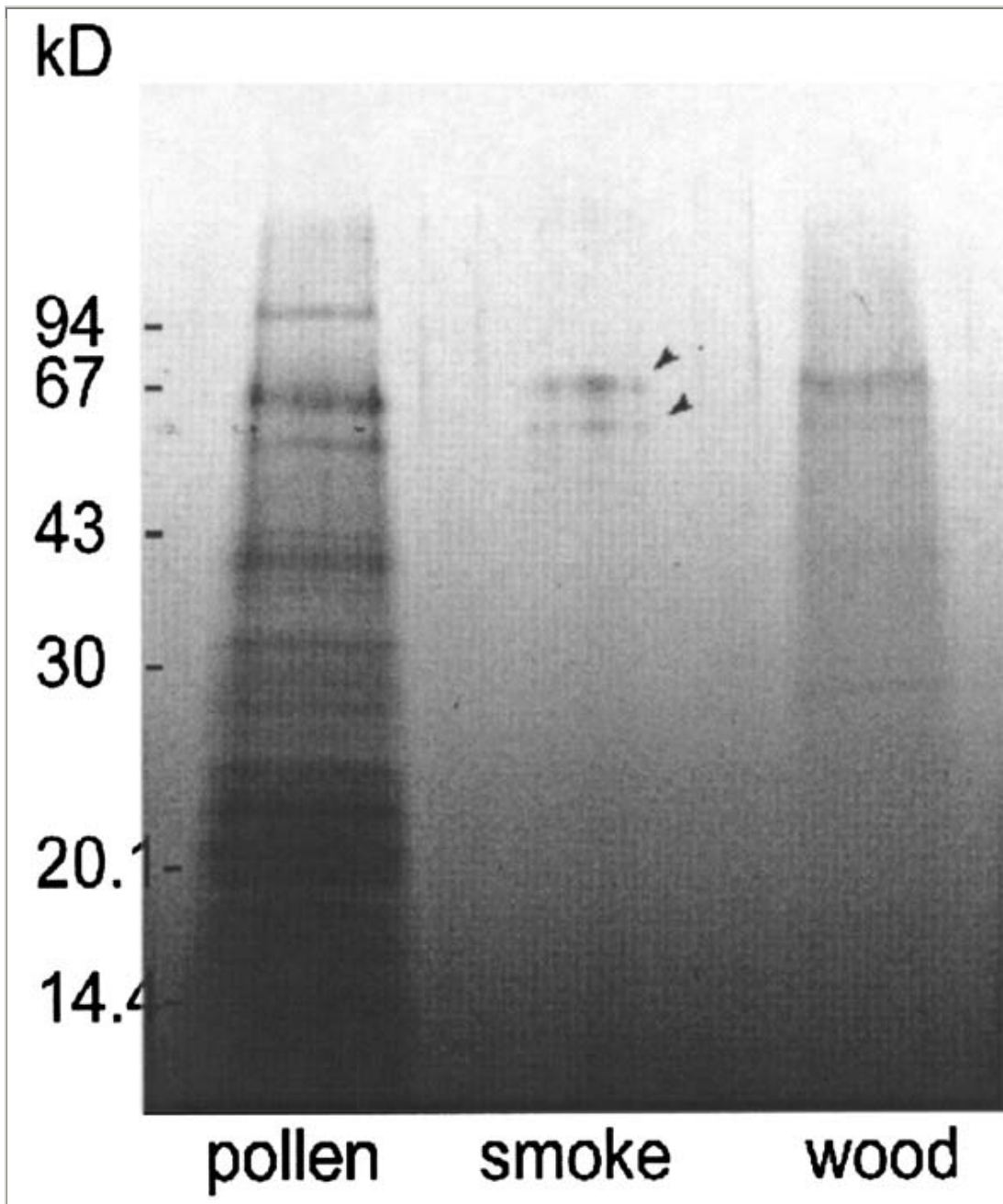
Gels were stained with 0.025 % Coomassie blue R-250. The molecular weight of each stained protein was calculated by comparison of the protein's gel position with a standard curve constructed from the molecular weight standards.

Immunoblots were accomplished as previously described.<sup>5</sup> In short, mesquite proteins from the 3 extracts were transferred from unstained SDS-PAGE gels to 0.45-µm nitrocellulose membranes. After transfer, the molecular weight standards were cut from the nitrocellulose membrane and stained with Coomassie blue. The nitrocellulose membranes were washed, blocked, and incubated overnight at room temperature with one of the following: (1) a 1:5 dilution of a mixture of the "smoke-sensitive" patients' sera in 10 % FBS; (2) a 1:5 dilution of a pooled sera sample from the mesquite pollen SPT-positive patients; or (3) 1:5 pooled cord sera used as a negative control. The membranes were washed and subsequently incubated for 6 hours at room temperature in mouse antihuman IgE monoclonal antibody (Sigma, St Louis, Mo) diluted 1:5000 in 10% FBS. Finally, the membranes were incubated overnight in alkaline phosphatase-conjugated goat antimouse IgG (Chemicon International, Temecula, Calif) diluted 1:2000 in 10% FBS. Membranes were developed, and the molecular weight of each allergen was determined from the molecular weight standards.

Inhibition immunoblots were accomplished as previously described.<sup>5</sup> The procedure duplicated that of the immunoblots, as described above, with preincubation of 10 mL of sera with 10 mg of mesquite pollen protein before the immunoblot procedure.

The SDS-PAGE of the mesquite pollen, wood, and smoke extracts is shown in Fig 1.

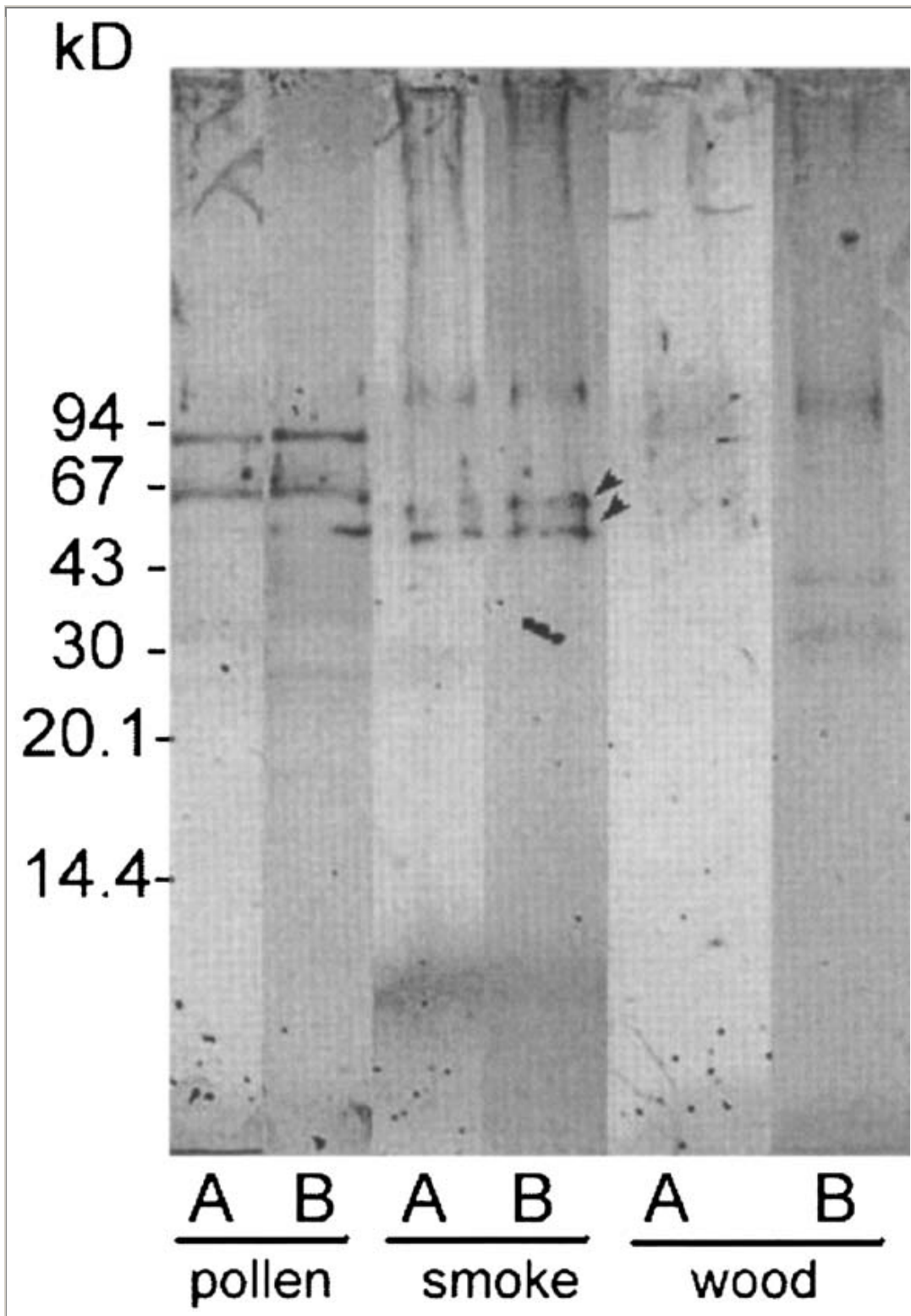
**Fig. 1.** SDS- PAGE of mesquite pollen, smoke, and wood extracts. Protein bands at 66 and 59 kd are shared by the 3 extracts (*arrowheads*) .



The smoke extract contains protein bands in the 66-kd and 59-kd ranges, which are shared by the pollen and wood extracts. The pollen and wood extracts have additional proteins not shared by the smoke extract.

IgE binding to the mesquite pollen and smoke extracts was demonstrated at the 66-kd and 59-kd proteins in both the "smoke- symptomatic" and mesquite pollen SPT –positive groups, as shown in Fig 2.

**Fig. 2.** IgE immunoblots to mesquite pollen, smoke, and wood extracts through use of pooled sera from smoke-sensitive patients **(A)** and pollen SPT–positive patients **(B)** . IgE to proteins at 59 and 66 kd is demonstrated in the mesquite pollen and smoke extracts in both groups (*arrowheads*) .



In addition, both groups demonstrated IgE binding to other proteins in the pollen and wood extracts. The negative control showed no IgE binding.

The inhibition blots performed with mesquite pollen extract partially inhibited IgE to selected bands in the smoke, wood, and pollen immunoblots (data not shown). The procedure was repeated to ensure that this was not due to laboratory error; the results were similar.

Nonpollen sources of airborne allergens have been implicated as possible causes of allergy symptoms during nonpollen seasons and at times when airborne pollen cannot be detected.<sup>6</sup> Although Goetz et al<sup>6</sup> and Steir and Plunkett<sup>7</sup> concluded that smoke collected from burning mountain cedar and grass, respectively, contained no significant allergens, Harper et al<sup>3</sup> determined that tobacco allergens survived combustion and remained immunologically active in smoke.<sup>3</sup>

We have proved that mesquite smoke does contain significant amounts of protein and that this protein elicits an IgE response in susceptible individuals. We describe 3 patients with mesquite smoke allergy and IgE to the 66-kd and 59-kd proteins present in mesquite smoke. Similar proteins were shared by the pollen and wood extracts. Surprisingly, the pooled serum from the mesquite pollen SPT–positive group also contained IgE to the shared 66-kd and 59- kd proteins in the extracts. This finding suggests that the sensitization to mesquite smoke is probably common, though the prevalence of clinical allergy to mesquite smoke is unknown. Therefore, the potential for allergy to mesquite smoke exists in individuals who have mesquite pollen sensitivity.

IgE to the 66-kd and 59-kd proteins shared among the extracts only partially inhibited with pollen extract. This does not necessarily imply that these proteins are different allergens, however. Bernstein et al<sup>8</sup> studied cross-reactivity of tree allergens with RAST inhibition and found that “tree pollen extracts . . . were poorer immunoabsorbents than ragweed or grass allergens” and “absorbed slightly more than 33% activity from their corresponding antibodies.”

Mesquite smoke might represent a new and important cause of occupational,<sup>2</sup> recreational, and food allergy manifesting as upper and lower<sup>2</sup> respiratory tract symptoms as well as oral allergy symptoms. The transfer of allergens to foods cooked over mesquite wood might lead to symptoms in individuals sensitized to shared proteins in mesquite pollen, wood, and smoke. Therefore, individuals with mesquite pollen allergy should consider avoidance of exposure to mesquite smoke and foods cooked over mesquite wood.

The views expressed in this article are those of the authors and do not represent the official policy or position of the United States Air Force, the Department of Defense, or the United States Government.

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