

Letter to the Editor

Mosquitoes as sources of inhalant allergens: Clinicoimmunologic and biochemical studies

To the Editor:

Emanations and detritus of mosquitoes released in air can be potent inhalant allergens causing IgE-mediated allergic respiratory disorders.^{1,2} Three common mosquito species occurring globally are *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. We investigated the allergenic significance of *C quinquefasciatus* in the cause of allergic respiratory diseases, characterized its allergenic proteins, and examined the presence of cross-reacting allergens among the 3 species of mosquitoes.

Two hundred patients with bronchial asthma, allergic rhinitis, or both from the outpatient department of the VP Chest Institute, Delhi, India, and 20 nonallergic healthy volunteers (NHVs) with no family history of allergic disorders were included in the study. The experimental protocol was approved by the institutional ethics committee. Written consent was obtained from each subject.

Three species of mosquitoes were reared in our laboratory, killed by quick freezing, lyophilized, and finely powdered. The powder was defatted with diethyl ether, extracted in 0.1 mol/L ammonium bicarbonate, dialyzed, and lyophilized. Protein contents in these whole-body extracts (WBEs) were as follows: *C quinquefasciatus*, 830 µg/mg; *A aegypti*, 720 µg/mg; and *A stephensi*, 640 µg/mg. The allergenic significance of *C quinquefasciatus* was evaluated by means of skin prick test (SPT), RAST, and immunoblot experiments. SPTs were performed with *C quinquefasciatus* WBE, 50% glycerinated PBS as a negative control, and histamine (5 mg/mL in 50% glycerinated PBS) as a positive control.³ An allergen-induced skin wheal response equivalent to the histamine response was graded as 2+. Skin responses of 2 mm less or 2 mm more than the histamine reaction were graded as 1+ and 3+, respectively. A wheal response of greater than 3+ was graded as 4+. Of the 200 patients undergoing skin tests, 35% (n = 70) elicited total positive (1+ to 4+) and 18.5% (n = 37) elicited markedly positive (2+ to 4+) responses. Only 1 of 20 NHVs had a 1+ reaction. *C quinquefasciatus* RASTs⁴ were performed to estimate allergen-specific IgE in patients' sera. Patients with increasing grades of SPT responses showed a concomitant increase in RAST positivity (24.2% in 1+, 69.2% in 2+, and 100% in 3+/4+ cases). Average RAST positivity was 52.9% in 1+ to 4+ and 78.4% in 2+ to 4+ cases. Only 1 of the 31 patients with negative SPT responses had a positive RAST result. Although homologous extract induced dose-related inhibition of *C quinquefasciatus* RAST, no significant inhibition was observed with 8 unrelated heterologous extracts (Fig 1). ANOVA showed a statistically significant difference when inhibition lines produced by the heterologous extracts were compared with those of the homologous extract ($P < .01$), establishing specificity of the *C quinquefasciatus* RAST.

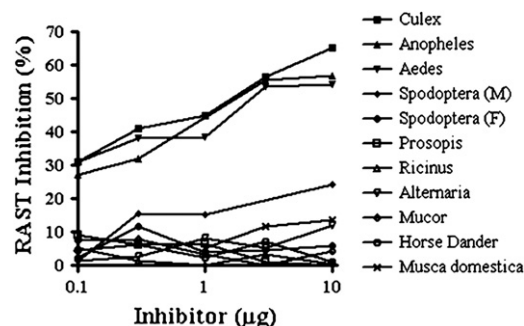


FIG 1. Inhibition of mosquito (*C quinquefasciatus*) RAST results with homologous (*C quinquefasciatus*) and the 2 heterologous mosquito WBEs (*A aegypti* and *A stephensi*) and 8 unrelated allergen extracts.

C quinquefasciatus allergen in air was measured by using an immunochemical technique,⁵ which confirmed the patients' exposure to the same. By using a volumetric sampler, airborne particles were collected on a polytetrafluoroethylene membrane at 20 m in height fortnightly for 1 year. The exposed membranes were soaked in 0.1 mol/L ammonium bicarbonate. The eluates were dialyzed, lyophilized, and reconstituted in appropriate diluent (1 mL) and were used as liquid-phase inhibitors (50-µL aliquots) in *C quinquefasciatus* RASTs. *C quinquefasciatus* allergen in these samples was calculated from the inhibition line generated with the homologous extract. There were day-to-day variations in the airborne *C quinquefasciatus* allergen (11-220 ng/m³). The pool of eluates showing high *C quinquefasciatus* allergen levels (n = 10) produced dose-related inhibition of *C quinquefasciatus* RAST results (data not shown). The slope of this inhibition line was parallel to that produced by homologous WBE. To the best of our knowledge, the presence of mosquito allergens in small-sized airborne particles is being reported here for the first time.

C quinquefasciatus, *A aegypti*, and *A stephensi* WBEs were subjected to SDS-PAGE.⁶ Several proteins (15-120 kD) were detected in the 3 mosquito WBEs, with variations in their protein profiles (Fig 2, A). Allergenic proteins in *C quinquefasciatus* WBE were identified by performing immunoblot experiments⁷ with pooled patients' sera (PPS [*C quinquefasciatus*]; Fig 2, B). PPS (*C quinquefasciatus*) was prepared by pooling sera from 20 patients with highly positive skin test and RAST results. Seven proteins of 24, 30, 33, 40, 48, 58, and 70 kD of *C quinquefasciatus* WBE bound the patients' IgE. In immunoblots with individual patients' sera (n = 6), IgE-binding protein profiles varied from patient to patient, showing heterogeneity of patients' IgE responses to different proteins of *C quinquefasciatus* WBE (Fig 2, B). Although IgE-binding protein of 33 kD was detected with all 6 patients' sera, 24- and 30-kD allergenic proteins were detected with 5 of the 6 sera. Thus proteins of 24, 30, and 33 kD were found to be the major allergens⁸ of *C quinquefasciatus* WBE.

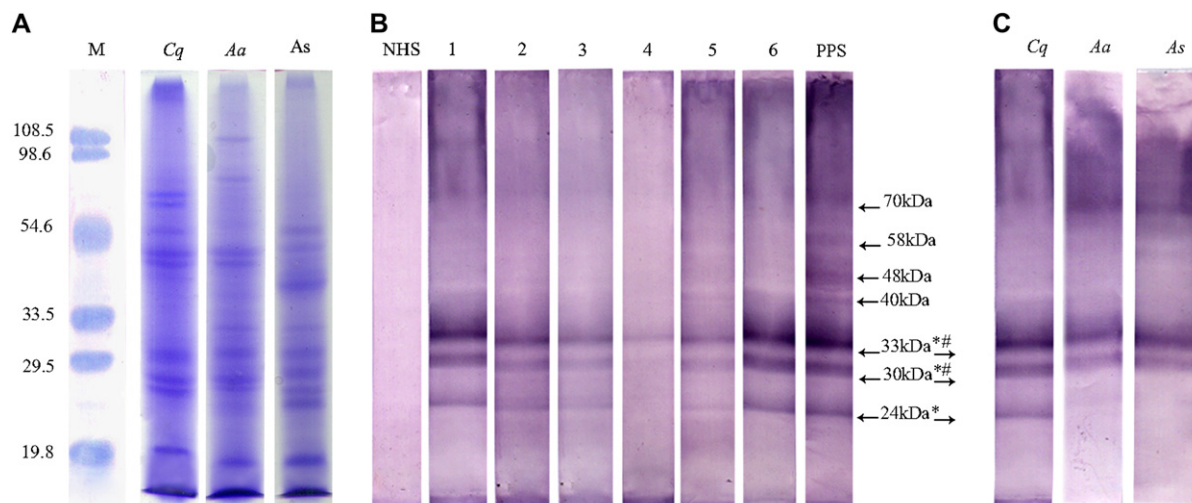


FIG 2. A, SDS-PAGE of 3 mosquito extracts. M, Molecular weight markers (in kilodaltons); Cq, *C quinquefasciatus* WBE; Aa, *A aegypti* WBE; As, *A stephensi* WBE. B, Immunoblots of *C quinquefasciatus* WBE with patients' sera. NHS, Normal human serum; 1-6, individual patients' sera; PPS, pooled patients' sera from patients with *C quinquefasciatus* allergy. C, Immunoblots of *C quinquefasciatus* (Cq), *A aegypti* (Aa), and *A stephensi* (As) WBEs with PPS (*C quinquefasciatus*). *Major allergen. #Cross-reacting allergens.

Immunoblots using sera from patients with negative SPT and RAST results, as well as NHVs, were uniformly negative.

Skin tests with *A aegypti* and *A stephensi* WBEs were performed on 26 *C quinquefasciatus*-sensitive patients. Nineteen (73.1%) patients had positive responses to both the heterologous extracts and 3 each to *A aegypti* and *A stephensi*. Only 1 patient had exclusively positive results to *C quinquefasciatus* WBE. These results suggested the presence of cross-reacting allergens in the 3 species of mosquitoes. This observation was further supported by the results of RAST inhibition and immunoblot experiments. In *C quinquefasciatus* RASTs, homologous, as well as the 2 heterologous, mosquito WBEs produced dose-related inhibition. Slopes of the inhibition lines obtained with the 2 heterologous extracts were not significantly different from that of the homologous extract ($P > .05$, Fig 1). WBE protein required for 50% inhibition of *C quinquefasciatus* RASTs was as follows: *C quinquefasciatus*, 1.08 μ g; *A aegypti*, 0.97 μ g; and *A stephensi*, 1.4 μ g. In immunoblot experiments using PPS (*C quinquefasciatus*) and the 2 heterologous mosquito WBEs, 2 IgE-binding major allergenic proteins of *C quinquefasciatus* extract (30 and 33 kD) were also detected in *A aegypti* and *A stephensi* WBEs (Fig 2, C). When PPS (*C quinquefasciatus*) was incubated with *C quinquefasciatus* solid-phase allergen, the supernatant failed to show any IgE-binding proteins in immunoblots with the 3 mosquito WBEs. These results provided evidence that 30- and 33-kD proteins are shared among the 3 mosquito WBEs.

Our results revealed that mosquito-derived allergens are present in air, which might serve as important inhalant allergens in type I allergic respiratory disorders. Three different-sized proteins found in the *C quinquefasciatus* allergen preparation have been identified that bind patient

IgE. Two of these proteins were also present in the other 2 mosquito species (cross-reacting allergens).

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