The prevalence of rat allergen in inner-city homes and its relationship to sensitization and asthma morbidity

Tamara Perry, MD,a Elizabeth Matsui, MD,a Barry Merriman, MA,b Trang Duong, BA,a and Peyton Eggleston, MDa Baltimore, Md

Background: Rat allergen has proved to be an important cause of IgE-mediated hypersensitivity in the occupational setting. The prevalence and significance of rat allergen in homes has not been studied.

Objective: The purpose of this study was to determine the prevalence of rat allergen in the homes of inner-city children with asthma and to examine the relationship between rat allergen exposure, sensitization, and asthma morbidity.

Methods: We developed a new monoclonal-based ELISA to determine the prevalence of rat allergen in dust samples from inner-city homes of the National Cooperative Inner-City Asthma Study population. Home characteristics were evaluated to detect variables that were associated with the presence of rat allergen. Data were also analyzed to assess the relationship between the presence of rat allergen, sensitization, and asthma morbidity.

Results: Thirty-three percent of inner-city homes had detectable rat allergen (Rat n 1). The presence of rat allergen was associated with reported rat and mouse infestation, as well as evidence of mouse infestation on home inspection. Twenty-one percent of the participants were sensitized to rat allergen; however, sensitization was not more common when rat allergen was found in the home. The number of hospitalizations, unscheduled medical visits, and days with slowed activity because of asthma were significantly increased in those individuals who were both sensitized and exposed to rat allergen.

Conclusions: Rat allergen sensitization and exposure are associated with increased asthma morbidity in inner-city children.

(J Allergy Clin Immunol 2003;112:346-52.)

Key words: Rat allergen, indoor allergens, inner-city asthma, sensitization, asthma morbidity

Rat allergen is a well-documented cause of IgE-mediated occupational disease; however, its role in allergic diseases outside of the work environment remains unclear. In the occupational setting, sensitization to rat allergen is common among those who work with rats in the laboratory, with prevalence estimates ranging from 12% to 31%. Rat and rat allergen exposure has been shown to be a risk factor for both sensitization and allergic symptoms. The significance of rat exposure and sensitization outside of the occupational setting has not been demonstrated in previous studies. In the National Cooperative Inner City Asthma Study (NCICAS), 19% of the study population was sensitized to rat allergen, suggesting that rat allergen exposure might play an important role in both the development and severity of asthma in children living in inner-city homes.

Studies in occupational settings have demonstrated an increased risk of sensitization to rat allergen with increasing exposure. In addition, exposure to increasing concentrations of rat allergen has been associated with increasing upper and lower airway responses in sensitized workers. It is uncertain whether these associations between rat allergen exposure, sensitization, and disease hold true outside of the occupational setting. To date, the major mouse allergen, Mus m 1, is the only rodent allergen studied in inner-city children with asthma. Phipatanakul and colleagues found that 18% of children in the NCICAS population were sensitized to mouse and 95% of homes had detectable mouse allergen. Exposure to Mus m 1 levels above the median (1.60 µg/g) was associated with sensitization to mouse allergen, but no statistically significant relationship between Mus m 1 exposure and asthma morbidity was found. Because of these findings and the already established role of rat allergen in occupational allergic disease, we sought to examine the prevalence of rat allergen exposure among the NCICAS participants. In addition to describing the distribution of Rat n 1, the major rat allergen, we examined the relationship between rat allergen exposure, sensitization, and asthma morbidity in this population.

METHODS

Study population and baseline evaluation

This study is based on a re-analysis of household dust samples collected in the NCICAS. The NCICAS population consisted of 1528 asthmatic children from 8 major inner-city areas. Participants had asthma and lived in neighborhoods where at least 30% of households had incomes less than the 1990 poverty level. The children...
dren and primary caretakers underwent a baseline evaluation that included extensive medical, environmental, demographic, and psychosocial interviews. Prick-puncture tests were performed to German and American cockroach, *D. pteronyssinus*, *D. farinae*, cat, dog, mouse, rat, *Alternaria tenuis*, *Penicillium* species, mixed grasses, orchard grass, white oak, maple, and giant and short ragweed in 50% glycerosaline (Greer Laboratories, Lenoir, NC), as previously described. We used previously published criteria to grade skin test results, and the skin test panel was considered valid if the wheal from the histamine control (1 mg/mL) was at least 1 mm larger than the negative control. The skin test response was considered positive if the panel was valid, and the mean wheal diameter of the allergen test was at least 2 mm larger than the negative control wheal.

The NCICAS protocol called for home evaluation on half of the study population. Home visits were conducted in the homes of 75 participants from each site, and a total of 663 visits from all study sites was carried out within 1 month of baseline according to protocol. Of the 663 dust samples, there were adequate samples for analysis for Rat n 1 from 602 bedrooms, 603 living rooms, and 556 kitchens. This study uses the previously collected data and dust samples of 489 children (Table I) from the original NCICAS population who had valid skin test results, adequate dust samples, and complete home characteristics data. Morbidity data were available for 480 of these records for analysis.

### Home evaluation and dust samples

At each home visit, trained personnel inspected the home according to a protocol, documenting construction; environmental conditions; and evidence of cockroach, mouse, and rat infestation. In addition, the family was interviewed regarding characteristics such as laundry methods, smoking habits, and infestations. Dust samples were collected from the child’s bedroom, the television/living room, and the kitchen using standard methods. Dust samples were sieved and stored at −30°C until extraction according to published methods. For this study, samples were thawed and analyzed for Rat n 1 using a monoclonal-based ELISA.

### Assessment of asthma morbidity

Asthma morbidity was assessed in terms of health care utilization, clinical symptoms, activities of daily life, and effect on caretakers. Caretakers were questioned during the baseline interview and by telephone interview at 3, 6, and 9 months. Recall of health care utilization and school days missed over the previous 3 months, clinical symptoms, daily activity, and effect on the caretaker over the prior 2 weeks was obtained at each interview. Health care utilization included the number of hospitalizations and unscheduled visits for asthma. For morbidity analyses, the averages of hospitalizations and unscheduled visits and percent of school days missed per session were obtained from the 3-, 6-, and 9-month interviews. In addition, the mean number of days per 2 weeks for clinical symptoms, activities of daily life, and the effect on the caretaker were obtained from these same interviews. Subjects were classified as sensitized to rat allergen if the skin test panel was valid, and the rat skin test response was positive. The exposed population included those subjects with detectable Rat n 1 in the bedroom dust sample.

### Preparation of monoclonal antibodies

Rat n 1 was purified from male Sprague-Dawley rat urine using dialysis against cold distilled water for 7 days followed by chromatography. AJ mice were immunized with purified Rat n 1 allergen, and immune spleen cells were fused with the mouse myeloma cell line Sp2/0-Ag14. Three hybridoma clones, 4D12 (IgG2b), 4F7 (IgG1), and 5C7 (IgG1), were selected on the strength of ELISA and ELISA against purified Rat n 1 and were grown in culture. Culture supernatants were enriched by affinity chromatography on recombinant protein G columns.

The 3 mAbs, 4D12, 5C7, and 4F7, were found to have slightly different specificities for Rat n 1. On Western blot, 4F7 recognized both 19 kDa Rat n 1 and a second protein at 30 kDa, whereas the other 2 mAbs recognized only Rat n 1 in the 19 kDa and 18 kDa state. This difference was also reported with the mAb described by Renstrom and colleagues and probably relates to the 2 isoforms of Rat n 1.

### ELISA procedure

Monoclonal capture antibody 4F7 (1 µg/mL) was coated on 96-well microtiter plates (Dynatech, Immunon IV, Chantilly, Va) overnight at 4°C in carbonate-bicarbonate buffer, pH 9.6, and blocked with PBS containing 1% BSA and 0.05% Tween 20. Purified Rat n 1 (0.5-20 ng/mL) or unknown samples diluted in PBS were added in duplicate and incubated for 1 hour at 25°C. Plates were then washed with PBS/Tween 20, and 100 µL of 1 µg/mL biotinylated detector mAb 5C7 was added. Purified mAb 5C7 was biotinylated using biotinyl-epison-amino caproic acid N-hydroxysuccinimide ester and blocked with N-HCl. After a second incubation of 2 hours at 25°C, the plates were washed again. Streptavidin-peroxidase (Sigma Chemical Company, St Louis, Mo) was added (125 ng/mL) for 30 minutes, the plates were washed again, and then they were developed with 1 mmol/L 2,2′-Azino-di-3-ethylbenzthiazoline-6-sulfonic acid in 70 mmol/L citrate phosphate buffer, pH 4.2, plus 0.03% hydrogen peroxide. The colormetric reaction was stopped with 1% SDS and read at 405 to 490 nm using SoftMax pro (Molecular Devices, Sunnyvale, Calif). The Mus m 1 ELISA was performed as previously described.
Environmental and occupational disorders

Statistical analysis

The study population was compared with the remainder of the NCICAS population using Student t test for continuous variables and chi-square analysis for categorical variables. Rat allergen and mouse allergen levels were compared using Spearman’s rank correlation. Odds ratios and chi-square tests were used to relate housing characteristics to detectable levels of rat allergen in the children’s bedrooms. Linear regression models were used to assess the relationship of bedroom rat exposure and rat sensitization to asthma morbidity. Averages for morbidity measures were calculated based on the 3-, 6-, and 9-month interviews. Covariates in the final model included sex, family history of asthma, Child Behavior Checklist score, tobacco smoke exposure, cockroach exposure, cockroach sensitization, and atopic status. Mouse exposure and sensitization were included in early models but were not included in the final model, because no effect was seen for either variable. For each analysis, the group that was both sensitized and exposed to rat allergen was compared with all other children as previously described.10

RESULTS

The dose-response curve for the ELISA for purified Rat n 1 is shown in Fig 1. Binding was maximal at a Rat n 1 concentration of 200 ng/mL, and the detection limit was 0.22 ng/mL, equivalent to 4.4 ng/g in settled dust. Mus m 1 was not reactive in the system at concentrations up to 200 ng/mL. In the NCICAS home samples, Rat n 1 was found in 129 of 602 (21%) bedrooms, 164 of 603 (27%) living/TV rooms, and 104 of 556 (19%) kitchens. For all homes with analyzable dust, 225 of 645 (33%) had detectable Rat n 1 in any room. The range of measurable Rat n 1 was 4.4 to 1413 ng/g in the bedroom, 4.4 to 3380 ng/g in the living room/TV room, and 4.4 to 4620 ng/g in the kitchen. The median level for all rooms was below detection. Because this analysis measured Rat n 1 in many of the same samples on which Mus m 1 analyses were performed,6 we plotted levels of rat allergen against mouse allergen measured in these samples. As demonstrated in Fig 2, there is no correlation between rat and mouse allergens in dust samples (r = .298). Specifically, increasing concentrations of Mus m 1 did not correlate with concentrations of Rat n 1, and in the 473 (79%) homes without detectable bedroom rat allergen, concentrations of Mus m 1 ranged from below detection (n = 79) to 153 µg/g.

The children in this study had characteristics similar to the entire NCICAS population3 (Table I). The mean age of the children was 6.2 years (range, 4-9), and 63% were males. Forty-two percent had a family history of asthma, 58% had at least 1 smoker in the home, 43% had inadequate social support, and 21% were sensitized to rat allergen. There were significant differences in the racial and income distributions of the 2 groups. In addition, a larger percentage of participants was sensitized to mouse in this study population than in the entire NCICAS population.3

Many home characteristics were examined, and these characteristics were similar to the original NCICAS population.3 Among the homes sampled, 46% of homes were older than 50 years, 46% had evidence of disrepair, 44% had evidence of trash or dirty dishes in the kitchen, and 51% had wall-to-wall or large bedroom carpeting (Table II). The homes consisted of 20% single-family dwellings, 22% row homes/duplexes, and 56% apartment houses. Certain home characteristics were related to the presence of rat allergen. As shown in Table II, reported infestation with rats or mice in the past year and evidence of mouse infestation, such as mouse droppings, on home inspection were associated with the presence of rat allergen in the
Although only 47% of homes with reported rat infestation had detectable rat allergen, these homes were more than 3 times as likely to have detectable rat allergen in dust samples than homes without reported rats ($P < .001$). Similarly, homes with reported problems with mice were more than 3 times as likely to have detectable rat allergen ($P < .001$). Homes with evidence of mouse infestation on bedroom inspection were more likely to have detectable rat allergen ($P = .03$). Evidence of cockroaches in the bedroom was not associated with the presence of rat allergen ($P = .92$). Similar results were seen in the kitchen and TV/living room (data not shown).
TABLE III. Relationship of bedroom rat exposure and asthma morbidity (n = 480)

<table>
<thead>
<tr>
<th></th>
<th>Negative skin test</th>
<th>Positive skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not exposed (group 1)</td>
<td>Exposed (group 2)</td>
</tr>
<tr>
<td>N</td>
<td>297</td>
<td>81</td>
</tr>
<tr>
<td>Hospitalizations in past year* (no.)</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Unscheduled medical visits in past year* (no.)</td>
<td>0.41</td>
<td>0.44</td>
</tr>
<tr>
<td>Days wheezing in past 2 weeks§ (no.)</td>
<td>3.34</td>
<td>3.46</td>
</tr>
<tr>
<td>Nights child lost sleep in past 2 weeks§ (no.)</td>
<td>1.63</td>
<td>1.99</td>
</tr>
<tr>
<td>Days with reduced activity in past 2 weeks§ (no.)</td>
<td>1.86</td>
<td>2.02</td>
</tr>
<tr>
<td>Days caretaker changed plans in past 2 weeks§ (no.)</td>
<td>2.52</td>
<td>3.46</td>
</tr>
<tr>
<td>Nights caretaker lost sleep in past 2 weeks§ (no.)</td>
<td>2.02</td>
<td>2.36</td>
</tr>
<tr>
<td>School days missed in last 3 months (%)¶</td>
<td>6.06</td>
<td>6.10</td>
</tr>
</tbody>
</table>

*Denotes average number per child per year.
†Denotes average number per child per year.
‡Denotes average number per child per year.
§Denotes average number per child per 2 weeks.
¶Denotes percent days missed while school in session.

 Linear regression models adjusted for family history of asthma, sex, smoke exposure, Child Behavior Checklist score, cockroach exposure, cockroach sensitization, and atopy.

This is the first study to examine the prevalence of rat allergen in the home environment. We found 33% of inner-city homes had detectable rat allergen, suggesting that exposure to this particular allergen is common in these homes, although not as common as mouse allergen, which was detected in 95% of the homes. Rat allergen was more common in the TV/living room (27%) than in the kitchen (19%) and bedroom (21%). This is in contrast to mouse allergen, which was more evenly distributed throughout the home and found in 87% of all TV/living rooms, kitchens, and bedrooms. It is likely that this dissimilarity in prevalence and distribution of rat and mouse allergens is due to the differences in the nesting habits of these rodents. Norwegian rats (Rattus norwegicus), the most common species in inner cities, are ground dwelling and build nests in underground burrows. Unless populations are very heavy, they do not nest in buildings. The Norway rats typically live in families and will forage for food and water at distances 30 to 50 m from the nest as long as food and water are easily accessible. Thus, the most likely site of allergen contamination would be in the rat’s nest rather than in homes, where they might forage.
Mice, on the other hand, are more likely to live indoors and nest near food stores, within cabinets, closets, walls, and in the voids of large appliances.10 For mice, contamination by urinary allergen is likely to be more intense and widespread in homes. This difference in ecology is compatible with the observation that 51% of families reported mice, whereas only 8% reported rats.

Reports of rat and mouse infestation, as well as evidence of mouse infestation on home inspection, were associated with detectable bedroom rat allergen. Surprisingly, both evidence of poor housekeeping, such as trash or dirty dishes in the kitchen, and the presence of carpeting seemed to protect against having detectable bedroom rat allergen. Consistent with this paradox, homes with a working vacuum cleaner were more likely to have detectable rat allergen, but this finding was not statistically significant. These findings were unexpected, prompting us to consider the possibility that these home characteristics were indicators of socioeconomic status (SES). However, analysis of the associations between these home characteristics and SES did not support the notion that SES was a confounder. In fact, the presence of a working vacuum cleaner and presence of carpeting were both associated with higher SES, whereas poor housekeeping was not associated with either higher or lower SES. We are left, then, without a satisfactory explanation for these findings.

Interestingly, only 47% of homes with reported rat infestation had detectable rat allergen. Because rats primarily live outdoors, they are commonly seen by inner-city residents outside of the home. The NCICAS questionnaire asked, “In the past year, have you had problems with rats?” but did not specify whether reported rats were seen inside or outside the home; therefore, it is possible that some reporting of rat infestation included rats that were seen outside. This could explain why more than half of homes with reported rat infestation had no detectable rat allergen. Another explanation might have been that the rat allergen assay was not sensitive enough to detect allergens in these samples, but the detection limit for the rat ELISA (4 ng/g) is similar to the detection limit of other animal allergen ELISA17 and only slightly higher than the detection limits for Mus m 1.18 We also found that significantly more row homes had detectable rat allergen than attached homes and apartments. Because virtually all of the row homes were located in Baltimore (95%), it is difficult to say whether this finding is related to the type of home or to the city.

The ranges of measurable rat allergen in inner-city homes were > 100-fold lower than levels of mouse allergen found in this same population.6 Because Rat n 1 and Mus m 1 allergens have > 80% homology,19 we considered that the low level of rat allergen represented only cross-reactivity to Mus m 1. The demonstration that the assay was not able to detect purified Mus m 1 at high concentrations (Fig 1) and that the measured levels of Rat n 1 in the same household dust samples did not correlate with Mus m 1 concentrations (Fig 2) suggest that cross-reactivity was not a factor. In fact, many homes with very high Mus m 1 levels had undetectable rat allergen.

The presence of rat allergen in the home was not associated with sensitization to rat. This finding is in contrast to mouse allergen, for which an association between home exposure and sensitization has been demonstrated.7 However, sensitization to rat was more common than sensitization to mouse (21% vs 18%), despite the increased prevalence and much higher exposure to mouse allergen in the home. This apparent disparity between exposure and sensitization prevalence could be due to a protective effect at higher levels of mouse exposure as has been suggested to occur with cat and dog allergens.20 Another, more plausible, explanation is that home exposure might be a more valid measure of true exposure for mouse than for rat allergen. For example, children might be exposed to mouse allergen primarily inside of homes where mice reside. For rat allergen, exposure might occur primarily outside of the homes, in yards or alleys or perhaps in school. Home rat allergen measurements might then be a less accurate measure of rat allergen exposure.

The most remarkable finding in this study was the relationship between rat allergen and morbidity in inner-city individuals with asthma. Although found in a minority of homes, the presence of rat allergen is associated with significantly higher asthma morbidity among rat-sensitized children. Sensitized and exposed individuals had significantly more hospitalizations, unscheduled medical visits, and more days with slowed activity because of asthma. This relationship remained significant after controlling for confounding psychosocial and environmental factors, including cockroach sensitization and exposure. A relationship between mouse allergen exposure and asthma morbidity was not found in earlier studies,7 because mouse allergen exposure was so closely associated with cockroach allergen exposure that any distinct effect of mouse allergen on morbidity could not be isolated from that caused by cockroach allergen. In this analysis, bedroom rat allergen exposure did not correlate with cockroach exposure; thus, the effect seen is likely due to rat exposure. Because this study population differed from the total NCICAS study population in terms of mouse sensitization, race, and income, we considered these variables in our analysis. There was no effect seen when mouse sensitization was included in earlier models, suggesting that mouse sensitization was not a significant confounder. Neither race nor income were significant predictors of morbidity and were therefore not included in the final model. We have demonstrated that rat allergen is not only commonly found in inner-city homes, but that it is a likely contributor to morbidity in inner-city children with asthma. This is a striking finding, because the association with morbidity remained significant after controlling for cockroach sensitization and exposure. These results suggest that rat allergen exposure is an important public health concern, and control measures should be implemented in inner-city neighborhoods. Rat allergen reduction measures might have a significant impact on asthma morbidity for inner-city individuals with asthma and reduce the overall
health care utilization for this high-risk population. To further explore and define the significance of rat allergen in the inner-city, future studies in this population should include measures of rat allergen exposure, sensitization, and the effect on morbidity.

REFERENCES

11. Longbottom JL. Purification and characterization of allergens from the inner-city, future studies in this population should include measures of rat allergen exposure, sensitization, and the effect on morbidity.

APPENDIX

In addition to the authors, the following investigators from the NCICAS participated in this study. Albert Einstein School of Medicine, Bronx, NY: D. L. Rosenstreicher, E. Crain, and L. Bauman; Children’s Memorial Hospital, Chicago Ill: R. Evans III, J. Lavigne, Y. D. Senturia, C. M. Weil, K. K. Christoffel, and H. J. Bins; Cook County Hospital, Chicago, Ill: M. Sullivan, J. H. Mayefsky, and M. F. McDermott; Rainbow Babies and Children’s Hospital, Cleveland Ohio: C. Kermcar, S. Redline, and S. Wade; Henry Ford Hospital and Medical Center, Detroit, Mich: D. Ownby, J. A. Anderson, F. E. Leicky, C. M. Joseph, and C. Johnson; Mount Sinai School of Medicine, New York, NY: M. Kattan, C. Lamm, M. T. Tin, G. Butts, E. Luder, and D. Baker; Washington University Medical School, St. Louis, Mo: H. J. Wedner and G. Evans; St. Louis University, St. Louis, Mo: R. G. Slavin; Howard University, Washington, DC: F. Malveaux, A. Thomas, S. Molock, and M. Richard; National Institute of Allergy and Infectious Diseases, Program Office, Bethesda, Md: P. Gergen, E. Smartt, K. Weiss, and R. Kaslow; Center for Occupational Environmental Health, Irvine, Calif: D. Baker; New England Research Institutes, Watertown, Mass: H. Mitchell, K. McNiff-Mortimer, H. Lynn, and S. Islam.